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Amirhossein Sahebkar
Thozhukat Sathyapalan *Editors*

Natural Products and Human Diseases

Pharmacology, Molecular Targets, and Therapeutic Benefits

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Preface

Natural products have a long history of use as folk medicines in several systems of traditional medicine. Extensive evidence from modern pharmacological studies has also confirmed traditional applications and unveiled the vast potential of naturally occurring compounds, particularly plant-derived phytochemicals, in the management of various human diseases. Of note, the past decade has witnessed a surge of findings from randomized controlled trials testifying to the safety and efficacy of natural products either as adjuncts or even alternative to standard-of-care medications for several illnesses. Biomolecular studies have unveiled hundreds of cellular and molecular targets for phytochemicals, including key transcription factors, receptors, enzymes, hormones, neurotransmitters, cytokines, lipids and non-coding RNAs.

Extensive research on the preventive and therapeutic effects of natural products necessitates regular updating of the literature regarding the potential roles of these compounds in different human diseases. This new book is distinctive in providing the most recent update on the pharmacological and clinical features of natural products and the role of phytopharmaceutical compounds in health and disease. The chapters are written by the authors with a long-standing of research on the health benefits of natural products. Iran has been a major source of research and publication on different aspects of natural products, especially medicinal plants. During the past decade and Iranian authors are among the world leaders of research on natural medicines based on the metrics provided by international indexing databases. Authoritative chapters are written by experienced authors and scientists well known for their contributions in their research topics, which makes this book suitable for researchers within the natural product research community and attractive to a broad audience including physicians, clinical scientists, and major drug companies. The chapters will collectively provide useful insights on the regulatory effects of phytochemicals and nutraceuticals on pathogenic molecular signatures associated with pathologies, disease biomarkers and ageing-related pathways.

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Effects of Curcuminoids on Systemic Inflammation and Quality of Life in Patients with Colorectal Cancer Undergoing Chemotherapy: A Randomized Controlled Trial

Yunes Panahi, Maryam Saberi-Karimian, Omid Valizadeh, Behzad Behnam, Alireza Saadat, Tannaz Jamialahmadi, Muhammed Majeed, and Amirhosein Sahebkar

Abstract

Background: Colorectal cancer (CRC) is the third and the fourth most common cancer in Iranian men and women, respectively. Curcuminoids are known to exertprotective effects against several kinds of cancers. We aim to assess the effects of curcuminoids on serum pro- and anti-inflammatory cytokines and quality of life in patients with colorectal cancer undergoing chemotherapy.

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Material and Methods: This study was a double-blind placebo-controlled trial in patients with CRC (stage 3) aged ≥20 years, who had chemotherapy after the surgery and were referred to Baqiyatallah Oncology Clinic. Patients were randomly assigned to the treatment group receiving curcuminoids capsules (500 mg/day) (n = 36), or the control group taking placebo capsules (n = 36) for 8 weeks. Erythrocyte sedimentation rate (ESR) and serum levels of C-reactive protein (CRP) and 12 pro- and anti-inflammatory cytokines including tumor necrosis factor (TNF-α), interleukin-1α (IL-1α), IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP-1), interferon γ (IFN-γ), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF)] were measured at baseline and at the end of the intervention. The EORTC-QLQ-C30 instrument was used to assess the quality of life before and after the intervention. Statistical analyses were performed using SPSS software.

Results: A total of 67 subjects completed the study as three and two subjects were lost to follow-up in the curcuminoid and placebo groups, respectively. A significant change in CRP (p = 0.002) and ESR (p = 0.0001) was observed in patients supplemented with curcuminoids at the end of 8 weeks compared to placebo. Moreover, IL-1α showed a decreasing trend after curcuminoid supplementation compared to placebo (p = 0.077). A significant improvement in functional (p = 0.002) and global quality of life (p = 0.020) scales was observed in the curcuminoid group.

Conclusions: The results showed that curcuminoids supplementation for a period of 8 weeks (500 mg/day) can improve ESR and serum levels of CRP in stage-3 CRC subjects and improve the global quality of life and functional scales compared to placebo.

Keywords

Colorectal cancer · inflammation · cytokines · growth factors · quality of life · curcumin

1 Introduction

Colorectal cancer (CRC) is a complex disease that occurs as a consequence of many genetic and epigenetic alterations in key oncogenes and tumor suppressor genes [1]. Lifestyle factors such as diet, exercise, and obesity have been linked to its risk and it is the fourth leading cause of cancer-related deaths in the world. Its burden is expected to increase by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030 [2]. In Iran, around 5000 new CRC cases are reported annually [3], and the incidence has been rising in recent years [4]. The five-year survival of patients detected at an early stage (stage 1) is more than 90% compared to 10% for late detection of the disease (stage 4) [5–7]. It has been shown that the cancer-related outcomes are associated with quality of life, and prognosis can be influenced by lifestyle factors [8, 9]. Investigating the quality of life effects in CRC screening has shown that screening does not have adverse emotional effects in the long term (>4 weeks) [10, 11].

There is an association between lifestyle and age with CRC. The adherence to diets such as consumption of processed meat and alcohol, obesity, smoking, and sedentary lifestyles is known to increase the risk of CRC [12–14]. Inflammatory bowel diseases including Crohn's disease and ulcerative colitis as well as familial adenomatous polyposis and hereditary non-polyposis CRC account for other hereditary risk factors for CRC observed in less than 5% of the patients. The disease usually begins with a benign tumor that eventually progresses to cancer [15]. The techniques of sigmoidoscopy and colonoscopy have been used for CRC screening. Any polyps found can be removed during the colonoscopy. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be associated with

a reduced risk for CRC in clinical trials, although these drugs are not recommended due to their side effects [16]. Generally, there are three therapeutic approaches for CRC, which are surgery, radiotherapy, and chemotherapy [17].

Curcumin is a phytochemical extracted from turmeric (*Curcuma longa* L.). There are several studies about its efficacy in the treatment of different pathologies and diseases [18–25]. Previous studies have shown that curcumin has protective effects in several kinds of cancers through its action on multiple targets and through different molecular mechanisms [26]. Curcumin exerts its potential anticancer effects through different biological pathways including apoptosis, cell cycle regulation, oncogene expression, mutagenesis, tumorigenesis, and metastasis, and acts as an adjunct therapy to improve the effects of chemotherapy and radiotherapy in cell carcinomas [27]. The effects of curcumin on systemic inflammation including cytokines and growth factors have been reported previously [28–30]. In the current study, we aimed to assess the effects of curcuminoids on serum pro- and anti-inflammatory cytokines and quality of life in patients with CRC undergoing chemotherapy.

2 Methods

2.1 Study Design

The current study was a double-blind placebo-controlled trial approved by the Ethics Committee at the BaqiyatallahUniversity of Medical Sciences (ID: IR.BMSU.REC.1396.1870). The protocol was explained to participants before the study and a written information consent form was completed by all subjects. Patients with stage 3 CRC aged ≥20 years-old, who were on chemotherapy and referred to Baqiyatallah Oncology Clinic, were recruited in the current study. Checklists of patient demographic and clinical history information were completed at the baseline. A quality of life questionnaire (EORTC-QLQ-C30) was recorded at baseline and after 8 weeks of intervention.

Patients were randomly assigned into the treatment or placebo groups. The treatment group received a curcuminoids capsule (500 mg/day; C3 Complex®, Sami Labs Ltd., Bangalore, India) for 8 weeks ($n = 36$), and the control group were given a placebo capsule for the same time period ($n = 36$). Each curcuminoid capsule also contained piperine (5 mg; Bioperine®, Sami Labs Ltd., Bangalore, India), which is a known bioavailability enhancer. Placebo capsules were prepared by the same company in capsules with the same shape and size. The sample size was calculated to be 32 subjects in each study group. A total of 36 volunteers were enrolled in this study in each group due to a probability of a 10% drop-out.

2.2 Biochemical Variables

Fasting blood samples (12 h fast) were taken from subjects individually into plain plastic tubes in the morning before and after the intervention. Serum was separated by centrifugation at 10,000 × g for 15 min and then, the serum aliquots were preserved frozen at −80 °C.

Erythrocyte sedimentation rate (ESR) and serum levels of C-reactive protein (CRP) and cytokines were measured in all samples at baseline and after the intervention. ESR was determined in whole blood samples. Serum levels of CRP were measured using Biosystem kits. The serum levels of 12 pro- and anti-inflammatory cytokines consisting of tumor necrosis factor (TNF-α), interleukin-1α (IL-1α), IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP-1), interferon γ (IFN-γ), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF)] were determined using an EV 3513 cytokine biochip array (Randox Laboratories, Crumlin, UK) by sandwich and competitive chemiluminescence immunoassays (Randox Laboratories, Crumlin, UK) [31, 32].

2.3 Quality of Life

To assess the efficacy of treatment on patient quality of life, the EORTC-QLQ-C30 questionnaire was applied before and after the intervention to analyze both single- and multi-item

measures. The questionnaire included five functional scales (Physical, Role, Cognitive, Emotional, and Social Functioning), nine symptom scales (Fatigue, Pain, Nausea/Vomiting, Constipation, Diarrhea, Insomnia, Dyspnea, Appetite Loss, Financial difficulties), and a Global Health Status/quality of life scale [33]. Every question had four choices for each scale: (1) not at all; (2) a little; (3) quite a bit; and (4) very much. However, the global health status and quality of life scale had options ranging from (1) very poor to (7) excellent [34]. Scores given to each item were linearly transformed to a scale from 0 to 100. Higher scores of functional scales and quality of life correlate with a better functionality and a more preferable global health status, and a high score for a symptom item represents a high level of symptomatology [35].

2.4 Statistical Analysis

The statistical analyses were performed using SPSS software version 16.0. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. The quantitative data were expressed as mean ± SD for variables with normal distributions or median and interquartile range for variables without a normal distribution. The chi-square, Student's *t*, and Mann-Whitney U tests were applied to compare the clinical and quality of life characteristics in the two groups. Bivariate correlations were assessed using Spearman's rho test. Data analysis considered $p < 0.05$ as statistically significant.

3 Results

A total of 67 individuals completed the study. Three and two subjects were lost to follow-up in the curcuminoid and placebo intervention groups, respectively. The mean age of the participants was 63.94 ± 10.40 and 58.68 ± 12.24 years in the curcuminoid and placebo groups, respectively (Table 1). There were no significant differences in baseline characteristics except for ESR ($p = 0.012$) and TNF-α ($p = 0.009$) concentrations between the two study groups (Table 1).

There were significant changes in CRP ($p = 0.002$) and ESR ($p = 0.0001$) from baseline to the end of the treatment at 8 weeks of intervention between the curcuminoid compared to the placebo group (Table 2). Moreover, IL-1α was decreased significantly in the curcuminoid supplementation group compared to the placebo but this was at trend level ($p = 0.077$) (Table 2).

Changes in quality of life scores based on curcuminoid supplementation are shown in Table 3. A significant improvement in functional scale ($p = 0.002$) and global quality of life ($p = 0.020$) scales was observed in the curcuminoid group compared to the control. However, symptom scales were increased significantly after curcuminoid supplementation compared with placebo ($p = 0.0001$). Bivariate correlations revealed that baseline values were significantly correlated with the magnitude of score changes in each of the quality of life scales in both curcuminoids and placebo groups (Table 4).

4 Discussion

The results indicated that 500 mg/day curcuminoid supplementation for a period of 8 weeks can decrease the ESR and serum levels of CRP in patients with CRC. However, there was no significant improvement in serum pro- and anti-inflammatory cytokines apart from a decrease at the trend level for serum IL-1α levels. Global quality of life and functional scale improved after 8 weeks of curcuminoids supplementation compared to placebo. The present trial was conducted with only 8 weeks of curcumin supplementation. It can be envisaged that a longer duration of supplementation could have resulted in a significant change in serum cytokine markers.

To the best of our knowledge, this was the first study that has assessed the effects of curcuminoids on an array of cytokines, as well as on the quality of life in CRC patients. Previous studies have reported that curcuminoids had a significant effect on inflammatory mediators including IL-6,

Table 1 Clinical and biochemical features in subjects at baseline

Variables		Curcuminoids	Placebo	*P*-value
Gender	Male (%)	48.5	44.0	0.795
	Female (%)	51.5	56.0	
Age (years)		63.94 ± 10.40	58.68 ± 12.24	0.091
CRP (mg/L)		13.10(7.45 to 28.15)	18.10(11.12 to 40.45)	0.093
ESR (mm/h)		43.00(35.50 to 50.050)	36.50(33.00 to 39.50)	**0.012**
Interleukin 2 (pg/mL)		ND	ND	–
Interleukin 4 (pg/mL)		0.00(0.00 to 0.74)	0.00(0.00 to 1.48)	0.282
Interleukin 6 (pg/mL)		8.65(1.51 to 39.67)	2.77(2.28 to 3.95)	0.194
Interleukin 8 (pg/mL)		14.41(6.31 to 43.97)	10.66 (6.60 to 19.96)	0.705
Interleukin 10 (pg/mL)		2.50 ± 8.93	0.12 ± 0.53	0.179
VEGF (pg/mL)		255.07 ± 234.111	221.97 ± 188.67	0.628
Interferon γ (pg/mL)		0.58 ± 1.85	0.10 ± 0.24	0.654
TNF-α (pg/mL)		1.23(0.00 to 1.91)	0.00(0.00 to 0.00)	**0.009**
Interleukin 1α (pg/mL)		0.00(0.00 to 0.42)	0.00(0.00 to 0.00)	0.244
Interleukin 1β (pg/mL)		0.00(0.00 to 2.82)	2.63(0.00 to 4.72)	0.086
MCP-1 (pg/mL)		260.77(167.85 to 499.26)	272.24(183.25 to 332.32)	0.924
EGF (pg/mL)		277.20 ± 286.36	334.55 ± 258.98	0.512

Values expressed as mean ± SD for normally distributed data, and median and interquartile range for non-normally distributed data. *P*-values in bold indicate significant differences between the curcuminoid and placebo groups
CRP high-sensitive C-reactive protein, *ESR* erythrocyte sedimentation rate, *EGF* epidermal growth factor, *INFγ* interferon γ, *MCP1* monocyte chemoattractant protein, *TNF-α* tumor necrosis factor, *VEFG* vascular endothelial growth factor, *ND* not detected

Table 2 Changes in clinical and biochemical features after 8 weeks of intervention, relative to baseline

Difference	Curcuminoids	Placebo	*P*-value
CRP (mg/L)	−3.80(−7.60 to −2.20)	−2.05(−3.25 to 2.20)	**0.002**
ESR (mm/hr)	−12.00(−14.50 to −6.00)	−4.00(−6.00 to 0.50)	**0.0001**
Interleukin 2 (pg/mL)	ND	ND	0.534
Interleukin 4 (pg/mL)	0.00(0.00 to 0.71)	0.00(0.00 to 0.06)	0.976
Interleukin 6 (pg/mL)	−0.11(−16.07 to 1.20)	−0.18(−1.13 to 0.49)	0.551
Interleukin 8 (pg/mL)	−0.51(−4.99 to 6.44)	0.75(−1.74 to 6.44)	0.685
Interleukin 10 (pg/mL)	−0.63 ± 4.37	0.21 ± 0.86	0.069
VEGF (pg/mL)	2.55 ± 69.25	16.38 ± 87.93	0.582
Interferon γ (pg/mL)	−0.35 ± 1.60	0.03 ± 0.35	0.581
TNFα (pg/mL)	0.00(−0.25 to 1.35)	0.00(0.00 to 0.00)	0.348
Interleukin 1α (pg/mL)	0.00(−0.42 to 0.00)	0.00(0.00 to 1.47)	0.077
Interleukin 1β (pg/mL)	0.00(−1.49 to 0.18)	0.00(−1.19 to 0.88)	0.659
MCP-1 (pg/mL)	30.10(−115.58 to 71.14)	−1.17(−44.32 to 74.02)	0.755
EGF (pg/mL)	28.86 ± 112.37	29.18 ± 68.01	0.992

Values expressed as mean ± SD for normally distributed data, and median and interquartile range for non-normally distributed data. *P*-values in bold indicate significant differences between the curcuminoid and placebo groups
CRP high-sensitive C-reactive protein, *ESR* erythrocyte sedimentation rate, *EGF* epidermal growth factor, *MCP1* monocyte chemoattractant protein, *TNF-α* tumor necrosis factor, *VEFG* vascular endothelial growth factor, *ND* not detected

Table 3 Changes in quality of life scores after curcuminoid supplementation in subjects with CRC

	Groups			
		Curcuminoids	Placebo	*P*-value
Functional scales*	**Before**	29.93 ± 3.84	39.95 ± 3.84	**0.0001**
	Changes	−1.42 ± 3.84	−7.95 ± 3.84	**0.002**
Physical scale (Mean ± SD)	**Before**	10.40 ± 3.84	14.08 ± 3.32	**0.001**
	Changes	−0.94 ± 3.13	−3.42 ± 2.89	**0.004**
Role scale (Mean ± SD)	**Before**	4.03 ± 1.89	5.37 ± 2.10	**0.017**
	Changes	−0.12 ± 1.54	−1.12 ± 2.07	**0.047**
Cognitive scale (Mean ± SD)	**Before**	11.64 ± 3.61	15.75 ± 4.88	**0.002**
	Changes	−0.03 ± 4.28	−2.04 ± 4.38	0.077
Emotional scale (Mean ± SD)	**Before**	7.85 ± 2.66	10.12 ± 3.85	**0.034**
	Changes	−0.03 ± 3.02	−1.29 ± 3.49	0.111
Social scale (Mean ± SD)	**Before**	6.48 ± 2.14	8.25 ± 1.62	**0.001**
	Changes	−0.76 ± 1.92	−2.71 ± 1.99	**0.001**
Symptoms scale (Mean ± SD)**	**Before**	27.15 ± 8.18	37.25 ± 6.43	**0.0001**
	Changes	−1.06 ± 6.81	−9.62 ± 6.30	**0.0001**
Fatigue (Mean ± SD)	**Before**	7.15 ± 2.01	9.25 ± 1.93	**0.0001**
	Changes	−0.72 ± 2.06	−2.66 ± 2.07	**0.001**
Nausea and vomiting (Mean ± SD)	**Before**	3.57 ± 1.85	6.0 ± 1.17	**0.0001**
	Changes	0.30 ± 1.82	−1.29 ± 1.39	**0.001**
Pain (Mean ± SD)	**Before**	4.45 ± 1.69	5.62 ± 1.55	**0.011**
	Changes	−0.51 ± 1.90	−1.70 ± 1.73	**0.016**
Dyspnea (Mean ± SD)	**Before**	1.45 ± 0.66	1.58 ± 0.88	0.750
	Changes	0.00 ± 0.50	−0.20 ± 0.93	0.216
Sleep disturbance (Mean ± SD)	**Before**	2.03 ± 0.88	2.79 ± 0.77	**0.002**
	Changes	0.00 ± 1.34	−0.58 ± 1.01	0.072
Appetite loss (Mean ± SD)	**Before**	2.21 ± 1.19	2.79 ± 1.21	0.065
	Changes	0.15 ± 0.97	−0.91 ± 1.24	**0.001**
Constipation (Mean ± SD)	**Before**	1.90 ± 0.94	2.70 ± 0.90	**0.001**
	Changes	−0.03 ± 0.98	−0.29 ± 0.80	**0.001**
Diarrhea (Mean ± SD)	**Before**	1.75 ± 1.03	3.00 ± 1.06	**0.0001**
	Changes	0.30 ± 1.13	−0.62 ± 0.92	**0.002**
Financial impact (Mean ± SD)	**Before**	2.60 ± 0.99	3.50 ± 0.51	**0.0001**
	Changes	−0.54 ± 1.17	−1.33 ± 0.76	**0.006**
Global quality of life*	**Before**	9.06 ± 2.52	11.25 ± 1.64	**0.0001**
	Changes	−4.84 ± 1.82	−6.50 ± 1.82	**0.020**
Overall health during the past week	**Before**	4.48 ± 1.41	5.50 ± 1.31	**0.011**
	Changes	−2.33 ± 1.88	−2.51 ± 1.56	**0.021**
Overall quality of life during the past week	**Before**	4.57 ± 1.45	5.75 ± 1.18	**0.004**
	Changes	−3.50 ± 1.85	−3.00 ± 2.08	0.299

Mann-Whitney U test is used. *Scores range from 0 to 100 with a higher score representing a higher level of functioning. **Scores range from 0 to 100 with a higher score representing a greater symptom burden. *P*-values in bold indicate significant differences between the curcuminoid and placebo groups

Table 4 Correlations[a] between baseline values and changes in quality of life scores in the placebo and curcuminoid groups

	Curcuminoid		Placebo	
Variables	Correlation coefficient	*P*-value	Correlation coefficient	*P*-value
Global quality of life	−0.82	**<0.001**	−0.60	**0.002**
Functional scales	−0.72	**<0.001**	−0.75	**<0.001**
Symptom scales	−0.80	**<0.001**	−0.64	**<0.001**

[a]Spearman's correlation coefficient

IL-8, TNFα, and high sensitivity (hs)-CRP in serum [36–39]. Quality of life and functionality in the treatment group improved after 8 weeks, indicating better health and functionality following curcuminoid consumption. On the other hand, symptom scores were increased in the curcuminoid group. One possible explanation for this might be gastrointestinal (GI) complications, which could describe the elevation observed in symptom scores as most of them were GI-related in nature. Moreover, patients in the curcuminoid group had a lower baseline symptom score compared to those in the placebo group. Finally, the effect of chemotherapy can vary with individuals, which could also explain this difference.

Adjunct therapy with a bioavailable curcuminoid preparation could significantly improve quality of life and decrease serum levels of IL-6, TNF-α, MCP-1, and hs-CRP in patients with solid tumors undergoing chemotherapy [40]. Recently, curcuminoid supplementation was found to be well-tolerated and safe with a potential to provide benefits in patients with metastatic CRC under folinic acid, fluorouracil, and oxaliplatin (FOLFOX) combination chemotherapy [41].

In another study, curcuminoid treatment was found to reduce IL-1, CRP, TNF-α, and polyisoprenylated protein methyltransferase (PPMTase) and produce a 55-point mean decrease in the Crohn's Disease Activity Index in CRC patients [42]. However, Howells et al. did not find any effects of curcuminoids on quality of life in CRC patients aged >18 years-old, although curcuminoid supplementation was found to be safe in patients with CRC undergoing chemotherapy [41].

Curcumin has been reported to exert its anti-inflammatory effects through the suppression of nuclear factor (NF)-kB signaling pathway which can affect CRP, IL-6, IL-1, and TNF-α levels [43]. Jeong et al. demonstrated the inhibition of NF-kB in CRC cell lines by curcumin [44]. Further, it has been suggested that the anti-inflammatory properties of curcuminoids in patients with CRC can be exerted by enhancing the expression of the tumor protein p53 in tumor tissue and modulating the tumor cell apoptotic pathway [45]. In an oxaliplatin/curcumin combination study in vitro, and using an animal model, Yin et al. found that curcumin could inhibit the phosphorylation of transcription factor p65 and Bcl-2 expression and prevent oxaliplatin resistance in CRC through the suppression of TGF-β/Smads signaling [46].

Although several in vitro studies have shown multiple molecular targets for curcumin in preventing cancer cell growth and metastasis, there is a lack of reproducible results with biomarker readouts in clinical trials [47]. This could be due to different dosages administered for variable time periods in the clinical studies. Curcuminoids have been used up to 8 g per day in clinics and further studies with escalated doses may result in a significant change in the pro- and anti-inflammatory markers in future studies.

The present study was limited by the small sample size as well as the short duration of follow-up which did not allow the assessment of survival and outcomes. Moreover, a single dose of curcuminoids was used in this study and the impact of dose escalation remains unclear.

5 Conclusions

The results showed that curcuminoid supplementation for a period of 8 weeks can improve the ESR and serum levels of CRP in patients with stage 3 CRC. Also, improvement in global quality of life and the functional scale were observed following curcuminoid supplementation compared to placebo. Although there was no significant improvement in serum pro- and anti-inflammatory cytokines, the levels of IL-1α showed a decreasing trend in patients receiving curcuminoids. Future studies employing larger sample sizes may help to resolve this issue. It is also recommended that future studies with longer follow-up periods be carried out to clarify the impact of curcuminoids on the survival of patients.

Conflict of Interest Muhammed Majeed is the founder of Sabinsa Corp. and Sami Labs Ltd. Other authors declare no competing interests.

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Curcumin and Piperine Combination for the Treatment of Patients with Non-alcoholic Fatty Liver Disease: A Double-Blind Randomized Placebo-Controlled Trial

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Abstract

Background: Experimental and clinical studies have revealed that curcumin may be an effective therapy for non-alcoholic fatty liver disease (NAFLD). Hence, the aim of this study was to assess the effect of curcumin plus piperine administration on NAFLD.

Methods: Adults 18–65 years-old diagnosed with NAFLD by liver sonography were randomly allocated to curcumin (500 mg/day) or placebo groups for 2 months. All participants received both dietary and exercise advice. Anthropometric and biochemical measurements as well as hepatic ultrasound were performed at baseline and final conditions.

Results: Seventy-nine participants were recruited and randomly allocated into the curcumin (n = 39) or placebo (n = 40) groups. There were no significant differences between placebo and curcumin groups for demographic

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and clinical characteristics and NAFLD grade at baseline. After the treatment period, the curcumin group exhibited lower alkaline phosphatase (−16.2 ± 22.8 versus −6.0 ± 22.5 mg/dL, $p = 0.04$) concentrations and severity of NAFLD compared with the placebo group ($p = 0.04$).

Conclusion: Results of this clinical trial suggest that short-term treatment with curcumin plus piperine administration improves NAFLD severity.

Keywords

Curcumin · Piperine · Fatty liver disease · Steatosis · Clinical trial

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent liver diseases which has been rapidly increasing in its incidence owing to different contributors such as obesity, sedentary lifestyles, and high-fat diets. The prevalence of NAFLD is 80–90% in obese adults, 30–50% in diabetic patients, 90% in hyperlipidemia, 3–10% in children, and 40–70% in obese children [1]. NAFLD comprises different hepatic disorders including simple steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [2]. The interaction of genes, hormones, nutrition, insulin resistance, lipotoxicity, and hepatic inflammation is involved in the complex pathophysiology of NAFLD [3]. Because there is no well-established pharmacological management for NAFLD, effective therapies are needed in order to treat this chronic liver disease.

Curcumin is a natural compound obtained from turmeric, a member of the Zingiberaceae family [4]. Owing to the biological effects of curcumin such as antioxidant, anti-inflammatory, immunoregulatory, hepatoprotective, antidiabetic, lipid-lowering, and anti-tumor effects [5–13], this nutraceutical has emerged as a promising therapeutic option for NAFLD. Even recent meta-analyses have revealed a favorable impact of curcumin supplementation on NAFLD [14–16]. However, low bioavailability and rapid biotransformation of this polyphenol have resulted in a limited application and controversial findings. In this regard, it has been demonstrated that piperine, an extract from black pepper, may improve the bioavailability and pharmacokinetics of curcumin in both animals and humans [17]. Therefore, the aim of this study was to assess the effect of curcumin plus piperine administration on NAFLD.

2 Method

2.1 Trial Design

This was a 2-month, double-blind, placebo-controlled, parallel-group trial with an allocation ratio of 1:1 for the two groups. It was performed in the Northeastern region of Iran, in Neyshabur City. The study was registered and certified by the Iranian Registry of Clinical Trials (IRCT registration number: IRCT2015052322381N1; http://www.irct.ir), the Institutional Review Board and the Ethics Committee of Mashhad University of Medical Sciences (Code: IR.MUMS.fm.REC.1395.303). Before any procedures were initiated, all patients enrolled in this clinical trial signed an informed consent document.

2.2 Randomization

The participants were distributed using a balanced block randomization technique into two groups designated for treatment with either curcumin or placebo. This was achieved through two steps, First, two letters were arranged and typed on two pieces of paper labeled "A" for "curcumin" and "B" for "placebo". The possible quad blocks were AABB, ABAB, ABBA, BBAA, BABA, and BAAB. Second, the number was selected randomly through a random number table. The randomization procedure was concealed to make sure the allocation sequence was

executed without the researcher having knowledge over which patient was in which group. In this way, the treatments were placed in boxes that were labeled with a serial number from 1 to 80 for all subjects in the two groups. Apart from the trial leader, the contents of each box were not known to the trial operators.

2.3 Study Population

All adults aged 18–60 who met the NAFLD criteria based on ultrasound evaluation and laboratory results were eligible for the study. A normal liver was determined if the liver parenchyma echogenicity was equal to or slightly higher than that of the renal parenchyma and NAFLD was determined on the basis of more liver echogenicity than that of the renal parenchyma due to fatty infiltration [18]. Patients were recruited from January 2017 to August 2017 at Bahman Hospital (Neyshabur, Iran). The conditions for inclusion were as follows: age between 18–65 years and ultrasound diagnosis of fatty liver. Exclusion criteria were pregnancy and/or lactation for women, the presence of acute or chronic liver disorders such as viral (hepatitis B and C) and autoimmune hepatitis, usage of anti-inflammatory drugs such as corticosteroids and liver enzyme inducer drugs, the presence of alcoholic liver disease, or metabolic liver disorders including Wilson's disease and hemochromatosis, Budd–Chiari syndrome, as well as other medical disorders such as hyper/hypothyroidism, cardiovascular diseases, and cancer. This procedure resulted in 80 patients with NAFLD being selected and 8 were excluded from the study.

2.4 Intervention

A combination of curcumin and piperine was used for intervention in this trial. Piperine is extracted from black pepper, which has been clinically proven to naturally enhance absorption of pharmaceuticals including the curcuminoids. In the treatment group, subjects received curcumin–piperine capsules [Curcumin C3 complex™ (500 mg) plus Bioperine™ (5 mg) patented extract obtained from black pepper fruits (*Piper nigrum*) standardized to a minimum of 95% piperine] or placebo capsules once daily. The capsules were consumed by the patients for two months as directed. In order to better follow-up medication use by the patients, the treatment bottles were allocated to the subjects at the beginning and at the end of the first month of intervention period and any remaining capsules were counted.

2.5 Assessment of Outcomes

The ultrasound examination and the biochemical and anthropometric measurements were the primary and secondary outcome measures, respectively.

2.6 Biochemical and Anthropometric Measurement

To measure biochemical and laboratory variables, venous blood samples were taken from each patient after an overnight fasting period at points before and after the intervention on days 0 and day 60. Blood samples were centrifuged at 1000 × g for 10 min for preparation of serum. Biochemical and laboratory measurements including fasting blood glucose (FBG), lipid profiles, and liver function tests were conducted immediately using serum aliquots in the BT-2000 Auto Analyzer machine (Biotechnica, Rome, Italy), using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran).

Anthropometrics and body mass were analyzed by the InBody 770 device (model: BPM040S12FXX, Seoul, South Korea) with an accuracy of 0.1 kg. All patients were shoeless with thin clothes during the tests, according to the manufacturer's recommended procedure. Body weight, fat mass, body mass index (BMI), waist:hip ratio (WHR), and other anthropometric measurements were again carried out by each device using standard protocols. Additionally, a

digital stadiometer (Model BSM 370, Seoul, South Korea) was used to measure height with an accuracy to the nearest 0.1 cm [19].

In addition to the intervention, all patients were advised based on the National Institutes of Health and the North American Association for the study of obesity to have an energy-balanced diet according to the clinical guidelines for identifying, assessing, and managing overweight and obesity in adults. According to the guideline, the diet should consist of carbohydrate (52–53% of the total energy value), fiber (20–30 g/day), total fat (≤30% of the total energy value, one-thirds saturated and two-thirds unsaturated), cholesterol (<300 mg/dL), and protein (15–18% of the total energy value). All patients were also advised to exercise for a minimum of 30 min, three times per week.

2.7 Statistical Analysis

For assessing the normality of variables the Kolmogorov–Smirnov test was used. Normal and non-normal distribution variables (parametric and non-parametric) were shown as the mean ± standard deviation (SD) and median (interquartile range (IQR), respectively. The independent T-test and the Mann–Whitney U test were performed for comparing characteristics of patients between groups of curcumin and placebo, for normal and non-normal distribution variables, respectively. The dependent t-test and the Wilcoxon signed-rank test were used to compare two related samples (before and after) for parametric and non-parametric variables, respectively. Additionally, categorical data such as sex and smoking were analyzed using chi-square and Fisher's exact test.

3 Results

Eighty-five patients with NAFLD were eligible for the study and 6 of these were excluded because they did not meet the inclusion criteria (Fig. 1). Thus, 79 participants were randomly allocated into the curcumin (n = 39) or placebo (n = 40) groups. During the follow-up period, there were 9 dropouts due to the side effects of curcumin or because the intervention was abandoned due to forgetful consumption of drug, travel, or inaccessibility in the follow-up period. Thus, the dropout rate was approximately 11%.

3.1 Characteristics of the Study Participants

Demographic and clinical characteristics of the study population are shown in Table 1. There were no significant differences between the placebo and curcumin groups for age, sex, smoking and drug consumption, history of diseases, anthropometric measurements, systolic blood pressure (SBP), diastolic blood pressure (DBP), and NAFLD grade at baseline.

3.2 Comparison of NAFLD Criteria Within Groups

Table 2 shows anthropometric, biochemical, and sonographic data before and after the intervention period. Regarding the anthropometric data, body fat mass, BMI, hip circumference, and waist circumference were significantly reduced in both treatment groups. In addition, weight and waist–hip ratio were significantly reduced in the placebo group but not affected in the curcumin group. According to the biochemical parameters, only HDL-C and ALP showed a significant decrease after the intervention in the placebo and curcumin groups, respectively. The comparison of liver sonography data within the groups revealed that the grade of NAFLD was significantly decreased after consumption of curcumin (P = 0.004) but no significant change was observed in the placebo group (P = 0.796).

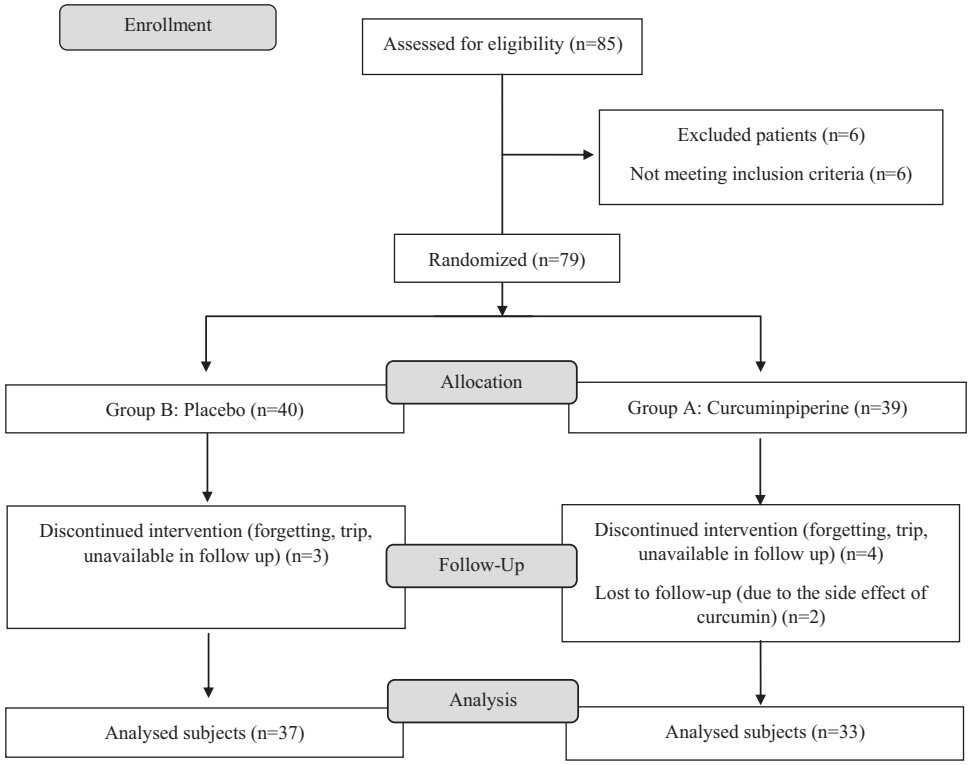

Fig. 1 Flow diagram of study population

3.3 Comparison of NAFLD Criteria Between Groups

Table 3 shows the comparison of anthropometric, biochemical, and NAFLD ultrasound data between the curcumin and placebo groups after the 2-month intervention period. Only ALP and NAFLD grade showed significant changes between the study groups. The curcumin group exhibited lower ALP concentrations and severity of the NAFLD compared with placebo group. There were no significant differences for any of the other variables.

4 Discussion and Future Perspectives

Results of this randomized placebo-controlled trial suggest that curcumin piperine supplementation exerts a hepatoprotective effect in patients with NAFLD. In agreement with our findings, a previous study reported a positive effect of curcuminoids plus piperine administration on NAFLD [20]. In this context, it has been described that curcumin therapy prevents hepatic steatosis by improving intestinal barrier function and reducing hepatic inflammation through down-regulation of toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [21]. Further, curcumin supplementation may protect against NAFLD by decreasing hepatic lipid accumulation and oxidative stress through modulation of fatty acid uptake [22]. Also, curcumin treatment improves histological changes of NAFLD including fibrosis and intrahepatic accumulation of CD4+ cells [23]. It has been suggested that curcumin may regulate endogenous and exogenous metabolism in NAFLD via the nuclear factor erythroid 2-related factor 2/farne-

Table 1 Baseline characteristics of patients in curcumin and placebo groups

Characteristics		NAFLD patients		
		Placebo (n = 37)	Curcumin (n = 33)	P-value[a]
Age, years		43.1 ± 11.6	45.6 ± 11.0	0.328
Male (%)		60	53.8	0.581
Smoker (%)		17.5	2.6	0.057
Ex-smoker (%)		60	65.8	0.718
Drug intake (%)		2.7	0	0.425
History of diabetes		15	17.9	0.724
History of hypertension		12.5	15.4	0.545
History of heart disease		12.5	2.6	0.096
History of myocardial infarction		2.5	0	0.368
History of kidney disease		27.5	12.8	0.105
History of liver disease		15	20.5	0.263
History of hyperlipidemia		37.5	30.8	0.473
History of weight loss		22.5	25.6	0.849
Height (cm)		165.7 ± 10.9	164.2 ± 10.1	0.529
Weight (kg)		80.0 ± 11.9	83.1 ± 10.6	0.243
BMI (kg/m^2)		29.2 ± 4.2	30.9 ± 4.3	0.093
SBP (mmHg)		112.5 ± 14.7	118.8 ± 18.8	0.104
DBP (mmHg)		79.9 ± 10.2	84.5 ± 12.0	0.077
NAFLD grade (%)	(1)	47.5	43.6	0.745
	(2)	47.5	46.2	
	(3)	5	10.3	

The continuous and categorical variables were described respectively, as mean ± SD and percentage

NAFLD nonalcoholic fatty liver disease, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

[a]The continuous and categorical variables were evaluated using the independent Student's t-test and chi-square/Fisher's exact test, respectively

soid X receptor/liver x receptor (Nrf2/FXR/LXRα) pathway [24]. In addition, curcumin might reverse hepatic steatosis by suppressing the expression of CD36 and peroxisome proliferator-activated receptor-gamma (PPAR-γ) via activation of the cAMP response element-binding protein [25]. In this line, curcumin attenuates fatty liver through the decrease in DNA methylation levels, increased PPAR-α mRNA and protein expression, and reduced hepatic lipid accumulation [26]. Additionally, curcumin mitigates hepatic steatosis by regulating hepatic lipid metabolism via 5′ AMP-activated protein kinase (AMPK) activation [27]. Thus, the aforementioned molecular mechanisms of curcumin could explain its beneficial effects on NAFLD.

On the other hand, a recent clinical trial found a significant improvement in lipid profile and hepatic enzymes after curcumin treatment in patients with NAFLD [28], which contrasts with our results. This inconsistency may be related to the short treatment period of our study, which might have been insufficient to induce significant changes in biochemical parameters. Nonetheless, it is noteworthy that the positive effects of curcumin are often found using doses greater than 1500 mg/day [29], while we only administered 500 mg/day. This could also explain the lack of effect of curcumin administration on anthropometric and biochemical parameters.

Although there were significant differences between the study groups at baseline, it is

Table 2 Comparison of important characteristics affecting the NAFLD within groups, before and after intervention

Characteristics		Placebo (n = 37)			Curcumin (n = 33)		
		Before	After	P-value	Before	After	P-value
Weight (kg)		80.0±11.9	76.4±11.0	0.021	83.1±10.9	82.0±10.4	0.106
BMI (kg/m^2)		29.2±4.2	28.6±3.8	0.023	30.9±4.4	30.2±4.7	0.001
HC (cm)		103.1±5.4	101.6±5.0	0.002	105.4±5.7	104.5±5.7	0.001
AC (cm)		99.5±11.1	97.0±9.7	0.001	102.0±9.6	100.5±10.0	0.002
WHR		0.9±0.1	0.9±0.1	0.040	0.9±0.1	0.9±0.1	0.074
Body fat mass		28.3±9.5	26.8±7.9	0.009	32.1±10.2	30.5±10.4	0.001
TG (mg/dL)		135.5(108.0-166.0)	130.5(100.0-177.7)	0.678	111.0(91.0-160.0)	121.5(93.2-171.7)	0.712
TC (mg/ dL)		194.0±36.2	188.7±36.0	0.304	185.5±41.7	180.8±33.8	0.510
HDL-C (mg/ dL)		45.6±10.6	43.5±8.9	0.033	43.8±9.8	42.9±10.7	0.381
LDL-C (mg/ dL)		105.6±25.2	107.5±32.1	0.696	99.4±23.6	104.1±27.0	0.304
AST (mg/ dL)		25.5±9.6	28.8±9.7	0.139	24.3±8.5	27.4±9.7	0.096
ALP (mg/ dL)		185.8±51.1	181.3±48.0	0.116	202.9±57.2	186.6±50.2	0.001
ALT (mg/ dL)		40.2±28.1	38.9±17.6	0.753	32.3±20.6	32.6±18.6	0.930
FBG (mg/ dL)		107.8±43.9	107.1±46.5	0.810	95.1±15.3	93.2±16.7	0.351
SBP (mmHg)		112.5±14.7	116.6±15.3	0.194	120.1±20.2	119.8±22.8	0.906
DBP (mmHg)		79.9±10.2	83.0±9.7	0.157	85.7±13.3	83.0±11.1	0.357
NAFLD grade (%)	(0)	0	5.4	0.796*	0	12.1	0.004*
	(1)	47.5	40.5		43.6	45.5	
	(2)	47.5	45.9		46.2	42.4	
	(3)	5	8.1		10.3	0	

Respectively, dependent Student's t and Wilcoxon tests were performed to compare normal and non-normal variables
Significant values are shaded in gray
For normal and non-normal distribution variables, values are expressed as mean ± SD and median (interquartile range (IQR), respectively
NAFLD nonalcoholic fatty liver disease, *BMI* body mass index, *HC* measured circumference of hip, *AC* measured circumference of abdomen, *WHR* waist–hip ratio, *TG* triglyceride, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *FBG* fasting blood glucose, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase
*Wilcoxon signed ranks test

important to note that those individuals in the curcumin intervention group were heavier in terms of weight, BMI, waist circumference, and body fat mass, which could explain the lack of effect of curcumin on anthropometric measurements. According to the biochemical parameters, lipids and hepatic enzymes were within the normal ranges in the curcumin group and, therefore, significant changes after the treatment period may not have been expected to occur.

There were a number of limitations in this study that should be taken into account. First, due to the short treatment duration of the present clinical trial, the long-term efficacy of curcumin plus piperine administration could not be evaluated. Second, NAFLD severity was only assessed by hepatic ultrasound, although this method has shown a high sensitivity, specificity, and accuracy for the diagnosis of fatty liver [29].

In conclusion, the results of this clinical trial suggest that short-term administration with curcumin plus piperine diminishes NAFLD severity. Although curcumin might be considered as a therapeutic option for the treatment of NAFLD, further clinical trials are mandatory to confirm the potential beneficial effects of this nutraceutical in both prevention and treatment of NAFLD. Such trials should explore different dosages and treatment periods.

Conflict of Interest None.

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Table 3 Changes of anthropometric, biochemical, and NAFLD ultrasound grading between groups of curcumin placebo

		Placebo (n = 37)	Curcumin (n = 33)	P-value
Weight (kg)		-1.9±4.3	-1.0±3.8	0.403
BMI (kg/m^2)		-0.3±0.9	-1.6±2.1	0.205
Body fat mass		-1.0±2.1	-1.6±2.1	0.317
WHR		-0.01±0.02	-0.01±0.02	0.877
AC (cm)		-1.8±2.4	-1.5±2.8	0.722
HC (cm)		-0.9±1.4	-0.8±1.4	0.835
TC (mg/ dL)		-5.7±40.0	-4.7±42.8	0.913
HDL-C (mg/ dL)		-2.7±7.7	-0.9±6.2	0.264
LDL-C (mg/ dL)		2.0±30.5	4.6±26.9	0.685
TG (mg/dl)		-0.5(-22.5-28.2)	-1.0(-22.7-31.2)	0.996
SBP (mmHg)		3.5±13.0	-0.3±12.8	0.306
DBP (mmHg)		4.0±13.5	-2.7±14.8	0.100
FBG (mg/ dL)		-1.0±26.8	-1.9±12.3	0.856
ALT (mg/ dL)		-1.3±25.5	0.2±16.9	0.759
AST (mg/ dL)		2.5±10.5	3.0±9.2	0.863
ALP (mg/ dL)		-6.0±22.5	-16.2±22.8	0.044
NAFLD grade (%)	(-2)	0	9.1	0.048*
	(-1)	18.9	21.2	
	(0)	67.6	69.7	
	(1)	10.8	0	
	(2)	2.7	0	

Independent Student's t and Mann–Whitney U tests were performed to compare normal and non-normal distribution variables, respectively. Values are expressed as mean ± SD and median (interquartile range (IQR)) for normal and non-normal distribution variables, respectively

Significant values are shaded in gray

BMI body mass index, *HC* measured circumference of hip, *AC* measured circumference of abdomen, *WHR* waist–hip ratio, *FBG* fasting blood glucose, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TG* triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *NAFLD* nonalcoholic fatty liver disease

*Mann–Whitney U test

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Evaluation of the Effects of Nanomicellar Curcumin, Berberine, and Their Combination with 5-Fluorouracil on Breast Cancer Cells

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Abstract

Introduction: Breast cancer is one of the main challenging areas in cancer treatment. Natural compounds such as curcumin and berberine have been approved with anticancer effects and are more favorable to people. Here, we investigated the potential synergistic anticancer effects of these two compounds in combination with the standard cancer drug 5-FU on the growth of MCF-7 breast cancer cells.

Materials and Methods: This study tested the effects of six different treatments on cancer cell growth: A) control; B) curcumin; C) berberine; D) 5-FU; E) curcumin + berberine; and F) curcumin + berberine + 5-FU. The IC_{50} concentration of each treatment on cancer cell growth was determined using the MTT assay. Invasiveness of cells grown in 3D culture was analyzed using the transwell chamber technique. Expression levels of genes involved in cancer cell growth and survival (*WNT1*, *APC*, *AXIN1*, *CTNNB1*, *TCF*, *MTOR*, *AKT1*, *MAPK1*, *PTEN*, *BIRC5*, *CCNG1*) were evaluated by real-time PCR.

Results: There was a reduction in cancer cell growth and invasion, and an increase in cellular decomposition across all treatment groups compared to the control with the strongest effects seen in the combined curcumin/berberine/5-FU group. The expression levels of all tested genes were altered in all treatment groups compared to the control, with that of *WNT1*, *CTNNB1*, *TCF*, *MTOR*, *AKT1*, *BIRC5,*

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and *CCND1* showing the most robust changes in the combined curcumin/berberine/5-FU treatment.

Conclusions
All treatment groups had anti-growth, anti-invasion, and pro-apoptotic effects on MCF-7 breast cancer cells in culture. In addition, all treatment groups showed changes in the expression of the genes involved in cancer cell growth and survival with the strongest effects found for the curcumin/berberine/5-FU combination. Therefore, curcumin and berberine may improve the anticancer effects of chemotherapy and these natural compounds should undergo further testing as potential adjuvants.

Keywords

Curcumin · Nano-curcumin · Berberine · 5-FU · Breast cancer · MCF-7 cells

1 Introduction

Breast cancer resulted in an estimated 40,920 deaths in 2018 in the United States alone, making it the cancer with the second highest mortality rate [1]. Moreover, breast cancer is the most common cancer in Iran with 14.2 deaths per 100,000 women and the age for disease onset has shown a decreasing trend [2]. Although there has been some progress, this cancer remains as one of the greatest challenges to health services. Therefore, the exploration of potentially efficacious novel treatments in combination with standard therapies has received considerable attention by research scientists in this field. Currently, surgery, radiotherapy, and chemotherapy are the mainstays of cancer treatments [3]. In addition, there has been the use of some hormonal and targeted therapeutics, each with specific limitations and benefits [4–6]. All of these show varying degrees of tolerability to the patients. For example, fluorouracil (5-FU) is a chemotherapy drug used in the treatment of cancers such as breast cancer, with common side effects such as diarrhea, nausea, and possible occasional vomiting, mouth sores, poor appetite, taste changes, metallic taste in mouth during infusion, watery eyes, and sensitivity to light. For these reasons, there has been recent interest in the use of natural compounds which display anticancer effects, but with fewer side effects and greater acceptability for patients [7, 8]. Curcumin is produced by *Curcuma longa* (turmeric) plants and is known for numerous salutary effects including its ability to regulate intracellular signaling pathways in inflammation, and cancer cell growth, invasion, and apoptosis [9–16]. Berberine is an isoquinoline alkaloid found in the roots, rhizomes, stems, and bark of various plants, which may have similar anticancer effects [17, 18].

As with most cancers, breast cancer cells exhibit over-proliferation and escape mechanisms that allow their continued migration and survival. These mechanisms can be mapped to molecular networks that regulate cell growth, invasion, and apoptosis, and constructing drugs to disrupt these pathways has been a key interest of researchers in this field [19, 20]. For example, the Wnt signaling pathway is a key and complex regulator that plays a role in various cellular processes such as embryogenesis and determination of cell fate, cell differentiation, migration, and apoptosis [21, 22]. Cyclin D1 is part of the molecular system involved in regulating the cell cycle to pass from step G1 to S, as well as other roles [23, 24]. Expression of the mammalian target of rapamycin (m-TOR) is essential in cell growth and survival, as well as processes such as transcription and protein synthesis, and alterations in such pathways have been found in some cancers, such as lung and breast cancer [25]. PTEN is a well-known tumor suppressor gene with a functional role in inducing apoptosis in cancer cells, and mutations and deletions in the *PTEN* gene have been detected in numerous cancers such as gliomas, prostate cancer, melanoma, endometrial cancer, and breast cancer [26–28]. Survivin is another member of the inhibitors of apoptosis family of proteins, which inhibits the activity of caspases and acts as a negative regulator, leading to apoptosis inhibition and regulation of cell division [29, 30].

In this study, we evaluated the effects of curcumin, berberine, and 5-FU as both mono- and combination treatments for potential synergistic effects on the growth, invasion, and survival of MCF-7 breast cancer cells. We also carried out real-time polymerase chain reaction (PCR) analysis of Wnt (*WNT1*), adenomatous polyposis coli (*APC*), axin-1 (*AXIN1*), β-catenin (*CTNNB1*), T-cell factor (*TCF*), m-TOR (*MTOR*), protein kinase B (*AKT1*), mitogen-activated protein kinase 1 (*MAPK1*), phosphatase and tensin homolog (*PTEN*), survivin (*BIRC5*), and cyclin D1 (*CCND1*) mRNA transcripts.

2 Materials and Methods

2.1 Chemicals and Reagents

Berberine, 5-FU, dimethyl sulfoxide (DMSO), trypsin, penicillin, streptomycin, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide (MTT), Transwell® Permeable Supports, and Matrigel® matrix were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nano-curcumin was obtained from Exir Nano Sina (Tehran, Iran). Each nano-curcumin soft gel contained 80 mg of curcumin [10]. Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) were obtained from GIBCO (Dublin, Ireland). Quantitative PCR reagents were purchased from Ampliqon (Odense, Denmark).

2.2 Cell culture

MCF-7 breast cancer cells were purchased from the Pasteur Institute cell bank (Tehran, Iran). These were cultured in RPMI medium containing 10% FBS, 1% penicillin (1% v/v), and streptomycin, and incubated under 5% CO_2 at 37 °C.

2.3 Determination of Cell Viability

The MTT colorimetric cell viability assay was used to evaluate toxicity and IC_{50} values (mg/mL) of each treatment group on the cancer cells. Media containing 1×10^4 cells/100 µL were added to each well of a 24-well plate followed by incubation for 48 h at 37 °C under 5% CO_2. After this, the cells were washed with phosphate-buffered saline (PBS), followed by the addition of nano-curcumin (0.67, 1.34, 2.5, 5, 10, 20, 40, 50 mg/mL), berberine (0, 1, 10, 20, 50, 100, 200, 300, 400, 500 µg/mL), or 5-FU (0.1, 1, 5, 10, 50, 100, 500, 1000 µM). After a further 48 h incubation period as above, 10 µL of 0.5 mg/mL MTT was added to each well followed by 3–4 h incubation. After this, the medium was removed and 100 µL DMSO added to each well, followed by mixing on a shaker for 15 min. Cell viability was measured by reading the optical density of each well at 570 nm in an ELISA reader (Organon Teknika, Boxtel, Netherlands).

2.4 Spheroid Analysis

Tumor spheroids were also analyzed as these are more similar to the pathophysiological structure of human tumor tissue. First, agar scaffolds were constructed using tissue engineering methods. Second, 5 g agar powder was dissolved in 20 mL of distilled water and autoclaved. From the autoclaved solution, 100 µL aliquots were added to the bottom of each well of the 96-well plate and this was placed at 4 °C. After this, 5000 cells were added to each well, followed by the addition of each treatment group at the IC_{50} concentrations determined from the MTT assay. The cells were placed in an incubator at 37 °C under 5% CO_2 for 7 days and the degree of cellular decay was investigated by microscopic examination on days 1, 3, 5, and 7.

2.5 Invasion Assay

The invasion assay was performed to determine the migration of cancer cells from the matrix as a measure of cell movement in the presence of drugs. To perform this test, 100 µL of a mixture of Matrigel and RPMI medium was added to the bottom of the chambers and then 700 µL RPMI medium was added to the wells of the TransWell

permeable supports and the chambers inserted. Then, 50 µL of the cells (100 cells/µL) were combined with 50 µL of the IC_{50} concentrations of the treatments and these mixtures were added to the chambers. After incubation for 48 h, 500 µL of 37% formaldehyde was added to each well, followed by incubation for 2 min at room temperature. Then they were washed with PBS and the plate left at room temperature until it was completely dried. The number of cells in each well and the corresponding chamber was counted using a microscope and the amount of invasion of the cells in the culture medium was determined.

2.6 Quantitative Real-Time PCR

Total RNA was extracted from cells using a serum/plasma kit (Qiagen Inc; Germantown, MD, USA). RNA content and purity were determined by optical density using the NanoDrop 2000 (Fisher Scientific; Schwerte, Germany). Primers were designed using the NCBI website (Table 1). Synthesis of cDNA from RNA was performed based on the manufacturer's protocol using the RevertAid First Strand cDNA Synthesis Kit Enzyme (ThermoFisher Scientific; Waltham, MA, USA). Relative quantitation was based on the determination of the ratio of target gene expression to that of the reference gene, using the ΔΔct method and log fold-change as the readout.

2.7 Statistical Analysis

Statistical analyses were performed using the SPSS software version 16 (SPSS Inc., Chicago, IL, USA). Statistical significant was set at $p \leq 0.05$.

Table 1 Sequences of primers designed from the NCBI website

Gene	Primer	Sequence (5′–3′)
Cyclin D1 (*CCND1*)	Forward	GCTGCGAAGTGGAAACCATC
	Reverse	CCTCCTTCTGCACACATTTGAA
Adenomatous polyposis coli (*APC*)	Forward	AAAATGTCCCTCCGTTCTTATGG
	Reverse	CTGAAGTTGAGCGTAATACCAGT
β-catenin (*CTNNB1*)	Forward	AAAGCGGCTGTTAGTCACTGG
	Reverse	CGAGTCATTGCATACTGTCCAT
T-Cell-Factor-7 (*TCF*)	Forward	CGAAGGTCAAGCTATGAGGACA
	Reverse	ATCTGCGATGCTGGCAATCT
Axin (*AXIN1*)	Forward	GGTTTCCCCTTGGACCTCG
	Reverse	CCGTCGAAGTCTCACCTTTAATG
Wnt (*WNT1*)	Forward	GTACGCCATCTCTTCGGCAG
	Reverse	GCGATGTTGTCAGAGCATCCT
Protein kinase B (*AKT1*)	Forward	TCCTCCTCAAGAATGATGGCA
	Reverse	GTGCGTTCGATGACAGTGGT
Mammalian target of Rapamycin (*MTOR*)	Forward	ATGCTTGGAACCGGACCTG
	Reverse	TCTTGACTCATCTCTCGGAGTT
Extracellular signal-regulated kinase (*MAPK1*)	Forward	TCACACAGGGTTCCTGACAGA
	Reverse	ATGCAGCCTACAGACCAAATATC
Survivin (*BIRC5*)	Forward	AAGAACTGGCCCTTCTTGGA
	Reverse	CAACCGGACGAATGCTTTT
Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG
Phosphatase and tensin homolog (*PTEN1*)	Forward	TGGATTCGACTTAGACTTGACCT
	Reverse	GGTGGGTTATGGTCTTCAAAAGG

3 Results

3.1 Evaluating Synergistic Cytotoxicity of Combination Nano-curcumin, Berberine, and Standard Drug Against MCF-7 Cells

The MTT assay was used to investigate the effects of the combination nano-curcumin, berberine, and 5-FU on the proliferation of MCF-7 breast cancer cells. The results of the cytotoxicity effects of nano-curcumin, berberine, and a nano-curcumin + berberine combination were published in our previous study [31]. The current analysis showed that co-treatment with nano-curcumin, berberine, and 5-FU had a cytotoxic effect on the MCF-7 cell line with an IC_{50} of 7.08 μM (Fig. 1).

3.2 Effects of Nano-curcumin, Berberine, and 5-FU in 3D Spheroid MCF-7 Cell Cultures

The spheroid culture analysis showed that cellular disintegration occurred from day 3 and reached a maximum level by day 7. This occurred for all 5 treatment groups in comparison to the control but was more marked in response to the nano-curcumin, berberine, and 5-FU combination treatment (Figs. 2 and 3).

3.3 Effects of Nano-curcumin, Berberine, and 5-FU in MCF-7 Cell Invasion Assay

The results of the invasion assay showed that fewer cells passed from the interstitial pores and reached the well chambers following the nano-curcumin, berberine, and 5-FU combination treatment (Fig. 4).

3.4 Real-Time PCR Analysis on Expression of WNT Pathway Genes

To evaluate the efficacy of the treatments on the wnt pathway genes, *APC*, *CTNNB1*, *AXIN1*, *WNT1*, and *TCF* expression levels were analyzed by real-time PCR. The results confirmed the reduction of *WNT1* gene expression in all treatment groups, with the most robust decrease observed following the nano-curcumin, berberine, and 5-FU combination treatment (6.6 log fold change lower than the control group) (Fig. 5). In addition, the expression of *TCF* in the 5-FU group was decreased by an approximate 1.8 log fold change in comparison with the control group, and the expression of the *AXIN1* gene was lower in all treatment groups (apart from the nano-curcumin group) compared to the control group. The expression of the *CTNNB1* gene in the combined nano-curcumin, berberine, and

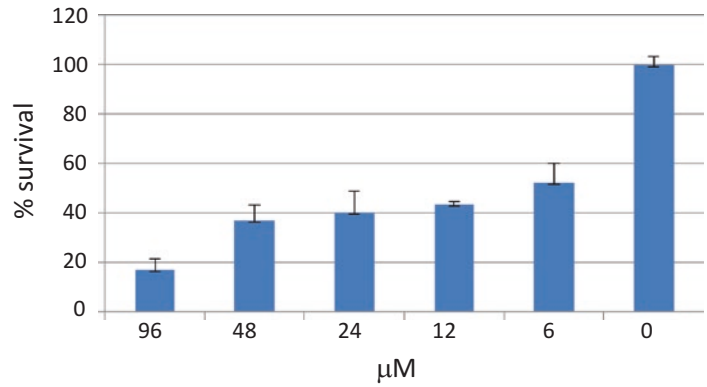

Fig. 1 Effects of combination of nano-curcumin, berberine, and 5-FU (6–96 μM) on cell viability of MCF-7 cell line

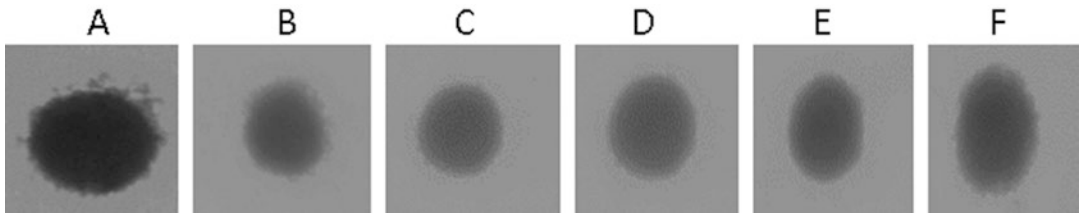

Fig. 2 The effect of each treatment group on MCF-7 cells using the spheroid model on the first day of treatment in six drug groups: (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination

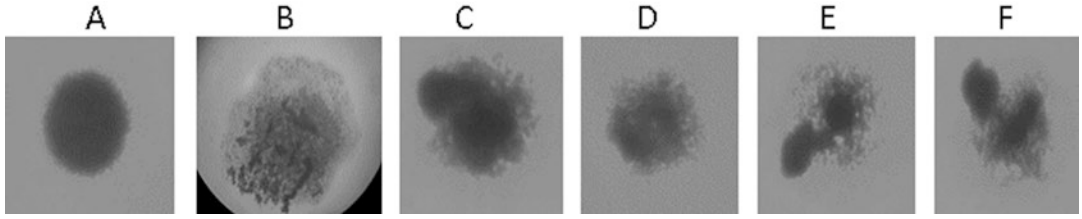

Fig. 3 The effect of drugs on MCF-7 cells using the spheroid model on the seventh day of treatment in six treatment groups: (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination

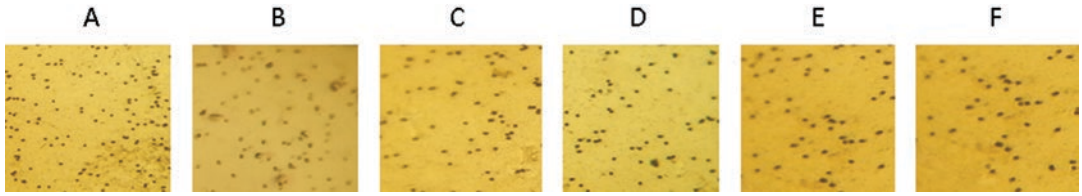

Fig. 4 The inhibitory effect of (**a**) control, (**b**) nano-curcumin, (**c**) berberine, (**d**) 5-FU, (**e**) combination of nano-curcumin and berberine, and (**f**) nano-curcumin, berberine, and 5-FU combinations on the invasion of MCF-7 cells stained with Gimsea. This showed a decrease in all five treatment groups with the highest decrease in invasion in group **f**, followed by groups **e**, **d**, **c**, and **b** sequentially

5-FU treatment group was 7.32 log fold change lower than the control group, while the reduction in expression in the other groups was not significant. The *APC* gene was also reduced by 3.58 log fold change in the combined nano-curcumin, berberine and 5-FU treatment group, compared to the control group ($p < 0.05$).

3.5 Real-Time PCR Analysis on Expression of m-TOR Pathway Genes

Real-time PCR analysis showed that expression of the *AKT1* gene was decreased with a 2.91 log fold change in the combined nano-curcumin, berberine, and 5-FU treatment group compared to the control group (Fig. 6). The expression of the *MTOR1* gene was more than 7 log fold change lower in the same combination group compared with the control ($p < 0.05$). In addition, *MAPK1* gene expression was decreased in both the nano-curcumin + berberine and nano-curcumin + berberine + 5-FU combination groups compared to the control.

3.6 Real-Time PCR Analysis on Expression of *BIRC5* and *PTEN* Genes

PCR analysis revealed decreased expression of the *BIRC5* gene in all treatment groups

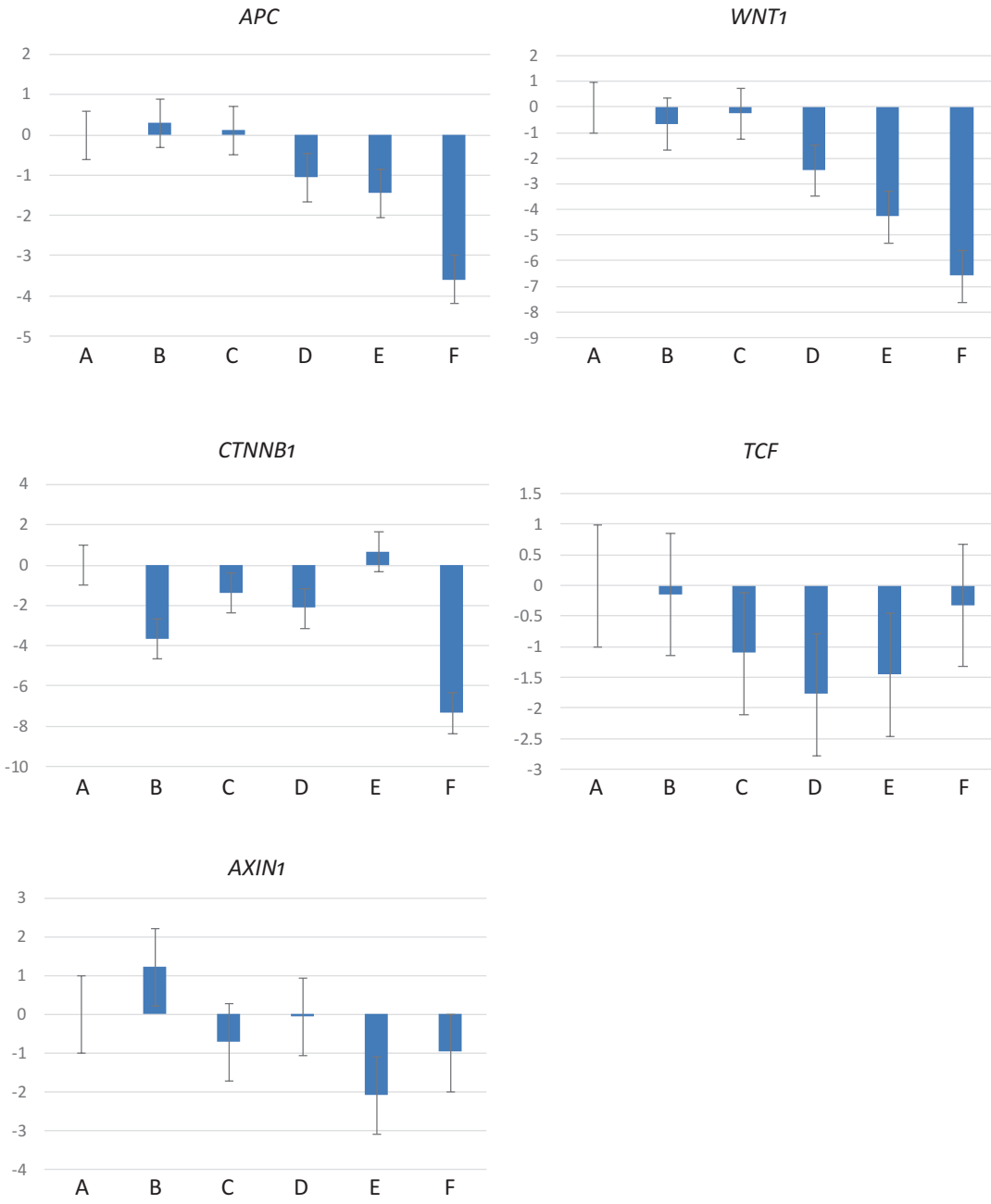

Fig. 5 Expression of Wnt pathway genes (*APC, CTNNB1, AXIN1, WNT1, TCF*) after treatment with (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination (*$p < 0.05$)

with the greatest reduction seen in the nano-curcumin + berberine + 5-FU combination group (log fold change > 3) (Fig. 7). There were no significant changes in the expression of the *PTEN* gene in any of the treatment groups.

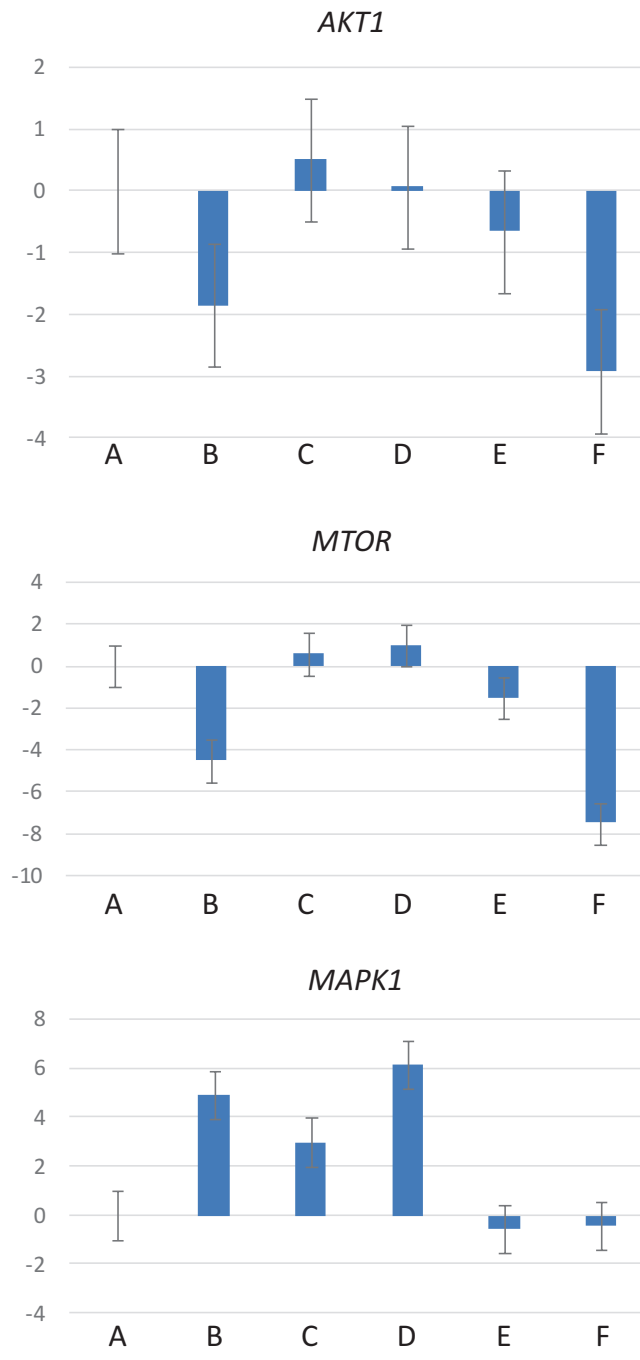

Fig. 6 Expression of m-TOR pathway genes (*AKT1, MTOR1, MAPK1*) after treatment with (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination (*$p < 0.05$)

3.7 Real-Time PCR Analysis on Expression of the *CCND1* Gene

Evaluating the results showed a reduction in the expression of Cyclin D1 gene in all of the treatment groups with a special focus on synergistic effects of combining drugs compared to the control group with a log fold change of −6.87 ($p < 0.05$) (Fig. 8).

4 Discussion

In a previous study, we found synergistic cytotoxic effects of nano-curcumin and berberine in combination against cancer cells [31]. Here, the standard cancer treatment, 5-FU, was added to this combination. The results showed the highest

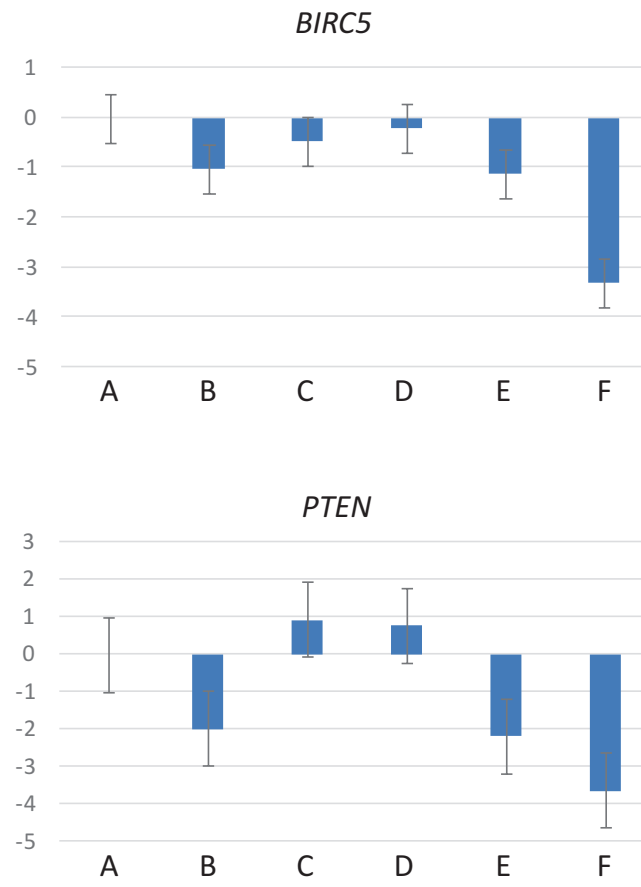

Fig. 7 Expression of PTEN (*PTEN1*) and survivin (*BIRC5*) genes after treatment with (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination (*$p < 0.05$)

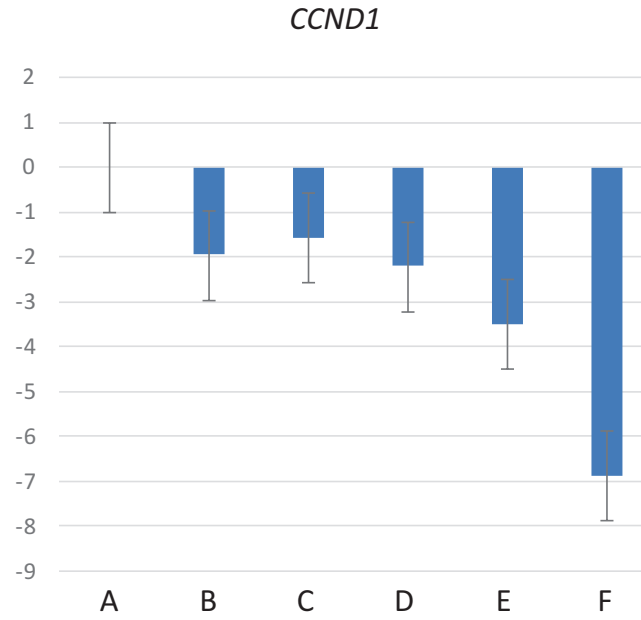

Fig. 8 Expression of Cyclin D1 gene (*CCND1*) after treatment with (**a**) control; (**b**) nano-curcumin; (**c**) berberine; (**d**) 5-FU; (**e**) nano-curcumin and berberine combination; and (**f**) nano-curcumin, berberine, and 5-FU combination (*$p < 0.05$)

synergistic effects in the combination of natural products with 5-FU. The findings of Pandey et al. were also in line with our findings. They showed successful synergistic effects in causing toxicity and reducing cell viability in gastric cancer cell line through the combination of berberine and curcumin with 5-FU [32]. Combination of curcumin with silibinin in the study of Nejati-Koshki et al. also showed synergistic effects in decreasing cell viability [33]. One possible interpretation of these findings is that these natural compounds can boost the anticancer effects of standard drug and may reduce harmful effects on normal cells, due to the lower required dosage of the chemotherapeutic agent.

Invasion and metastasis are the biological characteristics of malignant tumors. Two studies showed that inhibition of TGF-β signaling by berberine leads to a decline in migratory and invasive abilities of prostate cancer cells [34, 35]. Guo et al. also showed that the combination of curcumin and emodin was efficacious in the reduction of proliferative and migratory ability of breast cancer cells [36].

There are many tests to evaluate the cell invasion index in cancer studies, one of which is using the transwell chamber technique [37]. The current findings showed that all treatment groups were capable of inhibiting the invasion process and elicited synergistic effects in vitro. Another test on cell growth is through the use of a 3D cell culture model, in which an artificial environment is established to support cell growth in three dimensions, which comes closer to in vivo conditions [38]. Analysis of the results showed that on the seventh day, the decay of breast cancer cells reached its highest level and the combination of nano-curcumin, berberine, and 5-FU had the greatest effects on the disintegration of the cancer cells.

Apoptosis is important in regulating growth, cell proliferation, development, and the development of many diseases [39]. Cancers and viral infections are the result of poor performance of genes involved in controlling this process. We found that induction of apoptosis occurred in all groups in comparison to the control group and the greatest induction was seen in the case of the combination group of nano-curcumin, berberine, and 5-FU followed by the combination of berberine and nano-curcumin. Again, these findings suggest that this is due to a synergistic effect of these compounds. In line with this, we found reduced expression of apoptosis-related *BIRC5*, *PTEN,* and *MAPK1* genes. In a study performed in 2011, induction of apoptosis by a combination of curcumin and docosahexaenoic acid (DHA) in breast cancer cells was described through activation of genes involved in apoptosis, cell cycle arrest and inhibition of metastasis, and decreased expression of genes involved in cell cycle progression, cancer development, and metastasis [40]. Combining berberine and doxorubicin, Barzegar et al. reported induction of apoptosis and cell cycle arrest in T47D and MCF7 cells [41]. Siddiqui et al. also found that curcumin and DHA showed synergistic effects in reducing tumor growth and expression of survivin in a mouse breast cancer model [42]. In our study, the greatest reductions in the expression of the survivin gene were observed in the combination treatment groups.

One of the possible mechanisms for the observed induction of apoptosis, especially in the combination group of nano-curcumin, berberine, and 5-FU may be through the positive effects of the drugs in reducing the expression of *BIRC5* and *PTEN*. Loss of PTEN expression has been observed in breast cancer and may predict more aggressive behavior and worse outcomes [43]. Unlike previous studies, the expression of *PTEN* in this study was not significantly altered by any of the treatment groups. This finding might be due to the ineffectiveness of the doses used. Likewise, changes in the expression of *MAPK1* did not show significant reductions in the mono-treatments but the combination treatments led to a reduction in the expression levels. From selected apoptotic genes for this study, *MAPK1* and *BIRC5* were in line with previous studies and their reduction following the berberine, nano-curcumin, and 5-FU combination treatment at the mRNA level indicates the efficacy of this treatment group in the induction of apoptosis in the MCF-7 cell line.

In addition to expressing the selected genes in apoptosis, we investigated the effects of the five treatment groups on the expression of multiple genes involved in breast cancer using real-time PCR analyses. We first analyzed the Wnt pathway as this is known to be altered in breast cancer in association with reduced survival, and changes in the Wnt canonical pathway have been linked to poor prognosis [21, 22, 44].

In line with potential therapeutic effects in cancer, decreased levels of Wnt3a, β-catenin, and survivin were found following curcumin treatment [45]. Another investigation evaluated the effects of curcumin on the expression of the Wnt/β-catenin pathway in MCF-7 and MDA-MB-231 breast cancer cells [46]. This showed that the β-catenin (*CTNNB1*) and cyclin D1 (*CCND1*) genes were not expressed and the researchers suggested a negative role of the β-catenin pathway in the inhibition of cell proliferation and induction of apoptosis. Through binding of Wnt to its receptor, the conventional pathway is activated and high expression of this gene is associated with poor prognosis. In addition, the fact that it is an active component in cancer stem cells survival makes it important in tumor recurrence. In this study, we found reduced expression of this gene in all treatment groups with the highest decrease found in the combination groups. Our findings suggest that the use of selected combined natural compounds and standard chemotherapy treatment can prevent the cascade initiated by Wnt at the first step and at the level of gene expression. The *AXIN1* and *APC* gene products are expressed in various cancers, including breast cancer, and play an important role in regulating β-catenin stability in the Wnt cycle. In cancer, accumulated unphosphorylated β-catenin enters the nucleus and along with TCF and induces the expression of target genes. This study showed that the levels of β-catenin and TCF were decreased in all treatment groups, which may be important in the prognosis of this form of cancer [47].

Chemoresistance is a worldwide problem in breast cancer, which can lead to recurrence and metastasis. The anticancer effects of 5-FU in preventing cancer cell growth are achieved by targeting thymidylate synthase (TS) and long-term exposure to 5-FU can induce TS over-expression leading to 5-FU resistance [48]. Therefore, the findings of the previous studies showing that curcumin can reduce the effects of 5-FU resistance [49], combined with those of the current study showing that nano-curcumin, berberine, and 5-FU target the Wnt pathway warrant further investigation as potential means of reducing chemoresistance in breast cancer.

Increased activation of the MAPK/mTOR pathway has been shown to play a role in the increased cell growth and endocrine resistance in estrogen receptor-positive tumors found in breast cancer [50]. Studies have also shown that increasing the expression of Akt leads to increased tumor relapses and a poorer prognosis for the patient [51]. Considering the significant reduction in the expression of the *MTOR* and *MAPK1* genes in the treatment groups and elevated expression of these genes in breast cancer, it follows that successful reduction in the expression of these genes may reduce these adverse effects and poor prognosis for the patient at the level of gene expression. In a study done by Jiang et al., curcumin had the same anti-proliferative and pro-apoptotic properties and could arrest the cell cycle at G2/M and inactivate associated signaling pathways such as those involving the NF-κB and MAPK/mTOR signaling cascades [52].

CCND1 was the final gene investigated in this study. This protein has been implicated as a key factor involved in the regulation of the cell cycle [53, 54] and disruption of this pathway through activation of cyclin-dependent kinases is a central feature in cancer progression [55]. Hosseini et al. demonstrated the ability of curcumin to cause a significant reduction of *CCND1* expression in the MCF-7 cell line [56]. In the current study, we confirmed this finding and showed that the best results in all tests were achieved using the combination of nano-curcumin, berberine, and 5-FU. The effect of the treatment groups on the selective genes and their influence on each other are illustrated in Fig. 9.

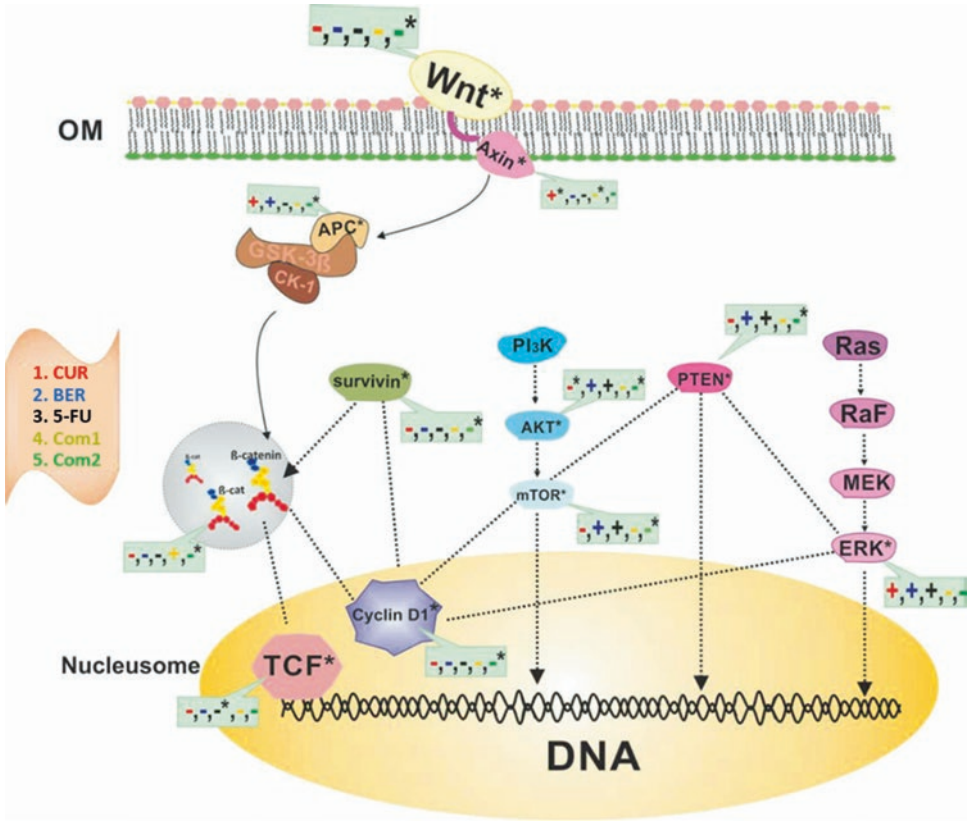

Fig. 9 Effect of five treatment groups on the expression of selected genes. Abbreviations: *CUR* curcumin, *BER* berberine, *DS* 5-FU, *Com1* combination of curcumin and berberine, *Com2* combination of curcumin, berberine, and drug standard

5 Conclusion

The results of this study showed that each of the treatment groups had anti-apoptotic effects and anti-invasive properties, along with the ability to decompose cells in a 3D environment. Also, most of the selected genes involved in tumorigenesis showed reduced expression in all treatment groups but the strongest impacts were observed in combination nano-curcumin, berberine, and 5-FU combination. Therefore, these two natural compounds may improve the anticancer effects of chemotherapy and possibly lower the consumption dose of these drugs, in turn may lead to lower side effects. The use of these two phytochemicals may be considered as potential adjuvants to standard chemotherapy treatment in breast cancer and warrant further clinical evaluation.

Conflict of Interest The authors have no conflict of interest to disclose.

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Data Availability Data associated with this study are available from the corresponding author upon a reasonable request.

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The Effect of Herbal Medicine and Natural Bioactive Compounds on Plasma Adiponectin: A Clinical Review

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Abstract

Noncommunicable diseases (NCDs) are one of the major public health concerns globally. Most of the NCDs including insulin resistance, metabolic syndrome, type 2 diabetes mellitus, fatty liver disease, and coronary heart disease are related to obesity and are called obesity-related NCDs (OR-NCDs). However, adipocytes can reduce OR-NCDs by secreting adiponectin. Adiponectin has an inverse relationship with body fat. Obese people have impairment in differentiating pre-adipocytes to adipocytes, the process facilitated by adiponectin. Adiponectin directly increases insulin sensitivity and reduces obesity-related insulin resistance by down-regulating hepatic glucose production and increasing fatty acid (FA) oxidation in skeletal muscle. Considering the various beneficial effects of adiponectin on health, increasing adiponectin might be a promising approach to prevent and treat OR-NCDs. Recent studies have shown that nutraceuticals

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and medicinal compounds isolated from plants could prevent and treat various diseases, particularly cardiovascular diseases (CVDs), diabetes mellitus, obesity, and non-alcoholic fatty liver disease. However, to our knowledge, the effect of these natural products, including herbal supplements and functional foods on adiponectin, has not yet been fully reviewed. The main aim of this review is to summarize the effects of nutraceuticals and herbal bioactive compounds on plasma adiponectin concentrations based on clinical studies. It can be concluded that medicinal plants, and herbal bioactive compounds, particularly curcumin, anthocyanins, resveratrol, soy, walnut, and dihydromyricetin can be used as adjunct or complementary therapeutic agents to increase plasma adiponectin, which could potentially prevent and treat NCDs.

Keywords

Adiponectin · Phytochemicals · Noncommunicable diseases · Obesity · Insulin resistance

1 Introduction

Noncommunicable diseases (NCDs) are one of the major public health concerns globally. It is estimated that about 71% of all deaths worldwide are currently related to NCDs, particularly cardiovascular diseases (CVDs), cancers, chronic respiratory diseases, and diabetes [1–3]. NCDs are prevalent in both developing and industrial nations and they are distributed across all age groups [2]. Improving the quality of health care and changing lifestyles (physical activity and dietary patterns) are the primary approaches to prevent and treat the progression of NCDs [4, 5]. Unhealthy diets as well as a low level of physical activity result in increased blood pressure, elevated blood glucose, raised blood lipids, and obesity. CVDs are the leading NCD in terms of premature deaths which stem from these metabolic risk factors [3, 6, 7]. Indeed, most of the NCDs are related to obesity including insulin resistance, the metabolic syndrome, type 2 diabetes mellitus (T2DM), fatty liver disease, and coronary heart disease, which are called obesity-related NCDs (OR-NCDs) [6, 7].

However, adipocytes can reduce OR-NCDs by secreting adiponectin. Adiponectin has an inverse relationship with body fat [8]. Obese people have impairment in their ability to differentiate preadipocytes to adipocytes, the process facilitated by adiponectin [8]. Adiponectin directly increases insulin sensitivity and decreased obesity-related insulin resistance by down-regulating liver glucose production and increasing fatty acid (FA) oxidation in the skeletal muscle [9–11]. Cardiac myocytes, endothelial cells, and skeletal muscle cells in addition to the adipocytes can produce adiponectin. Adiponectin has effects in the vasculature, skeletal muscle, kidney, heart, pancreatic β cells, and the liver [9–11]. Adiponectin inhibits the differentiation of monocytes into macrophages, the formation of foam cells, and the expression of adherent cells in the endothelium [8, 12, 13]. Adiponectin activates three receptors, namely AdipoR1, AdipoR2, and T-cadherin. The activation of AdipoR1 and R2 leads to increased production of skeletal muscle lactate, increased hepatic and skeletal muscle FA oxidation, reduced hepatic gluconeogenesis, increased cellular glucose uptake, and inhibition of inflammation and oxidative stress. Activation of T-cadherin reduces oxidative stress in vascular endothelial cells [8, 14–16]. Enhanced adiponectin may lead to the inhibition of the NF-kB pathway and downregulation of inflammatory cytokines such as IL-6 and TNF-α [17, 18]. Adiponectin also decreases blood glucose by increasing the adenosine monophosphate (AMP)–activator protein kinase [19–21].

Considering the numerous beneficial effects of adiponectin on health, increasing adiponectin might be a promising approach to prevent and treat OR-NCDs. Recent studies have shown that nutraceuticals and medicinal compounds isolated from plants could be used to prevent and treat various diseases, particularly CVDs [22–25], diabetes mellitus [26–28], hypertension [29–31], obesity [32–34], and non-alcoholic fatty liver disease (NAFLD) [35]. Although the effect of

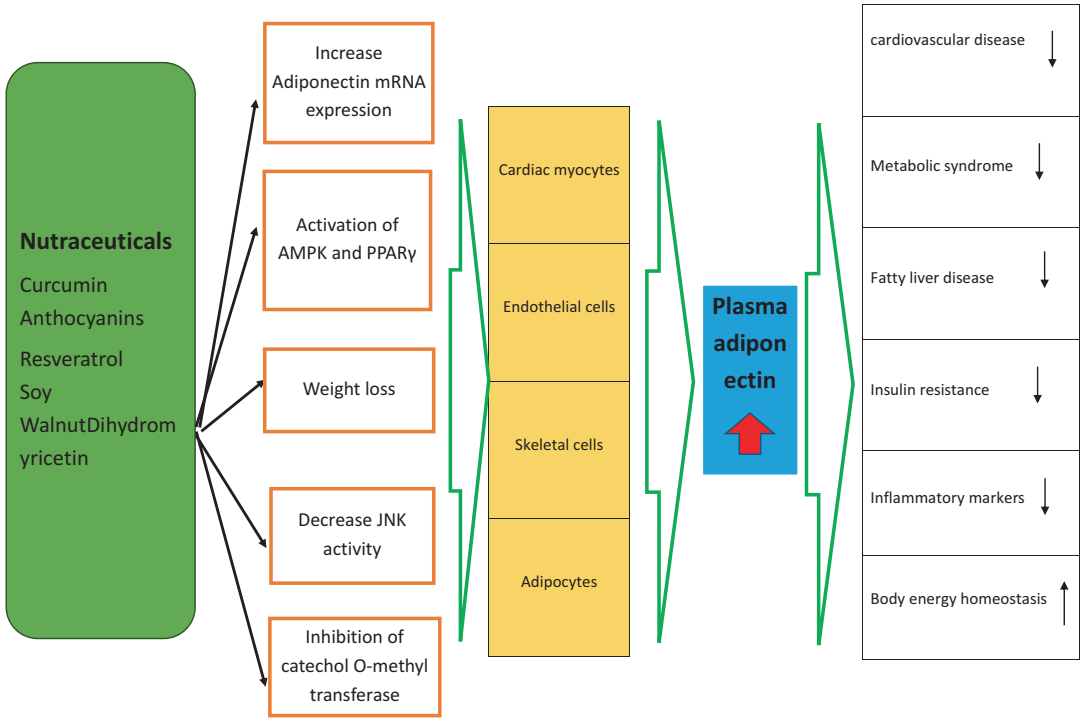

Fig. 1 The effect of herbal medicine and natural bioactive compounds on plasma adiponectin

nutraceuticals and herbal products on several NCDs [22–25] has been investigated previously [36–40], to our knowledge, the effect of these natural products, including herbal supplements and functional foods on adiponectin, has not yet been fully reviewed. Recent studies have shown that nutraceuticals and herbal medicines might affect adiponectin levels, which can indirectly have beneficial effects on OR-NCDs (Fig. 1) [41]. Thus, the main aim of this review is to summarize the effects of nutraceuticals and herbal bioactive compounds on plasma adiponectin concentrations based on clinical studies.

2 Flaxseed

Flaxseed is one of the best sources of alpha-linolenic acid (ALA) oil, lignans, high-quality protein, soluble fiber, and phytochemicals and is known as a functional food [42]. Studies show that flaxseed may have beneficial effects against metabolic syndrome and the development of type 2 diabetes by decreasing the concentration of lipids and glucose [43–46]. It seems that one potential mechanism regarding the beneficial effects of ALA on NCDs is its effects on adiponectin levels. In a previous single-blind clinical trial study, 27 men with cardiovascular risk factors were divided into two groups to receive a low carbohydrate (CHO) diet daily as (I) 35% of CHO and 60 g of raw rice powder, and (II) 32% of CHO and 60 g of flaxseed powder. After 42 days serum adiponectin significantly increased in both groups (the differences between the two groups were not reported) [47]. In another randomized controlled trial, 35 dyslipidemic and non-diabetic men were assigned into two groups: (I) 15 ml of flaxseed oil and (II) 15 ml of safflower oil daily for 12 weeks. At the end of the study, adiponectin levels did not change in the intervention group compared to the control group [48]. In one randomized, cross-over study, 25 pre-diabetics subjects (11 obese men and 14 postmenopausal women) consumed 0 or 13 or 26 gram flaxseed. Each period was 12 weeks with 4 weeks for washout between each

period. At post-intervention, no significant changes were observed on serum adiponectin levels between 3 doses of flaxseed [49]. In another clinical trial, 75 overweight adolescents were randomly allocated into three groups: (I) 28 g/d of brown flaxseed, (II) 28 g/d of gold flaxseed, or (III) 28 g/d wheat bran as the control group. After 11 weeks, serum adiponectin levels did not significantly change in both flaxseed groups and the control group [50].

3 Cinnamon

Cinnamon is a spice obtained from the dried inner bark of various trees. These trees are native to India and Sri Lanka and are used from time immemorial as an important traditional medicine against diabetes [51]. Some studies have shown that cinnamon could have a beneficial effect on insulin resistance, blood glucose, and lipid concentrations in type 2 diabetes mellitus (T2DM) [52–55].

One of the potential mechanisms behind these beneficial effects of cinnamon on type 2 diabetes mellitus might be its effects on adiponectin level; however, to date, this hypothesis was poorly investigated. As shown in Table 1, in a recent double-blind, randomized controlled study, 84 obese or overweight polycystic ovary syndrome (PCOS) were asked to consume 1.5 g cinnamon (3*500 mg capsules time) every day or placebo for 8 weeks. Results showed that serum adiponectin level did not significantly change in the cinnamon group compared with the placebo group.

4 Ginger

Ginger, as ancient herbal medicine, has a beneficial effect on vomiting and nausea. It is suggested that ginger could improve adipocyte dysfunction by inhibition of reduction in adiponectin expression in patients with metabolic syndrome [56, 57]. In one clinical trial, 40 breast cancer women were divided into four groups which respectively consumed a placebo, water-based exercise, ginger capsules (4*750 mg/per day), and water-based exercise + ginger (4*750 mg/per day) for six weeks. At the end of the study, adiponectin levels were increased in 3 intervention groups compared with baseline. Compared with baseline, these changes were significant in water-based exercise + ginger group and water-based exercise, but not in the ginger alone group [56]. In another study, 80 obese women were randomized into two groups to receive 2*1 g tablets of ginger powder (intervention group) or 2*1 g tablets of corn powder (control group) daily. After 12 weeks, results showed that adiponectin level did not significantly change in the intervention group compared with the placebo group [58].

5 Anthocyanin

Anthocyanin is a flavonoid pigment in many dark-colored fruits or vegetables [59, 60]. Studies show that anthocyanin and its sources can reduce the risk of T2DM in both preclinical and clinical studies [61–64]. Results of a recent systematic review and meta-analysis, which reviewed eight randomized, clinical trials with 390 participants, showed that supplementation with anthocyanin significantly increased plasma adiponectin levels (Table 2) [65]. As shown in Table 1, in one randomized double-blind controlled trial, 58 patients with diabetes were assigned into two groups, which received 160 mg of anthocyanin or placebo two times a day for 24 weeks. After the intervention, plasma adiponectin significantly increased in the anthocyanin group compared with the control group [66]

6 Curcumin

Curcumin is a dietary polyphenol with several salutary effects including antioxidant, anti-inflammatory, and immunomodulatory effects [67–73]. Several studies have shown that curcumin has beneficial effects on glycemic parameters, lipid profile, and other risk factors of CVDs, NAFLD, and the other OR-NCDs [35, 74–76]. Several studies assessed the effects of

Table 1 The effects of nutraceuticals and herbal bio-active compounds on adiponectin levels

Author, year	agent	Dose per day	Treatment duration	Subjects	Main outcomes	Final effects of flaxseed on plasma adiponectin
Cassani et al. 2015 [47]	Flaxseed	Group 1: 60 g of raw rice Group 2: 60 g of flaxseed powder	42 days	27 men with cardiovascular risk factor	Serum adiponectin significantly increased in both groups (the differences between 2 groups were not reported)	↑
Paschos et al. 2007 [48]	Flaxseed	Group 1: 15 ml of flaxseed oil Group 2: 15 ml of safflower oil	12 weeks	35 dislipidemic and non-diabetic men	No significant changes in adiponectin between two groups	No effect
Hutchins et al. 2013 [49]	flaxseed	1st period: 1 g 2nd period: 2 g 3rd period: 3 g	44 weeks	25 pre-diabetics subjects (11 obese men and 14 postmenopausal women)	No significant changes on adiponectin levels between 3 doses of flaxseed	No effect
Machado AM et al. 2015 [50]	flaxseed	(I) 28 g/day of brown flaxseed (II) 28 g/day of gold flaxseed (III) 28 g/day wheat bran	11 weeks	75 overweight adolescents	No significantly change in both flaxseed groups compared with control group	No effect
Borzoei et al. 2018 [154]	cinnamon	1.5 g cinnamon Or 1.5 g placebo	8 weeks	84 obese or overweight PCOS	Serum adiponectin level did not significantly change in the cinnamon group compared with placebo	No effect
Karimi N et al. 2013 [56]	Ginger	3 grams per day (4*750 mg capsules per day)	6 weeks	40 breast cancer women	At the end of the study, adiponectin was increased in 3 intervention groups. These changes were significant in water-based exercise (17.22%) and water-based exercise + ginger groups (31.03%) but was not significantly different in ginger (6.31%) group compared with baseline	No effect
Attari VE et al. 2016 [58]	ginger	2*1 g tablets of ginger powder or 2*1 g tablets of corn powder	12 weeks	80 obese women	Adiponectin levels had no significant change in the intervention group compared with the placebo group	No effect
Li D et al. 2015 [66]	Anthocyanins	160 mg twice per day	24 weeks	40 breast cancer women	Plasma adiponectin significantly increased (+23.4%) in anthocyanins group compared with the control group	↑

(continued)

Table 1 (continued)

Panahi Y et al. 2016 [80]	Curcumin	1000 mg/day	8 weeks	117 metabolic syndromes patients	A significant increase in adiponectin levels was observed in the curcumin group compared with the placebo group	↑
Chuengsamarn S et al. 2014 [81]	Curcumin	1500 mg/day 2*(3*250)	6 months	213 diabetic patients	Adiponectin levels significantly increased in the curcumin group (9.24 to 23.91 ng/ml) compared with the placebo group	↑
Chuengsamarn S et al. 2012 [82]	Curcumin	1500 mg/day 2*(3*250)	9 months	240 patients with sign of prediabetes	After treatment with curcumin, at the end of the study, significant increase was observed on adiponectin levels in comparison to the control group	↑
Campbell MS et al. 2019 [83]	Curcumin	500 mg/day	12 weeks	22 men	Adiponectin levels had no significant change in both groups and between them	No effect
Mirhafez SR et al. 2019 [84]	Curcumin	50 mg/day pure curcumin	8 weeks	56 NAFLD patients	Serum adiponectin levels were significantly increased in the curcumin group compared with the placebo group	↑
Adibian M et al. 2019 [17]	Curcumin	1500 mg/day	10 weeks	44 T2DM patients	Serum adiponectin levels were significantly increased in the curcumin group compared with the placebo group	↑
Gomez-Arbelaez D et al. 2013 [8]	Aged garlic extract (AGE)	1.2 g twice per day	12 weeks	46 metabolic syndrome patients	Adiponectin levels were significantly increased in the AGE group compared with the placebo group	↑
Sharifi F et al. 2010 [93]	Garlic	1.8g	6 weeks	40 adult women with metabolic syndrome + 10 healthy women	A significant increase in healthy participants and no effects in intervention groups	Healthy women: ↑ Metabolic syndrome patients: No effect
Xu C et al. 2018 [94]	AGE	3.6 g	6 weeks	51 obese healthy participants	No significant changes were observed in plasma adiponectin level between groups	No effect
Gulati S et al. 2014 [101]	pistachio nuts	20% standard energy	24 weeks	60 metabolic syndrome patient.	Adiponectin levels significantly increased in the intervention group compared with the placebo group	↑

Hwang HJ et al. 2019 [102]	Walnut	45 g walnuts	2* 16 weeks with 6 weeks washout	119 adult Korean metabolic syndrome patient	Adiponectin levels significantly increased in walnuts group compared with white bread	↑
Lozano A et al. 2013 [103]	Walnut	Walnut-enriched meal	8.5 hours	21 healthy white men	Serum adiponectin concentrations were higher at 3 and 6 hours in walnut group compared with butter group and it was higher at 6 hours in walnut group compared with olive oil group	↑
Aronis KN et al. 2012 [104]	Walnut	48 g walnuts	2* 4 days with 1 week washout	15 metabolic syndromes adult patient	Serum adiponectin level significantly increased in walnut group compared with baseline	↑
Shimada K et al. 2004 [112]	Oolong tea	1000 ml	2* 1 months with 2 weeks washout	22 coronary artery patient.	No significant changes were observed in adiponectin levels between groups	No effect
Chen T-S et al. 2009 [116]	Amla	450 mg (3 Amla extract tablets)	4 months	17 uremic patients.	No significant change was observed	No effect
Chen I-J et al. 2016 [121]	Green tea	High-dose green tea	12 weeks	102 women with central obesity	High-dose green tea significantly increased adiponectin levels compare with placebo	↑
Dostal AM et al. 2015 [122]	Green tea	Green tea extract including 843 epigallocatechin-3-gallate	12 months	121 overweight/obese postmenopausal women	Serum adiponectin levels did not change in both groups	No effect
Wu AH et al. 2012 [123]	Green tea	(I) Placebo (II) 400 mg EGCG (III) 800 mg EGCG	2 months	103 postmenopausal women	Adiponectin levels did not significantly change	No effect
Hsu C-H et al. 2008 [124]	Green tea	Receive green tea extract 400 mg capsule 3 times a day	12 weeks	100 obese women between16-60 years of age	Significant increase was observed in intervention group but it is not significant between groups	No effect
Liu C-Y et al. 2014 [125]	Green tea	500 mg of green tea extract 3 times a day	16 weeks	92 T2DM patients with abnormal lipid	A significantly increase was observed in both groups but there was no significant differences between groups	No effect
Basu A et al. 2011 [126]	Green tea	(I) 4 cups green tea (II) 2 capsules green tea extract and 4 cups water (III) 4 cups water	8 weeks	35 obese patient with metabolic syndrome	Serum adiponectin levels had no significant change in the intervention group compared with the control group	No effect

(continued)

Table 1 (continued)

Chen S et al. 2015 [129]	Resveratrol	2*150 mg resveratrol capsules twice a day	3 months	60 non-alcoholic fatty liver patients	Serum adiponectin levels significantly increased (1.22 (−0.37, 1.60)) in the intervention group compared with the control group	↑
Goh KP et al. 2014 [135]	Resveratrol	3 grams daily resveratrol	12 weeks	10 T2DM patients	No significant change in plasma adiponectin levels was observed in the resveratrol group compared with the placebo group	No effect
Bo S et al. 2016 [127]	Resveratrol	Placebo or 40 or 500 mg daily resveratrol	6 months	192 subjects with T2DM	No significant change in plasma adiponectin levels was observed in both resveratrol groups compared with placebo group	No effect
Arzola-Paniagua MA et al. 2016 [136]	Resveratrol	(I) orlistat 120 mg (II) resveratrol 100 mg (III) orlistat 120 mg + resveratrol 100 mg (IV) placebo	6 months	161 adult subjects (20–60 years old and $30 \geq BMI \leq 39.9$ kg/m^2)	No significant change was observed in adiponectin levels between groups	No effect
Christie DR et al. 2010 [147]	Soy	20g soy protein + 160mg isoflavones or shake + 20g casein protein without isoflavones	3 months	39 postmenopausal	No significant changes were observed in plasma adiponectin levels between groups	No effect
Charles C et al. 2009 [148]	Soy	20 g of soy protein (containing 160 mg isoflavones) or placebo	12 weeks	75 postmenopausal	Significant increase in the intervention group compared with control group	↑
Lozovoy MAB et al. 2012 [149]	Soy	(I) usual diet, (II) 29 g soy protein (kinako), (III) 3 g fish oil, or (IV) 29 g kinako+3 g fish oil	90 days	65 women	Adiponectin significantly increased in 2nd and 3rd group	↑
Chen S et al. 2015 [151]	Dihydromyricetin	2*(2*150) mg/day	3 months	60 NAFLD patients	Adiponectin levels in dihydromyricetin group were significantly increased compared with placebo	↑

Table 2 The effects of nutraceuticals and herbal bio-active compounds on adiponectin levels based on meta-analysis

Author, year	Type of the study	Number of included studies	Intervention/ control	Main outcomes	Statistical analysis
Fallah AA et al. 2019 [65]	Systematic review and meta-analysis	8 randomized clinical trials studies with 390 participants	Anthocyanins	Supplementation with anthocyanins significantly increased plasma adiponectin levels	(0.75 µg/ml, 95% CI: 0.23 to 1.26, $P = 0.004$)
Akbari M et al. 2019 [77]	Systematic review and meta-analysis	21 randomized clinical trials studies (18 articles) with 1604 metabolic syndrome participants	Curcumin	Curcumin significantly increased plasma adiponectin levels	(Standardized mean difference (SMD): 1.05; 95% CI, 0.23, 1.87; $P = 0.01$)
Clark CC et al. 2019 [78]	Systematic review and meta-analysis	6 randomized clinical trial studies	Curcumin	Supplementation with curcumin significantly increased circulating adiponectin. Greater effects on adiponectin were observed in trials lasting ≤10 weeks	(Weighted mean difference (WMD): 0.82 Hedges' g; 95% confidence interval (CI): 0.33–1.30, P < 0.001)
Simental-Mendía LE et al. 2019 [79]	Systematic review and meta-analysis	5 randomized clinical trial studies	Curcumin	Supplementation with curcuminoids significantly elevated plasma adiponectin concentration	(WMD: 6.47 ng/mL, 95% CI: 1.85, 11.10, p = 0.010; I2= 94.85%)
Darooghegi Mofrad M et al. 2019 [92]	Systematic review and meta-analysis	5 randomized clinical trial studies	Garlic	Supplementation with garlic had no significant effects on serum adiponectin levels	(WMD: 0.18 µg/L, 95% CI: −0.21, 0.57, $P = 0.35$, $I2 = 60.7\%$)
Haghighatdoost F et al. 2017 [120]	Systematic review and meta-analysis	14 randomized clinical trial studies	Green tea	Supplementation with green tea had no significant effect on plasma adiponectin concentration	WMD: −0.02 µg/ml, 95% confidence interval [CI], −0.41, 0.38; $P = 0.936$
Mazidi M et al. 2016 [100]	Systematic review and meta-analysis	20 randomized clinical trial studies	Nuts	Used tree nuts, peanuts and soy nuts had no significant effect on plasma adiponectin concentration	-0.18(mg/dL), (95% CI −1.24 to 0.88, I^2 9.3%)
Tabrizi R et al. 2018 [133]	Systematic review and meta-analysis	36 randomized clinical trial studies	Resveratrol	Supplementation with resveratrol had non-significant effect on serum adiponectin level	SMD: 0.08; 95% CI, −0.39, 0.55; $P = 0.74$; I2: 91.0)
Mohammadi-Sartang M et al. 2017 [134]	Systematic review and meta-analysis	9 randomized clinical trial studies	Resveratrol	Supplementation with resveratrol significantly improved adiponectin. Resveratrol was more effective when used 100 or more than 100 mg/d	(WMD: 1.10 _g/ml, 95%CI: 0.88, 1.33, $p < 0.001$); Q = 11.43, I2 = 21.29%, $p = 0.247$)

curcumin on adiponectin levels; therefore, to date, some systematic reviews and meta-analysis are published in this regard. Results of a recent systematic review and meta-analysis, which reviewed 21 studies (18 articles) with 1604 participants with metabolic syndrome, showed that supplementation with curcumin significantly increased plasma adiponectin levels (Table 2) [77]. Another recent systematic review and meta-analysis, which reviewed six clinical trial studies, showed that supplementation with curcumin significantly increased circulating adiponectin. Curcumin was more effective when used 10 weeks or more [78]. Results of another systematic review and meta-analysis on five clinical trial studies showed that supplementation with curcuminoids significantly elevated plasma adiponectin concentration [79].

In a double-blind, clinical trial study, 117 metabolic syndromes patients were divided into two groups to receive 1000 mg/day of curcumin (n = 59) or placebo (n = 58) for eight weeks. After the intervention, a significant increase in adiponectin ($p < 0.001$) level was observed in the curcumin group compared with the placebo group [80]. In another study, 213 diabetic patients were assigned to receive curcumin twice/day (3*250 mg capsules each time) or placebo for six months. At the end of the study, adiponectin levels significantly increased in the curcumin group (9.24–23.91 ng/ml) compared with the placebo group [81]. In another randomized, double-blinded, placebo-controlled trial, 240 patients with signs of prediabetes were assigned into 2 groups to receive placebo or 1500 mg curcumin (twice/day, each time 3*250 mg capsules). After nine months treatment with curcumin, a significant increase was observed on adiponectin levels in comparison to the control group [82]. In another study, 22 men were randomized into placebo groups (fenugreek soluble fiber) or intervention group (curcumin formulated and fenugreek soluble fiber) to receive 500 mg/day of these natural products for 12 weeks. At the end of the study, adiponectin levels did not significantly change in both groups [83]. In a previous clinical trial, 56 non-alcoholic fatty liver disease (NAFLD) patients were divided into two groups to receive phospholipidated curcumin (included 50 mg pure curcumin) or placebo daily. After 8 weeks, serum adiponectin levels significantly increased in the curcumin group compared with the placebo group [84]. In another clinical study, 44 T2DM patients were divided into two groups to receive 1500 mg/day curcumin or placebo for 10 weeks. At the end of the study, serum adiponectin levels were significantly increased in the curcumin group compared with the placebo group [17]. Results of the clinical trials were summarized in Table 1.

7 Garlic

Garlic is a herb with the beneficial effect on CVD because since it is a valuable source of nutrients and antioxidants. However, due to the bad odor and indigestion of fresh garlic, people may not consume this herbal medicine in high amounts [8, 85–87]. Results of several randomized clinical trials indicated the beneficial effects of garlic on glycemic indexes such as serum insulin, fasting blood sugar as well as other CVD risk factors such as lipid profile and inflammatory markers [88–91]. However, results of a recent systematic review and meta-analysis which reviewed 5 clinical trial studies with 12–3600 mg/day garlic doses and 2–52 weeks intervention duration showed that supplementation with garlic had no significant effects on serum adiponectin levels [92].

In a randomized, crossover study, 46 participants with metabolic syndrome were assigned to two groups to take four capsules of aged garlic extract (AGE), two times a day to receive a total of 1.2 g AGE/day or placebo capsules for 12 weeks without a washout period (Table 1). At the end of the study, results showed that adiponectin levels significantly increased in the AGE group compared with the placebo group [8]. In a recent double-blind, randomized controlled study, 40 adult women with metabolic syndrome were asked to consume 1.8 g garlic or placebo every day for 6 weeks. Ten healthy women were also asked to consume garlic at the same dose. After the intervention, there were no significant

changes in plasma adiponectin levels in the garlic group compared with the placebo group; however, a significant increase was found in healthy participants [93]. In still another clinical trial study, 51 obese participants were divided into two groups to receive 3.6 gram/day of AGE or placebo. After six weeks, no significant changes were observed in plasma adiponectin levels between the groups [94].

8 Nuts

Nuts are good sources of unsaturated fatty acids, micronutrients, protein, fiber, vitamins, and phytochemicals as well as have beneficial effects on total antioxidant capacity [95, 96]. Nuts consumption has beneficial effects on cardiometabolic factors such as lipid profile, and inflammatory markers but their effects on glycemic parameters are controversial [97–99]. Results of a recent systematic review and meta-analysis, which reviewed 20 clinical trial studies, showed that used tree nuts, peanuts, and soy nuts had no significant effect on plasma adiponectin concentration [100]. In a previous clinical trial study, 60 metabolic syndrome patients after three weeks standard diet and exercise were divided into two groups to receive standard diet and physical activity with pistachio nuts (containing 20% of energy) or standard diet and physical activity without pistachio nuts for 24 weeks. Results showed that adiponectin levels significantly increased in the intervention group compared with the placebo group [101]. In a randomized, crossover study, 119 patients with metabolic syndrome were recruited into two groups to consume 45 g walnuts or iso-caloric white bread. Intervention was conducted for 16 weeks, with a 6-week washout period to separate the interventions. At the end of the study, adiponectin levels significantly increased in the walnuts intervention group compared with the white bread intervention group [102]. In a randomized, crossover study, 21 healthy white men consumed a regular diet for 4 weeks then they assigned into three groups to receive three fat-loaded meals (1 g fat/kg body weight): (I) olive oil-enriched meal (22% saturated FAs [SFA], 38% monounsaturated FAs [MUFA], 4% polyunsaturated FAs [PUFA]), (II) butter-enriched meal (35% SFA, 22% MUFA, 4% PUFA), and (III) walnut-enriched meal (20% SFA, 24% MUFA, 16% PUFA, and 4% α-linolenic acid) and adiponectin were measured after 0, 3, 6, and 8.5 h. Serum adiponectin concentrations were higher at 3 and 6 h in the walnut group compared with the butter group and it was higher at 6 h in the walnut group compared with the olive oil group [103]. In another recent randomized crossover study, 15 metabolic syndrome patients were recruited to three groups to consume 48 g walnuts or placebo daily with an iso-caloric meal with identical macronutrient content. The intervention was conducted for 4 days, with one month washout period to separate the interventions. After the intervention, compared with baseline, serum adiponectin level significantly increased in the walnuts groups; however, it decreased (non-significantly) in the control group [104]. Results of the previous studies regarding the effects of nuts on plasma adiponectin are summarized in Table 1.

9 Oolong Tea

It is one kind of tea that is produced from *Camellia sinensis* by semi-fermented and enzymatic method. It is a good source of catechins; has more catechins (23.2%) than the black tea (4.3%) and less than the green tea (26.7%) [105]. It has been shown that oolong tea has antioxidant and antiobesity properties; it increases metabolic rate and fat oxidation, with beneficial effects on CVDs [106–111]. In a recent cross-over randomized study, 22 coronary artery patients were asked to consume 1000 ml oolong tea daily or 1000 ml water for one month with two weeks washout. After the intervention, no significant changes were observed in adiponectin levels between groups (Table 1) [112].

10 *Emblica officinalis* (Amla)

Emblica officinalis (Indian Gooseberry), a traditional and functional food, has physiological effects such as hepato-protection, cyto-protection,

and radio-protection, as well as hypolipidemic effects. In addition, amla often functions as an antioxidant because of the high level of ascorbic acid (from 1100 to 1700 mg per 100 g of fruit) in its fruit. According to previous preclinical and clinical trials, *Emblica officinalis* might have good effects on glycemic parameters [113–115]. In a previous clinical trial study, 17 uremic patients received Amla extract tablets (300 mg: 150 mg amla extract + 150 mg dextrin) three times a day for four months. At the end of the study, no significant changes were observed (Table 1) [116].

11 Green Tea

Green tea is a non-fermented tea and is a good source of catechin as a potent antioxidant. It has beneficial effects on inhibiting adipocyte differentiation and proliferation, thereby reducing body weight [117, 118]. Previous meta-analysis showed that that green tea catechins have a significant effect on fasting plasma glucose, but had no effects on fasting blood insulin (FBI), glycated hemoglobin (HbA1c), and homeostatic model assessment of insulin resistance (HOMA-IR)H [119]. Results of recent systematic review and meta-analysis, which reviewed 14 clinical studies, showed that supplementation with green tea had no significant effect on plasma adiponectin concentration [120]. As shown in Table 1, in a previous double-blind, randomized controlled study, 102 women with central obesity were asked to consume high-dose green tea every day or placebo for 12 weeks. After the intervention, a high dose of green tea significantly increased adiponectin levels compared with the placebo [121]. In one clinical trial study, 121 overweight/obese postmenopausal women were asked to take green tea extract (including 843 epigallocatechin-3-gallate, intervention group) for 12 months or placebo. After the intervention, serum adiponectin levels did not change in both groups [122]. In another clinical trial on epigallocatechin gallate (EGCG), the main catechin of green tea, 103 postmenopausal women were randomized to three groups: (I) placebo, (II) 400 mg EGCG, or (III) 800 mg EGCG. After two months, adiponectin levels did not significantly change [123]. In a previous clinical trial study, 100 obese women were divided into two groups to receive green tea extract (400 mg capsule) or placebo three times a day. After 12 weeks of intervention, there was a significant increase in adiponectin levels in the intervention group; however, this change was no significant compared with the changes in the control group [124]. In a previous clinical trial study, 92 patients with T2DM and abnormal lipid levels were divided into two groups to receive 500 mg of green tea extract or placebo three times a day for 16 weeks. They observed that adiponectin significantly increased in both groups but there were no significant changes between the groups [125]. In a recent randomized controlled study, 35 obese patients with metabolic syndrome were divided into 3 groups I) 4 cups/day green tea, II) 2 capsules green tea extract and 4 cups water per day, or III) 4 cups water/day for eight weeks. Results showed that serum adiponectin levels did not significantly change in the intervention groups compared with the control group [126].

12 Resveratrol

Resveratrol is a polyphenol component in grapes, peanuts, berries, and red wine and is commonly used as a supplement with 450 mg as an acceptable daily intake. Previous studies showed that it has beneficial effects on antioxidant capacity, inflammation, platelet aggregation, cardiovascular system, insulin resistance, aging activities, lifespan, body weight, and endothelial function [127–130]. Notwithstanding some reports that suggested the futility of resveratrol in modifying cardiovascular risk fators [131, 132], results of a recent systematic review and meta-analysis, which reviewed 36 clinical trial studies, showed that supplementation with resveratrol significantly reduced weight, body mass index (BMI), waist circumference and fat mass, and significantly increased lean mass; however, it had no significant effect on serum adiponectin and leptin levels [133]. Nevertheless, another recent sys-

tematic review and meta-analysis which reviewed nine clinical trials showed that supplementation with resveratrol significantly increased adiponectin. Resveratrol was more effective when used 100 or more than 100 mg/day [134]. In one clinical trial, 60 NAFLD patients were asked to take 2*150 mg resveratrol capsules twice a day as an intervention group for three months or 2*150 mg placebo as a control group. Results showed that serum adiponectin levels significantly increased in the intervention group than in the placebo group [129]. In another clinical trial study, 10 patients with T2DM were randomized into two groups to receive placebo or three grams daily resveratrol for 12 weeks. |After the intervention, no significant change in plasma adiponectin levels was observed in the resveratrol group compared with the placebo group [135]. In another study, 192 subjects with T2DM were randomized into three groups to receive placebo or 40 or 500 mg daily resveratrol for six months. At the end of the study, no significant changes in plasma adiponectin levels were observed in both resveratrol groups compared with the placebo group [127]. In another parallel randomized clinical trial, 161 adult subjects (20–60 years old and 30≥ body mass index (BMI) ≤39.9 kg/m^2) consumed the usual diet -500 kcal for two weeks. After these two weeks, they were randomly recruited to four groups (I) Orlistat 120 mg, (II) resveratrol 100 mg, (III) Orlistat120 mg + resveratrol 100 mg, or (IV) placebo. These supplements were consumed as single capsules three times a day before each meal. After 6 months, no significant changes were found in adiponectin levels between groups [136]. Results of the previous studies regarding the effects of resveratrol on plasma adiponectin are summarized in Table 1.

13 Soy

Soy is a traditional plant from the east of Asia. Soybean, soy milk, and tofu are the most well-known products of soy. Soy has advantageous effects on serum lipids, fertility, and menopausal symptoms [137–141]. It is a good source of fiber, plant sterols, the isoflavones daidzein, genistein, and some other phytochemicals [142]. Results of a previous meta-analysis indicated that soy protein had favorable changes in fasting glucose concentrations in studies that used whole soy foods or a soy diet [143]. It is suggested that consumption of soy protein instead of animal protein has a promising role in CVD reduction [144, 145]. The negative association between soy consumption and CVDs, stroke, and coronary heart disease risk was confirmed by a recent meta-analysis of observational studies [146].

In a previous clinical trial study, 39 postmenopausal women were divided into two groups to receive shake (120 calories, 2.5 g fat, 7 g CHOs, 600mg calcium, 500 mg phosphorus, 320 mg sodium, 560 mg potassium, and 3 mg iron) + 20 g soy protein + 160 mg isoflavones (intervention group) or shake + 20 g casein protein without isoflavones (placebo group). After three months of intervention, no significant changes were observed in the plasma adiponectin levels between the groups [147]. In a recent double-blind, randomized controlled study, 75 postmenopausal women were asked to consume 20 g of soy protein (containing 160 mg isoflavones) as an intervention group or placebo as a control group for 12 weeks. Results showed that serum adiponectin levels significantly increased in the intervention group compared with the control group [148]. In another clinical trial, 65 women were randomly recruited to four groups (I) usual diet, (II) 29 g soy protein (kinako), (III) 3 g fish oil, or (IV) 29 g kinako + 3g fish oil for 90 days. At post intervention, plasma adiponectin significantly increased in second and third groups (Table 1) [149].

14 Dihydromyricetin

Chinese traditionally used *Ampelopsis grossedentata* as a good source of dihydromyricetin. Dihydromyricetin had beneficial effects on health such as antimicrobial, anti-inflammatory, antioxidative, anticancer, lipid and glucose metabolism-regulatory activities, and cell death-mediating without or with minimum adverse effects on normal cells [150]. In a previous randomized con-

trolled trial, 60 NAFLD patients were assigned to receive two capsules dihydromyricetin (each capsule contained 150 mg) or placebo, two times daily for three months. At post-intervention, adiponectin levels in dihydromyricetin group were significantly increased compared with placebo (Table 1) [151].

15 Potential Mechanisms

The potential mechanisms regarding the effects of nutraceuticals and herbal medicine on adiponectin levels are summarized here.

1. Inhibition of catechol O-methyl transferase (COMT) enzyme by green tea catechins might lead to an increase in adiponectin levels [152, 153].
2. Anthocyanin increases high A molecular weight adiponectin expression [66].
3. Transcinnamic acid (tCA) increased secretion of adiponectin in 3T3-L1 adipocyte through activation of AMP kinase [154].
4. Cinnamaldehyde and ω3 stimulate adiponectin expression through increased expression of peroxisome proliferator–activator receptor gamma (PPARγ) in adipose tissue [149, 154].
5. Curcumin might have an effect on adiponectin through influenced on transcription factors (e.g., NFκB and activator protein 1), pro-inflammatory cytokines, acute phase proteins, growth factors, antioxidants, secondary messengers, nitric oxide (NO), hormones, and enzymes (cyclooxygenases) [80].
6. Curcumin has a positive effect on adipocytes to improve adiponectin expression [17].
7. In obese and overweight population weight loss may increase adiponectin which leads to decreased inflammatory markers such as TNF-α [47].
8. The activity of nitric oxide (NO) is increased by garlic due to the relationship between NO and adiponectin. As a result, adiponectin increases with garlic consumption [8].
9. In vitro studies show that ginger components can up-regulate the gene expression of adiponectin in the adipocyte. 6-gingerol increases adiponectin by inhibiting TNF-α, which is the mediator of c-Jun N terminal kinases (JNK) activity [58, 155].

16 Conclusion and Future Perspectives

This review assessed the effects of nutraceuticals and herbal bioactive compounds on serum adiponectin levels according to the clinical studies. Although some of the phytochemicals had no significant effects on adiponectin levels, some others particularly curcumin, anthocyanins, resveratrol, soy, walnut, and dihydromyricetin notably increased plasma adiponectin levels. However, for some of the above agents, there are currently very few studies. Furthermore, the total number of randomized clinical controlled trials in this regard is small. In almost all of the previous studies, adiponectin was assessed as a secondary outcome. Moreover, most of the studies did not assess whether improvement in adiponectin has led to an improvement in the disease or not. Adiponectin was assessed in different populations with different diseases. With regard to the salient role of adiponectin in human health and its beneficial effects on several obesity-related NCDs, including metabolic syndrome, T2DM, NAFLD, coronary heart disease, and CVDs, and considering the facts that these natural agents are generally safe, accessible, and inexpensive, larger clinical trials with plant-derived therapeutic agents are definitely warranted to accurately assess on plasma adiponectin to determine the optimal dose, and identify the correct dosing regimen (dosing frequency and the duration of treatment) to reap their full therapeutic potential. Finally, based on the results of the current review, which sum up the results of the existing clinical trials, medicinal plants and herbal bioactive compounds could be used as an adjunct or complementary therapeutic agents to increase plasma adiponectin, which might result in the prevention and treatment of NCDs.

Conflict of interests None

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The Effects of Nutraceuticals and Bioactive Natural Compounds on Chronic Periodontitis: A Clinical Review

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Abstract

The paper aims to review the current clinical evidence of various herbal agents as an adjunct treatment in the management of chronic periodontitis patients. Gingivitis and periodontitis are two common infectious inflammatory diseases of the supporting tissues of the teeth and have a multifactorial etiology. An important concern about chronic periodontitis is its association with certain systemic disease. New treatment strategies for controlling the adverse effects of chronic periodontitis have been extensively assessed and practiced in subclinical and clinical studies. It has been shown that the phytochemical agents have various therapeutic properties such as anti-inflammatory and antibacterial effects which can be beneficial for the treatment of periodontitis. The findings of this review support the adjunctive use of herbal agents in the management of chronic periodontitis. Heterogeneity and limited data may reduce the impact of these conclusions. Future long-term randomized controlled trials evaluating the clinical efficacy of adjunctive herbal therapy to scaling and root planing are needed.

Keywords

Chronic Periodontitis · Herbal Medicine · Inflammation · immune system · Dental Scaling

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1 Introduction

Periodontal disease, with the prevalence of about 20–50% of the global population, is one of the most significant public health concerns in both developing and industrial countries [1]. According to the National Health and Nutrition

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Examination Survey (NHANES) study, in 2009–2012, nearly half (45.9%) of the United States population aged 30 years and older had periodontitis [2].

One of the main concerns about chronic periodontitis is its association with some systemic diseases such as cardiovascular diseases (CVDs), diabetes, and adverse pregnancy outcomes [1]. It is estimated that 19% increase in the risk of CVDs is related to periodontal disease and this relative risk increases to about 44% among the elderly population [1]. In comparison between the patients with diabetes with no or mild chronic periodontitis, patients with type 2 diabetes suffering from severe periodontitis have 3.2 times greater mortality risk [1]. More interestingly, treatment of the periodontal disease may help in controlling glycemic level in patients with type 2 diabetes [3].

The main etiological agents of periodontal disease are periopathogenic bacteria in the subgingival area [4]. The colonization of microorganisms such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* (Pg), and *Prevotella intermedia* initiate the inflammation that can lead to tissues breakdown in the susceptible host [5, 6].

Scaling and root planing (SRP) is an essential and the most common treatment procedure for the management of periodontal infections [7, 8]. It should be considered that SRP may not provide optimal benefits in areas with complex anatomies such as furcations, deep pockets, and developmental grooves [9].

To overcome the limitations of conventional treatment, the use of antimicrobial therapy to complement the outcomes of mechanical debridement has been assessed in clinical studies. Concerns about the systemic application of antimicrobials, such as bacterial resistance, associated adverse effects, and drug interactions, provided the impetus for the development of local antibacterial delivery systems and also finding some alternatives for pharmaceutical agents [10–12].

On the other hand, in recent years, it has been shown that the immunological responses of host tissues can be considered as an important factor in progressing periodontal tissue destruction. In this regard, a new concept named "host modulation therapy" emerged in the scientific literature [13]. Based on this approach, the treatment plan should be focused on modifying the inflammatory response of the body with the aim of reducing the destructive aspects of the immune system [14].

Recently, a growing body of evidence showed that nutraceuticals and medicinal compounds isolated from plants have several health benefits to prevent and treat various diseases, particularly dyslipidemia and CVD [15–18], diabetes mellitus [19–21], hypertension [22–24], and non-alcoholic fatty liver disease (NAFLD) [25]. These health benefits of herbal medicine include lipid-modifying, anti-tumor, antioxidant, insulin-sensitizing, anti-steatotic, anti-fibrotic, anti-atherosclerotic, antithrombotic, antidepressant, and antirheumatic, anti-inflammatory, anti-stress oxidative, and antimicrobial activates [26–38]. In dental literature, recent preclinical studies showed anti-inflammatory effects of some of the herbal agents with respect to periodontal tissues [39–42]. Based on these properties, these phytochemical agents can be considered for host modulation therapy [43, 44]. Furthermore, several studies demonstrated the salient role of some nutraceuticals on decreasing bacterial load of dental and periodontal tissues [32, 45–47].

Selection of a right herbal antibacterial agent with an appropriate route of administration is the key point to achieve successful periodontal treatment. Reviewing clinical trials using herbal agents for the treatment of chronic periodontitis may be helpful for developing this concept. To the best of our knowledge, there is no study summarizing the results of the clinical studies regarding the effects of herbal medicine and nutraceuticals on periodontitis. With this background, the present review aims to summarize the current evidence on the application of herbal agents as an adjunct treatment in chronic periodontitis patients (Figs. 1 and 2).

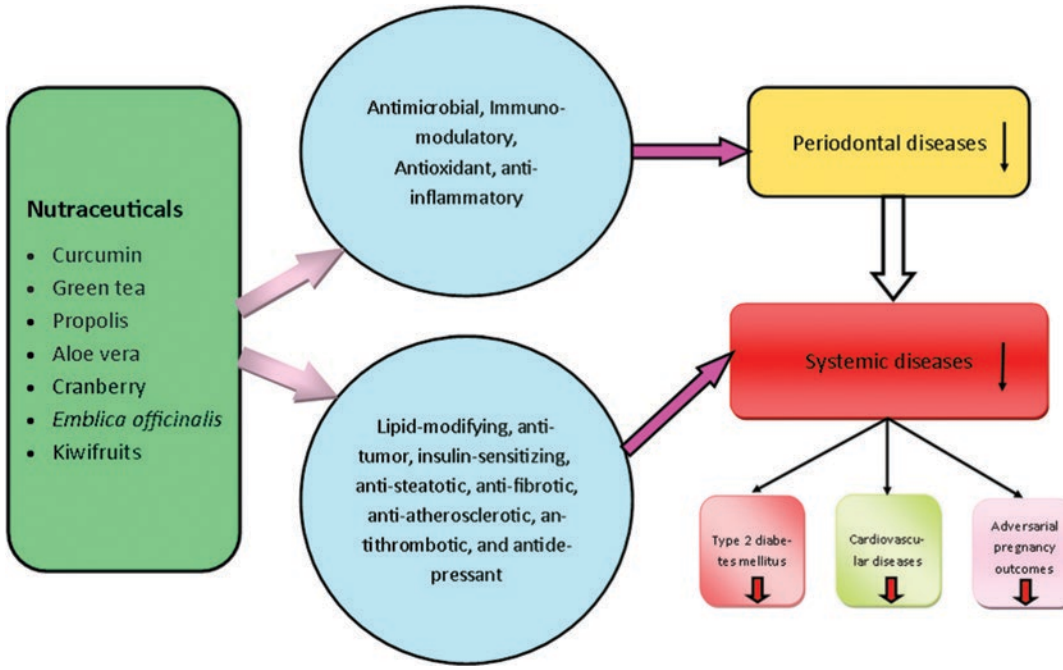

Fig. 1 Schematic summary of pathways of the effect of nutraceuticals and herbal bioactive compounds on periodontal diseases and systemic diseases such as cardiovascular diseases (CVDs), diabetes mellitus, and adversarial pregnancy outcomes and their potential related mechanisms

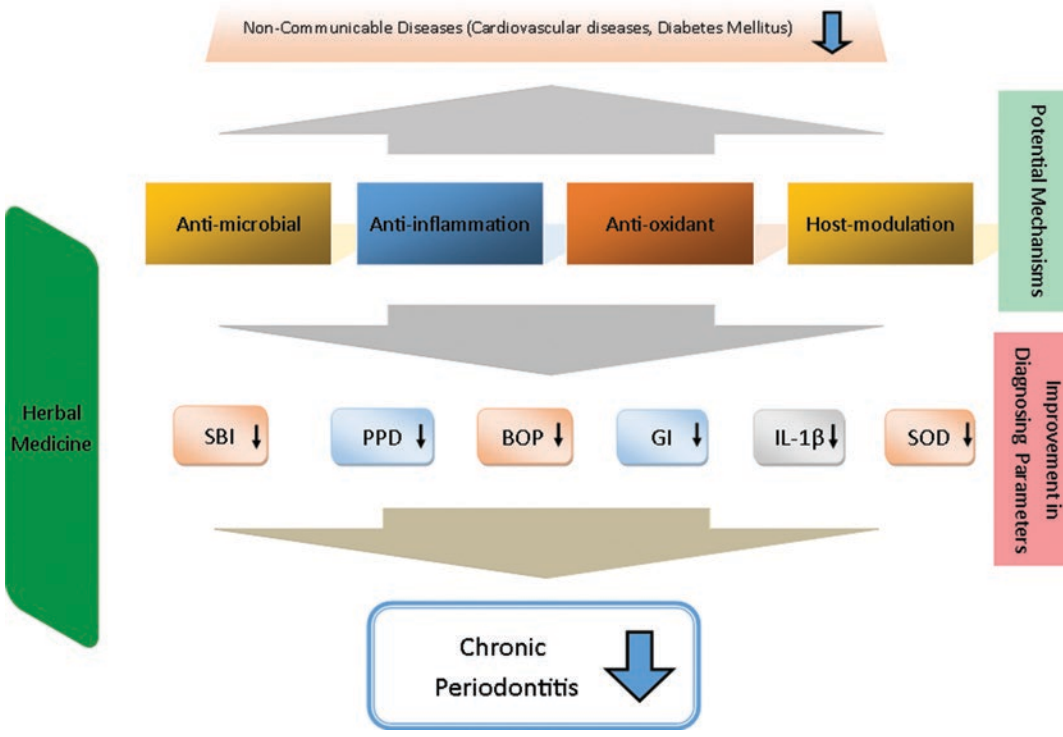

Fig. 2 Schematic summary of pathways of the effect of nutraceuticals and herbal bioactive compounds on clinical parameters of periodontal diseases and its potential related mechanisms. *SBI* Sulcus bleeding index, *PPD* Probing pocket depth, *BOP* Bleeding on probing, *GI* gingival index, *IL-1β* Interleukin 1β, *SOD* superoxide dismutase

2 Curcumin

Turmeric (*Curcuma longa*) is extensively used as an Indian spice and is derived from the rhizomes, a perennial member of the Zingiberaceae family. Lampe and Milobedzka identified and introduced curcumin (diferuloylmethane) as the main bioactive component of turmeric in 1910.

Curcumin has a wide spectrum of biological activities such as anti-inflammatory, antioxidant, anticarcinogenic, antiviral, and antimicrobial properties. Curcumin modulates the inflammatory response by down-regulating the activity of cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase enzymes and inhibits the production of the inflammatory cytokines. Moreover, there are some evidence regarding the effectiveness of curcumin in increasing collagen deposition and improving wound healing.

Based on the abovementioned features, many studies have been done to investigate the efficacy of curcumin in the treatment of periodontal disease. In a clinical trial in 2015, the efficacy of curcumin gel (10 mg/g) with and without photoactivation as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis was assessed. The results of this split-mouth clinical trial showed that the application of curcumin gel is an effective treatment modality as an adjunctive to conventional scaling and root planing. Moreover, the investigators showed that the effects were further enhanced by multiple applications of photodynamic therapy in addition to curcumin gel application [48]. The efficacy of treatment in this trial was evaluated based on clinical and microbiologic parameters. There was a significant reduction in clinical parameters such as the sulcus bleeding index (SBI), probing pocket depth (PPD), and clinical attachment level (CAL) in groups treated with curcumin gel. When compared for microbial parameters, there was a statistically significant reduction with respect to *Aggregatibacter actinomycetemcomitans* (Aa) and black pigment producing microorganisms (BPB) after 2 months and 3 months in quadrants in which curcumin gel was applied.

In another study with a larger sample size (30 cases), the efficacy of curcumin gel was compared with the efficacy of chlorhexidine (CHX) gel for the treatment of chronic periodontitis [49]. In this clinical trial, the patients were divided into two groups as control and experimental groups using a split-mouth design. At first, the standard SRP treatment was done for two groups. Following SRP, curcumin gel (2%) was applied in the experimental group and CHX gel (0.2%) in the control group. The main clinical criteria in this study were PPD, sulcus bleeding index (SBI), gingival index (GI), and plaque index (PI). These criteria were recorded at the day of treatment and subsequently after 1 month and 45 days.

Based on the statistical analysis in this clinical trial, all mentioned indices showed a significant reduction in both treatment groups. In comparison between two treatment modalities, the efficacy of curcumin gel was significantly better than CHX in reducing the pathologic parameters of periodontitis. Finally, the authors concluded that the curcumin gel has been shown to be more effective than the CHX gel in the treatment of mild to moderate periodontal pockets.

In another study conducted in 2016, the authors assessed the effect of 0.2% curcumin strip as a local drug delivery in conjunction with SRP for the treatment of chronic periodontitis [50]. In this study, the investigators not only registered the clinical parameters (PI, GI, SBI), but also they assessed the level of superoxide dismutase (SOD) enzyme, in gingival crevicular fluid (GCF). The results showed that the clinical parameters in both groups were improved and there was no statistically significant difference between groups. However, the level of enzyme in the group treated with the curcumin strip was significantly higher than in the control group. The SOD levels seem to be nearing to the healthy group when the curcumin strip was used as an adjunct to SRP.

In another study, the authors evaluate the level of IL-1β in saliva following treatment [51]. In this clinical trial, periodontal pockets of patients were randomly allocated to two treatment groups.

Control group was treated with SRP alone while the experimental group was treated with SRP followed by subgingival application of curcumin gel. The results of this study showed a single application of curcumin gel had limited added benefit over scaling and root planing in the treatment of chronic periodontitis. In this study, there was no significant difference between control and experimental groups regarding clinical and biochemical indices. None of the subjects who received curcumin gel in this study experienced any adverse effects.

In 2015 a clinical trial was performed to evaluate the effect of local application of curcumin on the "red complex" periodontal pathogens using polymerase chain reaction (PCR). In this split-mouth study 30 patients with chronic periodontitis were treated. The control side received the routine SRP treatment and the test side of the mouth was treated with SRP and application of 10 g curcumin gel subgingivally in the base of the pocket [52].

Based on the clinical results of this study, the mean PPD in the test site was significantly reduced when compared to the control site. However, there was no statistically significant difference between the two groups regarding the CAL parameter. The microbiologic assay (PCR) also shown a significant reduction in *P. gingivalis* (Pg), *Tanerella forsythia* (Tf), and *Treponema denticola* (Td) in the test group compared to the control group. The authors suggested that this significant reduction could be related to the antibacterial, anti-inflammatory, and antiplaque activity of curcumin.

In another innovative study, the curcumin extract was incorporated into Type I collagen chips and used for the treatment of periodontal pockets [53]. This clinical trial compared the efficiency of CHX chips and indigenous curcumin-based collagen as a local drug delivery system in the treatment of chronic periodontitis. The results showed improvement in all clinical and microbiological indices in both groups. However, at the end of the follow-up period (6 months), CHX group showed greater improvement in all of the clinical and microbiological parameters compared to the curcumin-collagen group.

3 Green Tea

Green tea is a natural product of tea (*Camellina sinensis*) leaves that is consumed as a beverage worldwide. The active ingredients of green tea are polyphenols. Most of them are catechins (flavan-3-ols), which can be classified into four main groups. The most common type (59%) is epigallocatechin-3-gallate (EGCG), followed by epigallocatechin (EGC, 19%), epicatechin-3-gallate (ECG, 13.6%), and epicatechin (EC, 6.4%) [54]. In addition, this compound has antibacterial, antioxidant, anti-inflammatory, and anticarcinogenic properties [55–58].

Green tea was found to be useful in oral health. In an epidemiological study conducted in 2009, it has been shown that there was a modest inverse association between the regular intake of green tea and periodontal disease [59].

Green tea catechins have an anti-oxidant and anti-bacterial effect on pathogens such as *Porphyromonas gingivalis* and *Prevotella intermedia*. The mechanism of action is through the inhibiting effect of EGCG and EGC on cysteine proteases of *P. gingivalis* [60, 61].

In a recent clinical trial, the effect of drinking green tea adjunct to SRP treatment in periodontitis patients has been investigated [62]. In this trial, 30 patients with chronic periodontitis were randomly divided into two groups. All the patients in the two groups received the first phase of periodontal treatment (SRP). The participants of group A were asked to drink commercial green tea 2 times a day (morning and night) for 6 weeks. The average reduction of PPD and bleeding on probing (BOP) were significantly greater in the intervention group than in the control group. However, there was no significant reduction in plaque index in interventional groups compared to the control group.

In another clinical trial, the adjunctive use of green tea dentifrice in periodontitis patients was assessed [63]. In this clinical trial, thirty patients with mild to moderate chronic periodontitis were randomly allocated into two treatment groups, "test" and "control" after initial SRP. The control group was given a commercially available fluoride and triclosan containing dentifrice, while the test group received green tea dentifrice with instructions on the method of brushing. All parameters were recorded at baseline and 4 weeks post-SRP.

In this study, not only the clinical indices were recorded, but also some biochemical parameters such as total antioxidant capacity (TAOC) and glutathione-S-transferase (GST) activity in the gingival crevicular fluid were assessed. At the end of the study period, the test group showed statistically significant improvements in GI, BOP, CAL, TAOC, and GST levels compared to the control group. It should be mentioned that GST activity was increased only in the test group. These results demonstrate the anti-inflammatory effect of green tea when used as an adjunct treatment of periodontitis.

Thermo-reversible sustained-release gel containing green tea was another form of this herbal agent which has been assessed for treatment of chronic periodontitis [64]. Thirty patients with two sites in the contralateral quadrants having a PPD ≥4 were included in this study. Total of 60 periodontal pockets from 30 patients was allocated in two groups. Following the completion of SRP treatment, green tea and placebo gels were applied to the periodontal pockets with a blunted cannula. In this study, the clinical parameters were recorded at the baseline and after 4 weeks of introducing the test or control gel into the pockets. The results showed a significant improvement regarding the clinical parameters (PPD, CAL, GI) in both groups. However, these improvements in all criteria were significantly greater in green tea groups compared to the placebo group.

The local effect of green tea for the treatment of periodontal pockets has been evaluated in another clinical trial. In this investigation, the green tea and placebo strips were randomly placed in the periodontal pockets of patients with diabetes and systemically healthy individuals [65]. The follow-up period in this study was 4 weeks. At the end of the study, the clinical indices (GI, PPD, and CAL) in the test sites of both groups were significantly improved compared to the placebo sites. Moreover, the prevalence of *P. gingivalis* in periodontal pockets of systemically healthy patients was significantly reduced from baseline (75%) to the fourth week (25%). However, the results showed no significant difference regarding microbiologic parameters in patients with diabetes before and after treatment.

Recently a systematic review has been conducted in this regard. In this review, four papers were included in the meta-analysis. All included studies performed SRP and an adjunct application of either a green tea catechin strip or gel on the test sites. Based on the conclusion of this review, the local application of green tea catechin may result in a beneficial reduction in PPD as compared to scaling and root planing with or without placebo [66]. However, there were high heterogeneity in the studies and some risk of bias related to the included studies. Hence, these data still need to be interpreted with caution.

4 Resveratrol

Resveratrol (3,5,4-trihydroxystilbene) is a polyphenol compound found in red wine, peanuts, apples, and several vegetables [67]**.** A well-known source of this component is *Polygonum cuspidatum*. From many years ago, the roots of *Polygonum cuspidatum* have been used in China and Japan as medicine [68]. Many preclinical and in vitro studies investigated the biological effects of resveratrol. These investigations showed anti-inflammatory, anti-carcinogenic, and antimicrobial properties for this agent [67]. Resveratrol may reduce the pro-inflammatory cytokines such as IL6, IL-1B, IL8, IL12, and TNF [68]. These anti-inflammatory properties

of resveratrol may influence the pathogenesis of periodontitis. Many animal studies assessed the influence of resveratrol administration on experimentally induced periodontitis showing promising results [69, 70]. However, this effect is not yet fully studied, making it difficult to incorporate resveratrol as a therapeutic/preventive agent clinically.

In a human clinical trial, the investigators evaluated the effects of resveratrol supplementation in adjunct with non-surgical periodontal therapy on inflammatory, antioxidant, and periodontal markers in patients with diabetes and chronic periodontitis. In this randomized, double-blind, and placebo-controlled clinical trial, 43 patients with diabetes suffering from chronic periodontitis were recruited. The subjects were randomly divided into control and intervention groups. In the first step, the phase one periodontal therapy was performed for all of the patients. Then the patients in the intervention and control groups received either 480 mg/d resveratrol or placebo capsules (2 pills) for four weeks. The results of this study showed that in the intervention group, the mean serum level of IL6 was significantly reduced post-intervention. No significant differences were seen in the mean levels of IL6, TNFa, TAC, and CAL between two groups post-intervention.

5 Propolis

Propolis is produced by honey bees from substances extracted from parts of certain plants, buds, and sap [71]. Propolis is a very complex mixture consisting of more than 230 constituents, including flavonoids, cinnamic acids and their esters, caffeic acid and caffeic acid phenethyl esters [72]. With regard to a wide range of biological constituents, propolis as a natural resin has several biological activities, including anti-inflammatory, antioxidant, antibacterial, antiviral, fungicidal, hepatoprotective, free radical scavenging, immunomodulatory, and anti-glycemic activities [73, 74]. For hundreds of years, propolis was used to improve the health status of numerous diseases, such as mucocutaneous infections of fungal, bacterial and viral etiology, and gastrointestinal disorders [75–77]. Caffeic acid phenethyl ester (CAPE) is recently introduced as an important active molecule of propolis; most of its therapeutic properties such as anti-inflammatory and antimicrobial properties are related to this component [78–81]. Several studies have investigated the effects of this natural compound on periodontal diseases and we have summarized their main outcomes in this review. In a recent randomized controlled clinical trial, a total of 50 patients with type 2 diabetes and moderate-to-severe chronic periodontitis were divided into two groups to receive one propolis capsule (400 mg/day) or a placebo capsule for 6 months. After the intervention, in the propolis group, hemoglobin A1C (HbA1C) was significantly reduced both 3 and 6 months after the SRP while there were no changes in the control group. In addition, in the propolis group, periodontal health improved as results showed that mean levels of CML significantly reduced in the intervention group though it did not notably change in the control group [82]. In another study, 20 patients with chronic periodontitis with at least 20 natural teeth were randomly either to the control (20 sites) or intervention (20 sites) groups. Control group treated by SRP alone and in the test group, subgingival placement of propolis was used after treatment with SRP. Local drug delivery was evaluated over SRP alone for a period of one month. Results showed that GI, bleeding index (BI), PPD, and CAL scores were significantly improved in the propolis plus SRP group; these changes were greater compared with the control group. Similar findings were obtained regarding microbiological parameters including *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Fusobacterium nucleatum* (Fn). These findings showed that subgingival delivery of propolis as an adjunct to SRP had beneficial effects on clinical and microbiological parameters in patients with chronic periodontitis [83]. In one study, 34 patients with moderate or severe periodontitis were randomly assigned into two groups to receive a polyherbal

mouthwash contained propolis resin extract (1:3), *Plantago lanceolata* leaves extract (1:10), *Salvia officinalis* leaves extract (1:1), and 1.75% of essential oils (intervention group) or a placebo mouthwash contained 2 ml of glycerin (sweetening agent), cinnamon and vanilla flavoring agents (control group) for 3 months. At the end of the study, in comparison to the control group, a significant reduction was observed in full-mouth bleeding score (FMBS) and full-mouth plaque score (FMPS). Compared with baseline, at post-intervention, PD and CAL significantly decreased in both groups, while differences between groups were not significant [84]. In another clinical trial, 30 patients with chronic periodontitis were assigned into two groups to receive 20% propolis hydroalcoholic solution 24 after SRP which was followed by one-stage full mouth disinfection (OSFMD) or control group in which patients received only SRP. After 12 weeks, probing depth reduction, reduction of microbiological counts of the periodontopathogens, and attachment gain were significantly higher in the treatment group compared with the control. In addition, PI, GI, BOP, PPD, and CAL significantly decreased in the propolis group compared with the control group [85]. In another study, 20 patients with chronic periodontitis, at first were subjected to scaling and root planing and after two weeks were treated with a hydroalcoholic solution of propolis extract twice a week for 2 weeks, or with a placebo twice a week for 2 weeks, or no additional treatment. At the end of the study, in comparison to the placebo and no-treatment groups, in response to propolis, the total viable counts of anaerobic bacteria were significantly reduced, and the proportion of sites with low levels ($\leq 10^5$ cfu/mL) of *Porphyromonas gingivalis* and the number of sites negative for bleeding on probing were significantly increased [86]. In their study, Tanasiewicz et al. evaluated the effects of propolis on the state of the oral cavity in 80 patients with periodontitis. Patients were assigned into 4 groups as follows: (i) Dental Polis DX toothpaste with propolis content (T), (ii) Dental Polis DX toothpaste without propolis content (G), (iii) Carepolis gel with propolis content (CT), (iv) Carepolis gel without propolis content (CG). After 8 weeks, results indicated that hygienic preparations with a 3% content of ethanol propolis extract efficiently support the removal of dental plaque and improve the state of the marginal periodontium [87]. In another clinical trial, 40 patients with chronic periodontitis were randomized into two groups to receive aloe vera tooth gel or propolis tooth gel. After 3 months of intervention, in aloe vera group, only *P. gingivalis* significantly reduced though in propolis group all the three red complex microorganisms significantly decreased. In addition, all the clinical parameters (PI, GI, Bleeding on Probing, PPD, and CAL) in both groups significantly reduced [88].

6 Aloe Vera

Aloe vera (*Aloe barbadensis*) belongs to the Liliaceae family, is widely used as a medicinal plant for medicinal and skin oral care properties for several years [89, 90]. It has a variety of minerals and vitamins and has several beneficial health effects such as immunomodulatory, antiviral, antitumor, and anti-inflammatory, anti-aging, and antioxidant properties [89–91]. In addition, aloe vera has a beneficial effect on wound healing and helps in treating various lesions in the oral cavity [91]. Totally, aloe vera has attracted significant attention in the field of dentistry as a natural and safe product in the treatment of a various oral and dental diseases including lichen planus, oral submucous fibrosis, recurrent aphthous stomatitis, alveolar osteitis, and periodontitis [89–94]. Due to the several unique properties of aloe vera, particularly anti-septic and anti-inflammation, anti-viral, and anti-fungal properties [90, 91], several studies assessed its effectiveness on patients with periodontitis, which we have summarized here. In a randomized, controlled clinical trial a total of 90 volunteers with moderate-to-severe chronic periodontitis were randomized to three groups to treat with (i) SRP+ placebo gel; (ii) SRP + 1% metformin gel; and (iii) SRP + aloe vera gel. After 12 months, a significant improvement was observed in GI, BOP, PPD, and CAL in all the

groups. However, compared to the placebo group, in the metformin and aloe vera groups, PPD reduction, CAL gain, and percentage of bone fill were greater [95]. In another study, 90 chronic periodontitis patients with class II furcation defects to three groups to treat with (i) SRP plus placebo gel; (ii) SRP plus 1% alendronate gel; and (iii) SRP plus aloe vera gel. After 12 months, a significant decrease in PD, relative vertical clinical attachment level (RVCAL), relative horizontal clinical attachment level (RHCAL), and gains were observed which were greater in the alendronate and aloe vera groups compared to the placebo group. Furthermore, a significantly greater change was also observed in Defect depth reduction (DDR) in the alendronate and aloe vera groups compared to the placebo group [96]. In their study, Moghaddam et al. assessed the effects of aloe vera gel as an adjunct to SRP for the treatment of chronic periodontitis. A total of 20 patients with moderate to severe chronic periodontitis were randomized to treatment with SRP (control group), or SRP combined with aloe vera gel (intervention group). After 60 days, the differences regarding PI were not significant between groups, GI and PD significantly reduced in both groups; however, the reduction was significantly greater in the intervention group than in the control group [97].

7 Other Nutraceuticals

Scrophularia striata is a plant species that belongs to Srophulariaceae family and has been used in traditional medicine from several years ago. Antimicrobial and anti-inflammatory effect of *S. striata* has been shown in previous studies [98, 99]. In a recent randomized clinical trial, the effect of *S. striata* mouthwash in the treatment of chronic periodontitis has been tested [100]. In this study, 50 patients with chronic periodontitis were randomly assigned in two groups. A group of patients used Irsha mouthwash (Iranian form of Listerine) and another group were given *S. striata* mouthwash and asked them to wash their mouth with 15 ml mouthwash for 30 s each night. The results showed that all the clinical parameters (plaque index, gingival bleeding, and probing depth) and also microbiological index (number of *Streptococcus mutans*) were improved in the test group compared with the control group.

The cranberry (*Vaccinium macrocarpon* Ait) is a native North American fruit that has recently received considerable attention in the treatment of infectious diseases [101–103]. The red cranberry extract is a rich source of various classes of potentially bioactive phenolic compounds which have biological properties and may be beneficial for the treatment of periodontal diseases [104]. There are several in vitro studies in the literature showing the antimicrobial effect of cranberry against periopathogens [105–108]. On the other hand, some in vitro studies showed anti-inflammatory effects of this pulpy and sour fruit which may be beneficial in controlling periodontitis [109–112]. In a randomized clinical trial, 41 patients who have both diabetes and chronic periodontitis were recruited. Results of this study showed that the consumption of cranberry juice adjunct with nonsurgical periodontal treatment could significantly improve periodontal status in patients with diabetes and periodontitis [113].

Āmla (*Emblica officinalis*) is another medical plant indigenous to tropical and subtropical regions of South-east Asia. This plant has various therapeutic effects. Previous studies, about *Emblica officinalis*, showed a wide array of biologic effects such as antibacterial, anti-inflammatory, analgesic, antioxidant, and immune-modulatory properties [114–117]. The antimicrobial property of *E. officinalis* fruit is attributed mainly to tannins, phenols, saponins, and flavonoids [118]. The effect of subgingivally delivered 10% *Emblica officinalis* gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis has been investigated in a randomized placebo-controlled clinical trial [119]. In this study, 46 patients suffering from chronic periodontitis (528 sites) were randomly assigned to control and test groups. Patients in the control group only received standard SRP treatment but the patients in the test group received both SRP and 10% *E. officinalis* gel applied in their periodontal pockets. The

results showed that locally delivered 10% *E. officinalis* sustained release gel used as an adjunct to SRP may be more effective in reducing inflammation and periodontal destruction in patients with chronic periodontitis when compared with SRP alone [119]. In another clinical study, the application of *E. officinalis* irrigation adjunct to SRP was tested. The result demonstrated significantly greater reductions in the mean PI, PPD, and BOP but a greater mean CAL at 3 months post-therapy in the test group than in the negative control group ($p < 0.05$) [120].

It has been shown that periodontitis is inversely related to plasma vitamin C levels [121–124]. Rich sources of vitamin C such as green fruits and vegetables may play an important role to treat periodontitis [122, 125, 126]. Kiwifruits are one of the richest dietary sources of vitamin C as green kiwifruit contains 93 mg of vitamin C per 100 g fruit. In a clinical trial study, this hypothesis has been tested [127]. In this single-centered randomized, parallel design, clinical trial with a 5-month follow-up, 48 patients with chronic periodontitis were assigned to two groups. The patients in the test group consumed two kiwifruits/day for 5 months and the control patients did not consume kiwifruits. After two months, all the patients received initial periodontal treatments. The results showed that the test group had significantly greater reductions of bleeding, plaque, and attachment loss than the control group. Systemic biomarkers and vital signs did not show clinically relevant differences between the test and control groups [127].

8 Conclusion

The results from animal and subclinical studies previously have shown a wide array of biological properties for herbal agents. The main biological properties of these agents are antimicrobial, antioxidant, and anti-inflammatory effects. Also, there are a large number of original investigations regarding the subclinical effects of natural phytochemicals on periodontal tissues, though the clinical studies in this regard are rare. The purpose of this paper was to review the clinical trials of herbal anti-inflammation and antibacterial agents used as an adjunct therapy for the treatment of chronic periodontitis (Table 1). Based on the results, some of the agents such as curcumin, green tea, propolis, and aloe vera have been shown significant clinical effects in good numbers of clinical trials. However, some other herbal agents such as resveratrol, cranberry, and *Emblica officinalis* have been tested in very few clinical studies. The main clinical parameters which have been measured in the studies were sulcus bleeding index (SBI), probing pocket depth (PPD), and clinical attachment level (CAL). All of these parameters have been changed positively in response to using these herbal remedies. For future studies, it would be better to investigate not only the clinical indices but also the immunological parameters of chronic periodontitis. Based on the proven biological effects of herbal agents, the hypothesis of applying them as host modulation therapy can be considered for the future. In this regard, larger randomized clinical trials are necessary for developing these concepts in the future. Altogether, the results of clinical trials have considered positive effects for using these natural agents as adjunctive therapy for the treatment of chronic periodontitis. However, more clinical trials are required for the investigation of the appropriate route of administration and optimal doses of the products for the treatment of various stages of chronic periodontitis.

Conflict of interests NoneFundingNone

Table 1 The effect of nutraceuticals on periodontal disease and its related factors

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Sreedhar et al. 2015 [48]	Curcumin	gel 10 mg/g	3 months	Sixty sites in fifteen chronic periodontitis patients	Significant reduction in clinical parameters [(SBI), (PPD), (CAL)] was observed in groups treated with curcumin gel. Statistically significant reduction with respect to (Aa) and (BPB) after 2 months and 3 months in quadrants which curcumin gel was applied.
Hugar et al. 2016 [49]	Curcumin	Gel 2%	45 days	30 patients with mild to moderate periodontitis	The efficacy of curcumin gel was significantly better than chlorhexidine in reducing PPD, SBI, gingival index (GI), and plaque index (PI)
Elavarasu et al. 2016 [50]	Curcumin	Strip 0.2%	21 days	Twenty subjects of age 35–55 years and 15 subjects with chronic periodontitis	SOD levels were significantly improved in test sites (using curcumin strip as adjunct to SRP) when compared with control sites (Just treated with SRP).
Kaur et al. 2019 (No full text) [51]	Curcumin	Gel	–	30 patients suffering from chronic generalized periodontitis with probing pocket depth ≥ 5 mm on at least 4 sites	Single application of curcumin gel has limited added benefit over scaling and root planing in the treatment of chronic periodontitis based on clinical and biochemical (IL-1β) parameters
Nagasri et al. 2015 [52]	Curcumin	Gel 10 g	4 weeks	30 patients aged 35–60 with chronic periodontitis	The local application of curcumin in conjunction with SRP has showed improvement in clinical (PPD, CAL) and microbiological (Pg, Tf, Td) parameters
Gottumukkala et al. 2014 [53]	Curcumin	Chips 50 mg/cm2	6 month	120 sites from 60 patients presenting with chronic periodontitis	Improvement in all clinical (PI, GI, PPD, CAL) and microbiological (BANA test, microbial colony count) indices in both groups. However, after 6 months follow-up CHX group showed greater enhancement in all parameters compared to curcumin group

(continued)

Table 1 (continued)

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Taleghani et al. 2018 [62]	Green Tea	Drink 2 times per day	6 weeks	30 patients with chronic periodontitis	Improvement in PPD and BOP average in intervention group compared to control group. No significant difference was observed regarding the plaque index between two groups.
Hrishi et al. 2014 [63]	Green Tea	Dentifrice Twice daily for a minimum period of 2 min	4 weeks	Thirty patients with mild to moderate chronic periodontitis	Statistically significant improvements in GI, BOP, CAL, TAOC, and GST levels compared to the control group
Chava et al. 2013 [64]	Green Tea	Thermo-reversible sustained-release Gel	4 weeks	Total of 60 sites (PPD $\geq$ 4) from 30 patients with chronic periodontitis.	Significant improvement regarding the clinical parameters (PPD, CAL, GI) in test group compared to control group.
Gadagi et al. 2013 [65]	Green Tea	Periodontal strips	4 weeks	50 patients with chronic periodontitis. Consisted of 25 systemically healthy patients and 25 diabetic patients.	Significant improvement in clinical parameters (GI, PPD, and CAL) in test group compared to control group. Significant reduction in the prevalence of *P. gingivalis* in sites treated with green tea strips in systemically healthy patients. No significant effect for periodontal treatment of diabetic patients regarding the microbiologic parameters.
Gartenmann et al. 2019 [66]	Green Tea	Strip or gel		Systematic Review	The local application of green tea catechin as an adjunct to SRP may result in a beneficial reduction in PPD.
Zare Javid et al. 2019 [128]	Resveratrol	Capsule 480 mg/d	4 weeks	43 patients diabetic patients suffering from chronic periodontitis	In the intervention group, the mean serum level of IL6 was reduced significantly post-intervention. No significant differences were seen in the mean levels of IL6, TNFa, TAC, and CAL between two groups post-intervention.

(continued)

Table 1 (continued)

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Borgnakke et al. 2017 [82]	Propolis	Capsule 400 mg/d	6 month	50 adult diabetic patients with moderate-to-severe chronic periodontitis	Propolis significantly reduced hemoglobin A1C (HbA1C) both 3 and 6 months after the SRP. The mean levels of CML significantly reduced in the intervention group
Sanghani et al. 2014 [83]	Propolis	~5 mg propolis adjunct to SRP	1 month	20 adult patients with chronic periodontitis	GI, bleeding Index (BI), PPD, and CAL scores were significantly improved in the propolis plus SRP group, these changes were greater compared with control group. Similar findings were obtained regarding microbiological parameters including *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Fusobacterium nucleatum* (Fn).
Pundir et al. 2017 [85]	Propolis	1 g of propolis powder to 3 ml of ethanol	12 weeks	30 patients with chronic periodontitis	Microbiological counts of the periodontopathogens, PI, GI, BOP, PPD, and CAL significantly decreased in propolis group compared with control group
Sparabombe et al. 2019 [84]	Propolis resin extract (1:3), *Plantago lanceolata* leaves extract (1:10), *Salvia officinalis* leaves extract (1:1) and 1.75% of essential oils	Mouthwash, Propolis 1:3	3 month	34 patients with moderate or severe periodontitis	Compared with control group, a significant reduction was observed in full mouth bleeding score (FMBS) and full mouth plaque score (FMPS). Compared with baseline, at post intervention, PD and CAL significantly decreased in both group, while differences between groups were not significant
Coutinho 2012 [86]	Propolis	3 mL 20% propolis hydroalcoholic solution twice a week	2 weeks	20 patients diagnosed with chronic periodontitis	Compared with the control group, the total viable counts of anaerobic bacteria significantly reduced, and the proportion of sites with low levels (≤10 5 cfu/mL) of *Porphyromonas gingivalis* and the number of sites negative for bleeding on probing were significantly increased

(continued)

Table 1 (continued)

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Tanasiewicz et al. 2012 [87]	Propolis	3% content of ethanol propolis	8 weeks	80 patients without the pathological changes of the periodontium and in the case of patients endangered with the occurrence of gingivitis caused by dental plaque	Hygienic preparations with a 3% content of ethanol propolis extract efficiently support removal of dental plaque and improve the state of marginal periodontium
Kumar et al. 2015 [88]	Aloe vera or propolis	tooth gel	3 months	40 patients with chronic periodontitis	All the clinical parameters (PI, GI, bleeding on Probing, PPD, and CAL) in both groups significantly reduced
Kurian et al. 2018 [95]	Aloe vera	1% Aloe vera gel	12 months	90 patients with moderate-to-severe chronic periodontitis	A significant improvement was observed in GI, BOP, PPD, and CAL in all the groups, however, compared to placebo group, in the metformin and aloe vera groups PPD reduction, CAL gain, and percentage of bone fill were greater
Ipshita et al. 2018 [96]	1) alendronate 2) aloe vera 3) placebo	1% alendronate and aloe vera gel	12 months	90 patients with moderate-to-severe chronic periodontitis	A significant decrease in PD, relative vertical clinical attachment level (RVCAL), relative horizontal clinical attachment level (RHCAL) and gains was observed which were greater in the alendronate and aloe vera groups compared to the placebo group
Ashouri Moghaddam et al. 2017 [97]	Aloe vera	%98 aloe vera gel concentration	60 days	20 patients with moderate-to-severe chronic periodontitis	The differences regarding PI did not significant between groups, GI and PD significantly reduced in both groups, however, the reduction was significantly greater in the intervention group than control

(continued)

Table 1 (continued)

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Abdelmonem et al. 2014 [129]	Aloe vera	Aloe vera (1 cc) 100% gel	Twice weekly for 3 weeks	40 patients with moderate-to-severe chronic periodontitis	A significant greater reduction in *P. gingivalis* and *P. intermedia* count was found in aloe vera gel + SRP in comparison to the sites treated with SRP alone. In addition, PI, GI, and papillary bleeding index (PBI) significantly reduced in both groups
Deepu et al. 2018 [130]	Aloe vera	2.5% Aloe vera gel	4 months	71 patients with chronic localized moderate periodontitis	After four months, no statistically significant difference was observed regarding PI, PPD, CAL, and GI between groups
Pradeep et al. 2015 [131]	Aloe vera	Aloe vera gel	3–6 months	60 type 2 diabetes mellitus patients with chronic periodontitis	A significant greater mean reduction in PI, modified sulcus bleeding index (mSBI) and PD and mean gain in clinical attachment level was observed in aloe vera group compared to those in placebo group from baseline to 3 months
Agrawal et al. 2019 [132]	Aloe vera	Aloe vera gel	1 month	20 patients with chronic periodontitis	PPD, GI, and PI significantly decreased in both groups
Sahgal et al. [133]	Placebo, chlorhexidine, aloe vera gel, pomegranate gel	98% Aloe vera gel	7 days	40 patients with chronic periodontitis	Bacteria count significantly reduced in chlorhexidine, or pomegranate gel compared with aloe vera gel or placebo groups
Virdi et al. 2012 [134]	Aloe vera	Pure aloe vera gel	6 weeks	20 patients with chronic periodontitis	GI and pocket depth significantly decreased in the aloe vera plus SRP compared to SRP alone
Ahmed et al. 2016 [135]	Aloe vera Metronidazole	Aloe vera gel Metronidazole gel 25%	90 days	20 patients with chronic periodontitis	GI and PI significantly decreased in both aloe vera and metronidazole plus SRP compared with SRP alone. PPD significantly reduced in Metronidazole plus SRP compared with SRP alone, but there was no difference between aloe vera plus SRP and SRP alone.

(continued)

Table 1 (continued)

Author, Year	Agent	Dose per day	Treatment duration	Subjects	Main outcomes
Kerdar et al. 2019 [100]	*Scrophularia striata*	Mouthwash 10 mg/100 ml	Four weeks	Fifty patients with chronic periodontitis (20–50 years old)	Clinical parameters (plaque index, gingival bleeding, and probing depth) and also microbiological index (number of *Streptococcus mutans*) have been improved in test group compared with control group.
Zare Javid et al. 2017 [113]	Cranberry	Drinking Juice 200 ml, twice daily	Eight weeks	41 patients with diabetes and periodontitis	Consumption of cranberry juice adjunct with nonsurgical periodontal treatment can significantly improve periodontal status (PPD)
Grover et al. 2016 [119]	*Emblica officinalis*	Gel 10%	Three months	Forty-six patients (528 sites) with chronic periodontitis	When test and control sites were compared, significantly more reduction in mean PPD, SBI, number of sites with PPD = 5–6 mm, PPD ≥ 7 mm, CAL ≥ 6 mm and greater CAL gain were achieved in test sites at 2- and 3-month post-therapy ($p < 0.05$).
Tewari et al. 2018 [120]	*Emblica officinalis*	Irrigation 10%	Three months	Sixty-six patients with chronic periodontitis	There were significantly greater reductions in the mean PI, PPD, and SBI but a greater mean CAL at 3 months post-therapy in the test group than in the negative control ($p < 0.05$)
Graziani et al. 2018 [127]	Kiwifruits	Consumption of two kiwifruits/day	Five months	Forty-eight patients with chronic periodontitis	Control group had significant greater reductions of BOP, PI, and CAL than the test group. Systemic biomarkers and vital signs did not show clinically relevant differences between test and control group

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The Multifaceted Actions of Curcumin in Obesity

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Abstract

Obesity remains a pervasive health concern worldwide with concomitant comorbidities such as cardiovascular diseases, diabetes, inflammation, and other metabolic disorders. A wealth of data validates dietary and lifestyle modifications such as restricting caloric intake and increasing physical activity to slow the obesity development. Recently, the advent of phytochemicals such as curcumin, the active ingredient in turmeric, has attracted considerable research interest in tracking down their possible effects in protection against obesity and obesity-related comorbidities. According to the existing literature, curcumin may regulate lipid metabolism and suppress chronic inflammation interacting with white adipose tissue, which plays a central role in the complications associated with obesity. Curcumin also inhibits the differentiation of adipocyte and improves antioxidant properties. In the present review, we sought to deliberate the possible effects of curcumin in downregulating obesity and curtailing the adverse health effects of obesity.

Keywords

Curcumin · Adiposity · Inflammation · Metabolism

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1 Epidemiology of Obesity

As one of the current world health concerns, obesity is listed among major health problems such as infectious disease, malnutrition, and even being underweight. The morbidity and mortality of obesity are exciding those of being underweight [1]. Obesity is not only the main problem of developed countries but also a rising issue in developing countries. United States of America, Pacific Islands, Europe, North Africa, and Australia are the geographical areas at the greatest risk of obesity epidemic due to progressive increases in prevalence rates [2]. The epidemiologic growth in the incidence of obesity is related to several factors such as important easy ways of worldwide trading, fast urbanization, and developments in economies which in turn result in lifestyle changing. This lifestyle changing means that the lower food prices result in higher food consumption, especially animal products and processed grains with added sugar that along with the lower daily activity causes positive energy balance and thus obesity.

There has been a notable increase in obesity prevalence over the last ten years and it has nearly doubled worldwide over the last 3 and half decades. The World Health Organization (WHO) reported more than 1.1 billion overweight adults and 312 million of obese cases worldwide. Among adults aged 18 years or older, 10.8% of men and 14.9% of women were obese in 2014 [3]. In the United States, the prevalence of obese cases among adults over the age of 20 is approximately 34.2% (34.9% and 33.6% among women and men, respectively) [4]. Obesity or being overweight has been found in more than 155 million children that accounts for 42 million under five years-old cases. In the United States, the prevalence of obesity among children was 17% in 2014 [5].

Although adipose tissue as a whole increases the risk of obesity-related diseases, fat distribution has a dramatic differential impact on disease status. Thus, visceral fat can cause metabolic syndrome which is associated with a higher risk of cardiovascular disease (CVD), hypertension, and type 2 diabetes than peripheral adipose tissue [6]. The mortality of overweight and obese patients due to vascular disease and diabetes is increased by 41% and 210%, respectively. Furthermore, the overall mortality is increased by 29% [7]. Development of cardiovascular and metabolic diseases is associated with central fat mass which can be estimated by measuring waist circumference, but not only an elevated BMI.

2 Comorbidities Of Obesity

The risk of dyslipidemia-associated problems such as type 2 diabetes mellitus, cardiovascular, gallbladder, and respiratory disorders, obstructive sleep apnea (OSA), degenerative osteoarthropathy, and the number of malignancies is increased with the presence of obesity. Several lifelong problems have also been reported with increased risk in obesity such as rheumatoid arthritis. Furthermore, drug categories have also an impact on weight gain that includes antidepressants, atypical antipsychotics, antiepileptic drugs, sulfonylureas, thiazolidinediones, insulin, corticosteroids, and beta-blockers. In addition, obesity presents an economic burden to society as it can cause lower school performance and even attendance, and cause further economic burden on the health system.

2.1 Cardiovascular Disease

Many of the underlying cardiovascular risk factors that is associated with a higher rate of CVD can be found in obese patients including insulin secretion and response problems, overt diabetes, hypertension, dyslipidemia (high triglycerides, apolipoprotein B, small and dense LDL, low HDL), upregulated cytokines (TNF-alfa, IL-6, PAI-1, adipocyte fatty acid-binding protein, lipocalin-2, chemerin, leptin, visfatin, vaspin, resistin), and hyperthrombotic state. These can result in coronary heart disease (from angina to a myocardial infarction that can lead to even heart failure or sudden death), peripheral vascular disease, and ischemic stroke [8]. Even further obesity-associated conditions including OSA, hyperco-

agulability, changes in the cardiovascular, and also other unknown mechanisms can increase the risk of CVD as intermediate risk factors [9].

Although obese patients are at higher risk of CVD development, their overall survival is higher than the normal population which is illustrated in the term "obesity paradox" [10].

2.2 Metabolic Syndrome and Type 2 Diabetes Mellitus

The metabolic syndrome is defined as a group of disorders that accompanies an increased risk of cardiovascular disease and diabetes. The common feature in metabolic syndrome patients that is associated with waist circumference and increased fat mass is central adiposity. The main components of metabolic syndrome are raised triglycerides (≥150 mg/dl), reduced HDL-cholesterol (<40 mg/dl M, <50 mg/dl W), raised blood pressure (≥130/≥85 mmHg), and raised fasting plasma glucose (110 mg/dl), all of which are the result of weight gain, specifically intraabdominal fat accumulation and a widened waist circumference (≥102 cm M, ≥88 cm W) [11]. Therefore, the higher the prevalence of global obesity, the greater the prevalence of metabolic syndrome development as it has involved 10 to 30 percent of especially adult population in Europe [12]. National Health and Nutrition Examination Survey reported that metabolic syndrome prevalence from 28% in the 1988–1994 had risen to 34% in the 1999–2004 [13]. Metabolic syndrome is found in 42% of the adults in their seventies [14].

Moreover, the rise in the prevalence of type 2 diabetes can be nearly associated with the increased epidemicity of obesity and it is estimated that about 90% of this disease is attributable to excess weight [15]. The pathophysiology connecting obesity and diabetes is chiefly attributed to two factors: insulin resistance and insulin deficiency [16].

Thus, some kind of prevention from metabolic syndrome can be achieved by timely weight loss and lowering the waist size. Reducing weight by 5–10% by a healthy diet, lifestyle modifications such as controlled caloric intake, increased physical activity, with or without anti-obesity drugs, lowers all metabolic syndrome components and risk of type 2 diabetes and cardiovascular disease [17].

2.3 NAFLD

As addressed in other parts metabolic syndrome can affect different parts of body as it presents itself in the liver in the form of non-alcoholic fatty liver disease. The underlying pathology of this disease is steatosis with the presence or absence of steatohepatitis. Hypertension, type two diabetes, impaired lipid profile, episodes of recurrent weight gain, and weight loss are risk factors for "primary" NAFLD. Furthermore, it is found that higher BMI accompanies a higher prevalence of NAFLD. Assessments of liver autopsies and biopsies estimated prevalence rates of steatosis and steatohepatitis as 15% and 3%, respectively, in non-obese persons, 65% and 20%, respectively, in persons with class I and II obesity (BMI 30.0–39.9 kg/m^2), and 85% and 40%, respectively, in extremely obese patients (BMI ≥40 kg/m^2) [18]. Imaging studies also estimated the prevalence of NAFLD in adults as 14–31% [19]. The disease pathology severity ranges from a mild steatosis to intermediate liver lesion and even cirrhosis at end-stage disease. It is estimated that only 5% of the patients with NAFLD progress to the end-stage cirrhosis; however, the rising prevalence of the disease in the background of increased obese patients has turned it into one of the major causes of chronic liver disease [20]. It has been proposed that NAFLD can be addressed as an independent risk factor for CVD besides other risk factors. Obviously, NAFLD leads to causes morbidity and mortality; however, understanding the natural history of this disease can help for risk stratification of the patients [21].

2.4 Obstructive Sleep Apnea Syndrome

Intermittent closure of the upper airway during sleep due to the presence of collapsibility is termed as OSA. The higher fat mass particularly in central parts of the body causes mechanical and physiologic effects such as a rise in collapsibility of pharynx and neuromuscular changes due to the rise of adipokines [22]. This condition involves around 2% of females and 4% of the males and it is associated with epidemic obesity in Western society [23]. OSA, even in mild or moderate cases, can cause hypertension, type 2 diabetes, and CVD. As obesity is becoming epidemic, OSA develops much more cases of alveolar hypoventilation during the daytime, cor pulmonale, and frank respiratory failure. As a prevalent and ailing condition, OSA is associated with high socioeconomic burden for Western society [24].

2.5 Polycystic Ovary Syndrome

As it is obvious from the term, PCOS is characterized by ovarian cysts that causes hormone-related abnormalities such as disturbances in menstrual periods, infertility, acne, hirsutism, oily skin, melisma, and obesity. American College of Obstetrics and Gynecology proposes that around 80% of women with PCOS suffers from obesity and the condition causes 6- to seven-fold higher morbidity in obese females than in the normal population. The high fat mass in obese females is responsible for increased metabolic risk through producing higher levels of androgen and thus obesity screening is recommended for women with PCOS. Weight loss can further normalize the hormonal imbalances in these. The effects of weight loss on PCOS symptoms and hormone imbalances have been assessed in patients with morbid obesity. It has been reported that around 50% of premenopausal women who are candidates for bariatric surgery have comorbid PCOS. After a post-surgery period of one year, with a significant mean weight loss of 41 ± 9 kg (95% CI, 36–47 kg; $P < 0.001$), patients had a much more normalized hormonal state (free testosterone, androstenedione, and dehydroepiandrosterone) and reported less hirsutism. Patients were more sensitive to insulin and showed higher circulating sex hormone-binding globulin (SHBG) [25].

2.6 Cancer

Several malignancies such as colorectal, esophageal, endometrial, renal, hepatic, biliary, pancreatic, hematopoietic, prostatic, and breast cancers are related to overweight and obesity. There are many mechanisms that underlies carcinogenicity of obesity including using food with carcinogen additives along with more energy intake; decreased energy output due to reduced physical activity which implies the loss of cancer protective effects, such as antioxidant and protective cytokines; increased adiposity which results in growth hormone and factor release (chronic hyperinsulinemia, IL-1, TNF-alfa, PAI-1, high circulating levels of leptin) and this can complicate signaling pathways that underlies in inflammation, oxidative stress, cell proliferation which in turn results in carcinogenesis; secondary associations [26]. Colon cancer is connected with central obesity, processed meats with nitrites, and carbon derivates, and artificial sweeteners. Furthermore, downregulation of SHBG due to insulin and IGF-1 high levels that results in higher levels of active estradiol and testosterone, but also excessive adipose tissue aromatase, which in turn facilitate the conversion of estrogen to androgen, and is linked to increased endogenous estrogen production; gastroesophageal reflux and cholelithiasis are more common among overweight and obese people which is a result of high intra-abdominal pressure, stasis and imbalance of bile acids respectively, both pathologies result in chronic inflammation that can be a causative of esophageal adenocarcinoma and gallbladder carcinoma [27].

2.7 Hypothyroidism

Obese patients are reported to have changes in thyroid function tests like higher levels of thyroid-stimulating hormone (TSH) that is an evidence of obesity-related changes in the hypothalamic-pituitary-thyroid axis just like the condition seen in primary hypothyroidism [28].

2.8 Cushing's Syndrome

Patients with Cushing's syndrome usually presents several conditions besides obesity such as hypokalemia, hypertension, osteoporosis, and type 2 diabetes; however, study results propose that around one out of ten patients that refer with obesity suffers from Cushing's syndrome and thus it should be kept in mind to screen patients with obesity for this syndrome [29].

2.9 Major Depressive Disorder

Six-year follow up of otherwise healthy obese patients reported a higher risk of depression development and thus it can be addressed as a predisposing factor for the major depressive disorder [30]. However, obesity cannot predict the persistency of depression. Obesity somehow connects with anxiety.

2.10 Osteoarthritis

The most known obesity-related musculoskeletal disorder is osteoarthritis (OA) which is a chronic degenerative disorder with a disabling pain and reduced range of motion that affect the quality of life in the patients. BMIs over 30 kg/m^2 are related with around 7 times more risk of developing Knee OA compared to the normal population. Thus, it is advisable to alleviate this risk factor in OA patients [31, 32].

OA pathogenesis relates to both increased weight-bearing on joints and altered biomechanical patterns together with hormonal and cytokine dysregulation; pro-inflammatory factors are secreted by inflamed synovial membranes of the articular capsules under excess weight-bearing, resulting in cartilage damage and breakdown. Obesity is associated with the incidence and progression of OA of both weight-bearing and non-weight-bearing joints, to the rate of joint replacements as well as operative complications [33]. Weight loss in OA can impart clinically significant improvements in pain and delay the progression of joint structural damage.

3 Immunometabolic Changes Associated With Obesity

Immunometabolism is an emerging field of research that studies the relationship between immunology and metabolism.

Immune system plays an important role not only in infectious and neoplastic disease but also in supposedly non-immune pathologies, such as neurodegeneration, cardiovascular function, and metabolism. In these pathologies it has been found an increase in activation of the innate and adaptive immune systems and, particularly in obesity, this facilitates metabolic abnormalities, determining an increased susceptibility to type 2 diabetes, cardiovascular diseases, cancer, and neurodegeneration [34].

If several studies demonstrate a relationship between obesity and a pro-inflammatory state, the molecular mechanism that could explain this phenomenon is still unclear [35]. However, two molecular mechanisms have been speculated: first, the release of inflammatory cytokines by the immune cells infiltration (macrophages, monocytes, T cells, and b cells) of adipose tissue [36], second a production of adipocytes themselves of inflammatory mediators [37]. In this regard, adipocytes not only are energy storage but also they have endocrine properties with body weight regulating effects.

Therefore in obesity, we can observe on one side an overexpression of adipokines (such as leptin, TNF, and IL-6, resistin) that, promoting a pro-inflammatory state, increases the development of obesity-linked metabolic diseases and on the other side, there is a reduction in anti-

inflammatory factors production, like adiponectin [38].

In mammals within the immune system, we can distinguish two types of immune responses: innate and adaptive. The macrophages, part of the innate response, change from anti-inflammatory type (M2) to pro-inflammatory type (M1).

In obesity, we can observe an expansion adipose tissue (AT), principally determined by hypertrophy of adipocytes with a consequent increase in fatty acid flux through pro-inflammatory state increasing, rise in leptin secretion vascularization, hypoxia, and adipocyte cell death [39].

The uncontrolled fatty acid flux acts as pro-inflammatory factor and recruits macrophages. The rapid expansion of adipose tissue can cause his partial hypoperfusion determining an increase in adipocyte death rate and, in turn, this may be a sufficient trigger to stimulate macrophage infiltration and induce AT inflammation interacting with toll-like receptors (TLRs) in order to phagocytize cellular debris. Moreover, the increase in adipocytes is connected with increased leptin levels that promotes chemotaxis by overexpressing endothelial adhesion molecule and causing chemoattraction [39, 40].

In one study on human primary adipocyte, Meijer et al. (4) investigated the effects of lipopolysaccharide stimulation in order to probe their capacity of generating an inflammatory reply [37]. After this stimulation adipocytes demonstrate an immune-cell-like behavior, increasing the production of cytokines, chemokines and cell adhesion molecules, and facilitating T cell activation.

Like T cells, upon cytokine polarization stimulation, macrophages differentiate into classically activated macrophages (M1) and alternatively activated macrophages (M2) and different activators, markers, and function characterize them. M1 macrophages are attracted by inflammatory stimuli and secrets pro-inflammatory cytokines, while it is IL-4 and IL-13 cytokines that activate M2 macrophages and they secrete anti-inflammatory cytokines. When the person is thin, the body adipose tissue is full of natural killer (NK) cells and M2 type anti-inflammatory macrophages; thus M2 macrophages provide an insulin-sensitive state through IL10 secretion. Contrary, obese cases are provided with lipolysis and free fatty acid release that provide inflammatory medium and cytokines such as TNF-α and CCL2 that produce M1 macrophages through chemotaxis of blood monocytes. The recruited and stimulated M1 macrophages cause insulin resistance in adipose tissue through producing IL-1β, IL-B4, IL-6, TNF-α, nitric oxide (NO), and resistin [41].

In addition, as aforesaid, obesity can cause a chronic state of inflammation and this inflammatory state is shown to make the intestine more permeable in a high-fat diet or genetic models. This highly permeable intestine provides the body with an increased translocation of bacteria produced endotoxins such as LPS. It is found in the obesity models that Bacteroidetes and even microbial diversity are reduced and Firmicutes are increased in these models.

In general, intestinal microbiota and their changes through food and antimicrobial agents can change metabolism in obese patients [42].

4 Metabolic Effects of Obesity

Obesity is associated with CVD and metabolic diseases risk factors including hypertension, diabetic and pre-diabetic states, dyslipidemia, OSA, and non-alcoholic fatty liver disease (NAFLD) [43].

In obesity we can often observe alterations in lipid metabolism (dyslipidemia in around 2/3 of the cases) and, in particular, hypertriglyceridemia is a typical find in association with an increase in LDL, VLDL, apolipoprotein B, and a decrease in HDL.

Several risk factors contributing to dyslipidemia in obesity have been described; for example, adipose tissue macrophages cause pro-inflammatory state and induce insulin resistance. In obesity, we can observe an increase in free fatty acid explained by three main factors: first, the enlarged adipose tissue results in augmented delivery of fatty acids to the liver. The increase in FFA in the liver determines an

increase in triglycerides synthesis that, preventing the intrahepatic degradation of Apo B-100, allows an increase in VLDL formation and secretion. Additionally, whereas insulin resistance in obesity determines a decrease in lipolysis with a consequently increased delivery of fatty acid to the liver; on the other hand, hyperinsulinemia stimulates sterol regulatory element-binding protein (SREBP-1c) activity that acts as a transcription factor to overexpress fatty acid producing enzymes determining an increase in de novo fatty acid synthesis. Finally, in animal models, an increase in intestinal chylomicrons synthesis has been observed with an increase delivery of FFA to the liver.

In parallel, reduced clearance lipoproteins enriched in triglycerides rich lipoproteins may result in serum triglycerides rise (by reduced LPL activity at adipocyte and skeletal muscle level). In addition, hypertriglyceridemia stimulates cholesterylester-transfer-protein (CETP), which in turn increases the exchange of cholesterylesters and TG between VLDL and HDL and LDL. This mechanism affects TG and HDL-C metabolism and levels, and shifts LDL towards higher density. Furthermore, the removal of TG and phospholipids by hepatic lipase from LDL results in triglyceride-depleted forms of small dense LDL.

In obesity, HDL levels are typically low and serum triglycerides high while normal ranged LDL levels and increased small dense LDL can be found [43, 44].

In obesity, energy intake exceeds energy expenditure determining an increase both in subcutaneous adipose tissue and visceral adipose tissue. There is a clear relationship between obesity and insulin resistance confirmed by several studies in which lean subjects without previous history of obesity or diabetes on a fatty diet develops insulin resistance [45].

The mechanisms on the basis of insulin resistance arrive at three different levels: prereceptorial (FFA excess), receptorial (insulin receptor downregulation), and postreceptorial (inhibition of the intracellular cascades by increased FFA, impairment in adipokines and/or cytokines secretion) insulin pathway defects [46].

First, according to some authors [46], the increase of nonesterified fatty acids (NEFA) typical of obesity can initiate insulin resistance in obese patients.

Second, impaired secretion of inflammatory cytokines/adipokines (e.g., decreased adiponectin, increased FFA, TNF-alpha, and IL-6) and increased circulating insulin could determine a down-regulation of insulin receptors substrates (IRS-1 or 2).

Finally, together with pro-inflammatory state, the excess in NEFA decrease activity of phosphatidylinositol-3-kinase (an enzyme involved in GLUT4 translocation to the plasma membrane) and consequently insulin-mediated glucose transport which impairs glucose uptake, reducing hepatic glucogenesis, increasing glycogenolysis, and de novo lipogenesis. The excess in NEFA determines also an increase in some toxic metabolites (DAG and ceramides) and a possible damage on pancreatic β-cells [46, 47]. However, more recently, Karpe et al. [48] revised the literature showing that not necessarily obesity is associated with the increase in NEFA.

5 Curcumin and Obesity

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], alternatively named as diferuloylmethane, is one of the principal healthful components and the main natural polyphenol derived from the grounded rhizome of the turmeric or *Curcuma longa* and other *Curcuma* spp. Curcumin has recently been the subject of increasing attention owing to its numerous pharmacological actions against numerous pathological states [49–57].

As curcumin has anti-oxidants and anti-inflammatory effects, it is widely studied for obesity and metabolic diseases treatment. This molecule promotes weight loss, through promoting basal metabolic rate and reducing the incidence of obesity-related diseases, because it has anti-inflammatory activity that counteracts against the chronic low-grade metabolic inflammation of obesity [58]. The direct action of curcumin is posed on the white expanded adipose

tissue in the obese where inhibits inflammatory macrophage infiltration: they become more abundant and activated, so they exert their pro-inflammatory effects through inflammatory signals and cytokines, including TNFα and IL-6, which are nontraditional novel risk factors for cardiovascular diseases. As a systemic factor of inflammation, TNFα increases fat mass and insulin resistance; IL-6 increased the expression of high-sensitivity CRP and this is particularly associated with abdominal obesity. So curcumin suppresses inflammatory adipokine secretion from white adipose tissue, reduces the expression of TNFα, PAI-1, MCP-1, and increases the production of adipose tissue-related anti-inflammatory factor known as adiponectin. Moreover, curcumin downregulates transcription of AP-1 and NF-kB and promotes ROS scavengering and suppresses mitogen-activated protein kinases. Finally, this molecule may further suppress differentiation preadipocyte to adipocytes and promote cytoprotective antioxidant expression [59].

Obesity-related disturbances including insulin resistance, secretion of cytokines and adipocytokines, transcription factors, and sex hormones can result in higher risks of malignancy development through disturbing proliferation-apoptosis cycle. Therefore, curcumin can prevent obesity and malignancies due to the obesity through inhibiting signaling pathways related to adiponectin, leptin, and inflammatory mediators. It inhibits lipid aggregation and fatty acid synthase expression. Furthermore, the rise in the low-density lipoprotein receptors expression, oxidation blockage of low-density lipoprotein, bile acid secretion, cholesterol usage in metabolic pathways result in modified lower cholesterol levels [60].

6 Immuno-Metabolic Effects Of Curcumin: Focus On Adipose Tissue

Several natural anti-obesity agents are on study acting at different levels in the pathogenesis of obesity, suppressing adipose tissue growth, inhibiting preadipocytes differentiation, stimulating lipolysis, and inducing apoptosis of existing adipocytes with consequent reduction of adipose tissue mass [61]. Most of these pathways are either directly or indirectly targeted by curcumin.

In this regard, curcumin has several molecular targets, such as transcription factors, enzymes, cytokines, and growth factors [62], thus exerting anti-inflammatory, anti-oxidant and effect on the regulation of hunger, on glucose homeostasis, and on lipid metabolism.

6.1 Anti-inflammatory Effect

Some of the inflammatory genes that are regulated by NF-kB play a crucial role in several inflammatory pathways of obesity and insulin resistance. In adipose tissue, the expression of several signaling molecules involved in inflammation (like TNF-α, adiponectin, resistin) is regulated by NF-kB activity and vice versa. Moreover, NF-κB is involved in the development of insulin resistance [63, 64]. The regulatory factor of NF-kB activation is I-kB that makes a complex with NF-kB and leaves it alone in the cytoplasm and therefore make it inactive [58]. Following cytokine stimulation, a subunit of I-kB is phosphorylated and rapidly degraded. Subsequently, NF-kB is activated and translocated to the nucleus and activates a cascade of kB-dependent genes (more than 200) which in turn regulates innate and adaptive immunity, inflammation and cell survival, cellular transformation, proliferation, antiapoptosis, angiogenesis, invasion, and metastasis [65, 66]. Curcumin exercises part of his anti-inflammatory effects down-regulating the inflammatory transcription factor NF-κB by the reduction of the activation of I-κB kinases and of the dissociation of I-κB complexed to NF-κB [66, 67]. In C57BL/6 J mice, which are susceptible to diet-induced obesity, curcumin diet (4 g/kg diet added 2 days/week for 28 weeks) reduced inflammatory response in rats on fat saturated diet by the inhibition of the rise in hepatic NF-κB expression level in mature adipocytes [67]. If we know the role of NF-kB in the murine model, little is known about his function in human adipocytes, because of the lack of

selective and specific inhibitory compounds. However, Laurencikiene et al. [68] showed the role of NF-kB in TNF-α-mediated lipolysis in a study on human lipocytes. In this study, the incubation of human adipocytes with an inhibitor of NF-kB (a 28 amino acid long peptide derived from the γ subunit of IkB kinase sequence) determined a reduction in TNF-α-induced lipolysis.

Cyclooxygenase 2 (COX-2) is an enzyme able to convert arachidonic acid to prostaglandins. Induction of COX-2 expression is mediated by various factors such as cytokines, ROS, mitogens, and various tumor initiators [69].

In the murine model, COX2 demonstrated to have a role in the differentiation and inflammation of adipose tissue [70]. In an in vivo experiment, Sprague-Dawley rats, on a high fat diet, were randomly classified into three groups cotreated with vehicle or 30 mg/kg/day celecoxib (as a selective COX-2 inhibitor) or 30 mg/kg/day mesulid. Study on homeostasis model assessment of insulin resistance (HOMA-IR) and hepatic triglyceride contents showed that inhibition of COX-2 determined a reduction in adipocyte hypertrophy [71]. Curcumin reduces the expression of TNFα, IL-6, IL-1β, and COX-2 through Inhibiting NF-κB. In adipocytes derived from 3 T3-L1 (a fibroblast line able to differentiate into adipocytes under appropriate conditions) stimulated by TNFα, curcumin (from 0 to 20 μM) reduced expression of IL-1β, TNFα, IL-6, and COX-2 genes in a dose-dependent manner downregulating NF-κB and consequently cytokine expression [69]. COX-2 gene is not only upregulated in adipose tissue but its transcription is abundant in adipose tissue [71].

TNF-α is another important mediator of inflammation and inflammation-related diseases [64]. In obesity, we can observe an increase in TNF-α both in fat tissue of obesity murine model [72, 73] and in human adipocyte [74, 75] and a dysregulation contributing to the pathogenesis of insulin resistance [72]. The expression of TNF-α in adipocytes has been implicated in the inhibition of adipogenesis, lipogenesis, and in the induction of lipolysis with an increase in blood FFAs and hepatic lipid stores. Moreover, TNF-α further modulates adipocytes endocrine function and insulin resistance as a result of obesity in obese mice. Curcumin inhibits the production and action of TNF-α in vitro. On this regard, curcumin can downregulate the production of TNF-α and block intracellular signaling pathways (including JNK, MAPK, PI3K/Akt) and reduce expression of various cell surface molecules or binding directly to TNF-α. There are numerous reports in vitro and in vivo that demonstrate the relationship between curcumin and TNF-α [64]. In mesenteric adipocyte from male high-fat diet, C57BL/6 murine model on curcumin (from 0,1 to 10 μM) notably suppressed the cellular production of inflammatory factors including NO and TNF-α [76]. Yekollu et al. studied the effects of intraperitoneal injection with curcumin-containing liposomes (curcusomes) on macrophage activation of NF-kB and production of cytokine in ob/ob mice with insulin resistance and steatosis [77]. The authors observed a reduction in TNF-α level both in dendritic cells of the liver and macrophages in fat tissue with a consequent improvement of insulin resistance, lowered activation of classic NF-κB and in the TNF-α levels. In human subjects, the administration of oral curcumin reduced TNF-α expression both in patients affected by colorectal cancer (360 mg thrice/day) [78] and type 2 diabetes mellitus patients (300 mg twice/day) [79].

Adiponectin and leptin belong to the adipokines family and their dysregulation can be observed in metabolic syndrome. Particularly adiponectin has demonstrated a beneficial effect on the cardiovascular system by his anti-inflammatory properties, thereby improving the metabolism of lipid and glucose, alleviating insulin resistance, and preventing atherosclerosis. In various experiments [80, 81] it has been demonstrated that adiponectin is inversely associated with body weight, serum lipids, insulin resistance, and blood pressure and is positively associated with HDL-C blood concentrations [82]. Adiponectin, an insulin-sensitizing hormone, modulates several signaling pathways in the cells stimulating AMPK, PPAR-γ, and MAPK. Serum levels of this adipokine are lower in obese than in healthy people. In this regard, curcumin is able to increase the expression of adiponectin reducing

the risk of atherosclerosis [60]. Qu et al. in a vitro experiment on human adipose tissues studied the effect of different dosages of curcumin (10–100 μg/ml) on the release of IL-6 and adiponectin. After 6 hours, the level of adiponectin was significantly increased and IL-6 level was notably decreased in treatment adipocyte culture (100 μg/ml curcumin) compared to the control group ($p < 0.05$) of [83].

On the other hand, leptin, secreted by adipose tissue, is an adipokine that, modulating the sensation of satiety, regulates energy intake and, consequently, lipid metabolism. There is a direct correlation between serum leptin levels and body fat. Plasma leptin levels increase with adipose tissue and correlate with insulin resistance. Moreover, plasma leptin concentration is proposed as a risk factor that independently predict coronary artery disease [82]. In 3 T3-L1 adipocytes, curcumin supplemented diet at different concentration (2.5, 5, and 10 μg/ml for a period of one day) reduced significantly leptin levels and increased expression levels of adipose triglyceride lipase and hormone-sensitive lipase mRNA [84]. In Sprague-Dawley rats on high fructose diet curcumin treatment dose-dependently (15, 30, and 60 mg per kg) reduced fructose-induced serum concentrations increase of VLDL, TG, and TNF-α and hepatic triglicerid concentrations [68].

The effects of curcumin on leptin and adiponectin blood levels of metabolic syndrome patients were investigated in a double-blind randomized placebo-controlled ($n = 58$ and 59 respectively) trial. In this study, the authors observed that a supplementation diet with curcumin (1000 mg/d) during eight-week notably increased adiponectin serum levels ($P < 0.001$) and a reduction in serum leptin concentrations ($P < 0.001$) and improved the ratio of serum leptin to adiponectin [82].

Monocyte chemoattractant/chemotactic protein-1 (MCP-1) is a chemokine with a fundamental role in regulating migration and tissue infiltration of monocytes/macrophages [85]. Woo et al. [76] suggested that curcumin is able to inhibit MCP-1 serum from 3 T3-L1 adipocytes and suppress underlying inflammatory response due to obesity firstly by inhibiting macrophage recruitment in fat mass and secondarily by inhibiting the production of several adipocytokines such as, MCP-1, TNF-α, and NO. The further advantage of MCP-1 production inhibition by curcumin in adipose tissue is prevention from atherosclerosis and insulin resistance [85].

AP-1 [86] (activator protein-1) is a transcriptional factor that is activated due to stress, cytokines, growth factors, and infections. In a population of obese patients with NASH, the researchers [87] observed an overexpression of liver DNA binding of NF-κB and AP-1 and, consequently, an increase in oxidative stress and IR contributing to NASH. Curcumin antioxidant properties can suppress the activation of AP-1 due to stress by a direct interaction with its DNA binding motif [88]. Curcumin and its metabolite, tetrahydrocurcumin (0.5 g/100 g) for four weeks, can improve oxidative stress (induced by ferric nitrilotriacetate) induced renal injury in mice [89].

6.2 Effects on Lipid Metabolism

Cholesterol concentration changes by curcumin are mainly due to suppressing LDL oxidation, expression of LDL receptors, bile acid over secretion and excretion of cholesterol through metabolic pathways, and decreasing fatty acid synthase expression [60].

Asai et al. [90] showed that if rats on a high fat diet were supplemented with curcumin, they would experience a reduction in TG content of VLDL but not of chylomicrons. For this reason, we can think that curcumin improves rises of TG clearance in the liver without interfering with TG absorption in the intestine. In addition, curcumin and its metabolites can act as peroxisome proliferator-activated receptor (PPARγ)-activating ligands increasing its hypolipidemic effect.

Furthermore, curcumin inhibits acyl CoA cholesteryl acyltransferase (ACAT) HMG-CoA reductase as liver enzymes that reduces liver cholesterol content, non-high-density lipoprotein (HDL)-C, and total cholesterol. Also, it is proposed that diet supplemented with curcumin suppresses fatty acid synthase (FAS) in the liver and

increases fatty acids beta oxidation [78]. Curcumin lowers fat accumulation downregulating FAS. However, the exact effect of curcumin on FAS is still unclear.

Jang et al. [91] found a notable rise in plasma level of HDL, Apo-A-1, and paraoxonase (PON) in hamsters on high-fat diet. Paraoxonases are hydrolytic enzymes with several isoforms associated with HDL and prevention of the formation of oxidized LDL.

In a vivo experiment, male Sprague-Dawley rats following two studied diets, protection (80 mg/kg, rosiglitazone −1 mg/kg – their combination, or vehicle – in control groups – were given for a duration of −60 days – besides fat saturated diet) and treatment (15 days of high fat diet after 60 days of high fat diet to induce insulin resistance and type 2 diabetes). In this study, curcumin lowered TNF- α and free fatty acid plasma levels in male Sprague-Dawley rats with fat saturated diet and antihyperglycemic effect and anti-insulin resistance effects were found [92]. Peschel et al. [93], using the human hepatoma cell line HepG2 as a model system, examined the influence of curcumin on hepatic gene expression. The authors incubated HepG2 at different concentrations of curcumin from 50 to 2 μM and control medium. After curcumin exposure, LDL-receptor mRNA was increased by sevenfold dependent on the concentration of curcumin, and expression of HMG CoA reductase and farnesyl diphosphate synthase slightly increased but there was also a reduction in cell viability. In a randomized, double-blind, crossover trial, 30 obese individuals received curcumin 1 g/day for four weeks and a reduction in serum triglycerides concentrations, but not other lipid profile indices, was observed compared to the matched control group [94].

6.3 Glucose Homeostasis

According to experimental and clinical studies, curcumin has a fundamental action in improving insulin resistance acting on four main pathways: inflammation, lipid metabolism, insulin pathways (through activating the insulin receptor and its downstream pathways), and oxidative stress [95].

Protein-tyrosine phosphatase 1B (PTP1B) may play a central role in the regulation of post-receptor insulin signaling pathway, regulating the steady-state balance of the tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and the docking of Src homology2 (SH2) domain-containing adaptor proteins to IRS-1 [96]. On this issue, in male Sprague-Dawley rats characterized by fructose-induced insulin resistance an oral curcumin integration (15, 30, and 60 mg/kg) promoted activity of insulin receptor through phosphorylation of tyrosine in homogenates of hepatocytes, perhaps inhibiting protein-tyrosine phosphatase 1B (PTP1B) [97].

In parallel, curcumin exerts its hypoglycemic effect regulating glycolytic enzymes, increasing hepatic glucokinase (GK) activity and glycogen storage, and downregulating the gluconeogenic enzymes via suppressing phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). GK converts glucose into glucose-6-phosphate, and protein kinase A (PKA) suppresses and AMP-activated protein kinase (AMPK) activates GK [95]. In Male Wistar rats (a type of diabetic rat) fed on a diet containing 50, 150, or 250 mg/kg, Na et al. [98] observed an increase of GLUT4 transporters and of phosphorylation of AMPK in differentiated skeletal myoblasts. So curcumin determined in these cells an increase in the uptake and oxidation of fatty acids and glucose in skeletal muscle.

Heme oxygenase-1 (HO-1) is characterized by cytoprotective effects in pancreatic β-cells as it is an antioxidant enzyme. In a vitro model, mouse islets (MIN6 murine cell line of beta cells) growth in a culture medium with demethoxy curcuminoids was associated with increased expression of phase 2 enzyme HO-1 through activation of nuclear factor-E2-related factor (Nrf2), reducing oxidative stress and xenobiotic insult.

We can observe an increase in pro-inflammatory cytokines in insulin-resistant obese and diabetic patients. One of the main mechanisms that underlie insulin resistance is the activation of nuclear factor kappa B (NFkB) and other signaling mechanisms by free fatty acid [86]. In 3 T3-L1 adipocytes incubated in a growth medium at different concentrations of curcumin

for a period of one day (5 μmol/L,10 μmol/L, 20 μmol/L), Wang et al. observed a downregulation of IL-6 and TNF-α expression [99].

Male C57BL/6 J ob/ob mice possess a knockout of the leptin gene that produces hyperphagia, low metabolic state, obesity, and diabetes, that finally leads to pancreatic β-cell hyperplasia and hyperinsulinemic state. In an experimental model, wild-type and ob/ob male C57BL/6 J mice received a fat-saturated diet with or without a supplementation of 3% by weight of curcumin. Male C57BL/6 J murine model on fat saturated diets slowly develop obese and diabetic. In this study, the results of HBA1c percentage and glucose and insulin tolerance tests showed that diet saturated with curcumin can alleviate diabetes in high-fat diet-induced obese and leptin deficient ob/ob male C57BL/6 J mice [100].

In a double-blinded, placebo-controlled trial, 240 patients satisfying criteria of prediabetes according to American Diabetes Association (ADA) practice guidelines were included. Cases should receive three capsules (each one with 250 mg of curcuminoids) of curcumin or placebo bid (total of six capsules per day) for a nine-month period. Curcumin supplementation significantly reduced the development of T2DM in prediabetic individuals and it seems to promote the overall activity of β-cells [101].

In another double-blind, randomized, placebo-controlled trial, one hundred patients with comorbid obesity and diabetes were randomly grouped to curcuminoids (300 mg/day) or placebo for a three-month period. Curcuminoids significantly lowers HbA1c ($p = 0.031$), fasting blood glucose ($p < 0.01$), and insulin resistance index (HOMA-IR) ($p < 0.01$) in patients with diabetic. Curcuminoids further influenced lipid metabolism reducing total TG ($P = 0.018$), FFAs in serum ($p < 0.01$), a rise in LPL activity ($p < 0.01$) [102].

6.4 Differentiation of adipocytes

Wingless-type integration site family members (WNTs) are a group of released glycoproteins involved in autocrine and paracrine signaling in order to modulate homeostasis of tissue and remodeling and cell proliferation through two main pathways: the 'canonical' and 'non-canonical' pathways to manage cell behavior, proliferation, survival, and fate. In this regard, cell culture studies and gain-of-function mouse models suggested WNT signaling in regulating mesenchymal stem cells proliferation, maintenance, fate determination, and differentiation of preadipocyte, and thereby, adipose tissue expansion [103, 104].

Curcumin is able to reduce fat adipose tissue acting on preadipocyte differentiation by the regulation of WNT signaling pathway in preadipocytes. Wnt signaling determines the growth arrest of preadipocytes and their terminal differentiation into mature adipocytes [58, 105]. Curcumin not only reduces lipogenesis, but can also induce brown-like phenotype adipose tissue, strengthening his possible role in the obesity treatment. Experimental 3 T3-L1 cell line and premature white adipocytes in a growth medium with various curcumin concentrations (1–20 μM) notably induce browning of adipose tissue (increasing mitochondrial biogenesis, increasing lipolysis and suppressing of lipogenesis and increasing expression of the uncoupling protein 1, UCP1) [106].

Ejaz et al. [107] assessed the effect of curcumin (in 3 T3-L1 mouse embryonic fibroblasts of mice on fat saturated diet (22%) with curcumin supplementation of 500 mg/kg for a twelve-week period) on adipogenesis, angiogenesis, differentiation, and apoptosis in 3 T3-L1 cells. This study showed that curcumin blocks preadipocytes differentiation and initiated apoptosis; it also suppresses vascular endothelial growth factor-α expression and thus inhibited adipokine-related angiogenesis in endothelial cells of human.

6.5 Autophagy

Autophagy is an adaptive mechanism to starvation and other types of stress in which cells throughout a self-digestion process into autophagosomes recycle their contents (cytoplasm, macromolecules, and organelles) in order to provide energy and building blocks for renewal [108].

The fundamental inhibitory regulator of autophagy is the mammalian target of rapamycin (mTOR), when it is suppressed there is the induction of autophagy by dephosphorylation of the nuclear transcriptional factor EB (TFEB, a nuclear transcription factor that is important for autophagy management and biogenesis and action of lysosome) which translocates to nuclei promoting gene transcription. On this topic, it is not clear if curcumin can induce autophagy through inhibition of the Akt-mTOR pathway.

Zhang et al. [108] first found that treatment with curcumin can increase autophagic flux (in human colon cancer HCT116 cell line and MEFs as murine embryonic fibroblasts), stimulates lysosomal function, downregulates mTOR, and activates transcription factor EB (TFEB) (after the binding TFEB curcumin stimulates his translocation to the cell nucleus, increasing transcriptional activity).

7 Conclusions

Curcumin is a phytochemical isolated from the ancient spice turmeric that has been shown to target potential therapeutic activity in treating obesity and obesity-related metabolic disorders.

Curcumin has beneficial effects such as anti-inflammatory, anti-adipogenesis, and antioxidant potency with underlying diverse mechanisms. Obesity is associated with chronic low-grade inflammation, metabolic disturbances, and insulin resistance.

Curcumin suppresses the obesity-related inflammation by subsiding macrophage infiltration and blocking NF-κB activity in adipose tissue. Anti-inflammatory features of curcumin are also linked with the inhibition of pro-inflammatory cytokines including TNFα, PAI-1, and MCP-1.

Curcumin mediates suppressive effect on pre-adipocyte differentiation and initiates apoptosis with Wnt signaling pathway within WAT. Also, adipokine-related angiogenesis is inhibited by suppression of vascular endothelial growth factor-α expression. Furthermore, curcumin significantly promotes insulin sensitivity and glucose metabolism.

This phytochemical has direct effects on lipid metabolism such as reducing TG synthesis and increasing beta oxidation of free fatty acids with raising metabolism rate and releases cytokines that are effective in weight loss. Moreover, consuming food item containing curcumin suppresses fatty acid synthase (FAS) and increases fatty acids beta oxidation [78]. Recently, several cellular pathways have been extended in the context of elucidating the basic mechanism for obesity and obesity-related metabolic diseases. These findings may introduce curcumin as the novel phytochemical treatment for obesity-related chronic diseases.

Conflict of interest Muhammed Majeed is the founder of the Sabinsa-Sabinsa group. The other authors have no other conflicting interests to disclose.

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Antiviral Plants in View of Avicenna's *The Canon of Medicine* and Modern Medicine Against Common Cold

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Abstract

Common cold is known as a serious clinical problem worldwide. Coronaviruses have long been identified as respiratory pathogens causing "common cold" in healthy people. The pandemic of 2019 novel coronavirus as a serious public health problem and concern has resulted in severe illness and death especially in the elderly. COVID-19 is picking up pace around the world and has spread to more than 219 countries. Due to the very easy spread of COVID-19 and its lack of recognized appropriate treatments and vaccines as well as potential therapeutic effects of several traditional herbal remedies, we decided to gather, evaluate, and compare the potential pharmacological effects of medicinal herbs from Avicenna's perspective and modern medicine with antiviral properties which may lead to the discovery of suitable traditional treatments to prevent or reduce the adverse symptoms of common cold.

Keywords

Common cold · Coronavirus · COVID-19 · Antiviral · Medicinal plants · Avicenna

Abbreviation

ADV Adenoviruses
AFP α-fetoprotein
ALT Alanine aminotransferase

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ARV	Antiretroviral
AST	Aspartate aminotransferase
AuNPs	Gold nanoparticles
BeA	Betulinic acid
cAgNPs	Citrate-coated silver nanoparticles
CCID50	Cell culture infectious dose 50%
CI	Confidence interval
COVID-19	Coronavirus disease
CoVs	Coronaviruses
CpHV-1	Caprine herpesvirus 1
CVB1	Coxsackievirus B1
DG	Diammonium glycyrrhizin
EC50	50% effective concentration
EEE	Ent-epiafzelechin-(4α→8)-epiafzelechin
EGFP	Enhanced green fluorescent protein
EO	Essential oil
EV71	Enterovirus 71
18β-GA	18β-glycyrrhetinic acid
GEO	Ginger essential oil
GL	glycyrrhizinate
GMK	Green monkey
GRA	Glycyrrhizic acid
HA	Hemagglutination
HBV	Hepatitis B virus
HCoVs	Human coronaviruses
HCV	Hepatitis C virus
HEp-2	Human epithelial type 2
HGG	Honey ginger and garlic
HIV	Human immunodeficiency virus
H1N1	Hemagglutinin type 1 and neuraminidase type 1
HRSV	Human respiratory syncytial virus
HSV-1	Herpes simplex virus type 1
IAV	Influenza A virus
IBV	Infectious bronchitis virus
IC50	Half maximal inhibitory concentration
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
IFN-β	Interferon beta
LC3	light chain 3
LiCl	lithium chloride
MDBK	Madin-Darby bovine kidney
MDCK	Madin-Darby canine kidney
MERS-CoV	Middle East respiratory syndrome coronavirus
MeV	Measles virus
NDV	Newcastle disease virus
NHC	National Health Commission
NS5B	Nonstructural 5B
N/V	nausea and vomiting
PBMCs	Peripheral blood mononuclear cells
PHS	*P. harmala* seeds
PI-3	Parainfluenza type 3
PRV	Piscine orthoreovirus
RSV	Respiratory syncytial virus
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TMV	Tobacco mosaic virus
USA	the United States of America
WHO	World Health Organization

1 Introduction

1.1 Common Cold

Common cold is an acute self-limited viral infection of the upper respiratory system and known as a significant clinical problem over the world [1, 2]. The hallmark symptoms are sore throat, nasal congestion, rhinorrhea, sneezing, cough, low-grade fever, discharge, and headache [1, 3]. The term common cold is defined as a heterogeneous group of diseases caused by numerous pathogens including rhinoviruses, respiratory syncytial virus (RSV), *influenza viruses*, parainfluenza viruses, *coronaviruses*, and adenoviruses. Based on numerous studies, on average, adults usually experience two to four and children depending on age have more (six to eight) colds per year [1, 3]. The rates of common cold per year depend on many factors including age, season, day-care attendance, psychological stress, smoking, heavy physical activities, and genetic factors [1, 3]. The most common causes among all causal factors in all age groups are rhinoviruses and *coronaviruses* with 50–70% of cases [1, 3, 4]. Virus incubation period varies significantly from 12 h for influenza

B to 5.5 days for adenovirus. Studies have determined that the peak of symptoms occurs at 2–3 days postinoculation and generally lasts for 7–11 days. Evidence has revealed that rhinoviruses do not cause notable cytopathic effects on upper respiratory tract airway epithelial cells, but have the potential to infect the lower airways. Overall, it is assumed that symptoms of common cold are triggered by the inflammatory response [1]. Various drugs are used to control the clinical complications of common cold; however, side effects, such as respiratory depression, renal and hepatic damage, gastrointestinal disturbances, tolerance, sedation, spasm, bone marrow depression, constipation, and suppression of response to infection or injury, and osteoporosis have been documented in studies [5].

1.2 Influenza

Influenza is considered as a main threat to global public health because of 290,000–650,000 deaths every year over the world [6–8]. Influenza virus belongs to the family of *Orthomyxoviridae*, which are classified into five serotypes of influenza viruses: influenza A virus (IAV), influenza B virus, influenza C virus, isavirus, and *Thogotovirus* [4, 6, 7]. There have been four influenza pandemics in human over the past 100 years: 1918 hemagglutinin type 1 and neuraminidase type 1 (H1N1) Spanish flu, 1957 H2N2 Asian flu, 1968 H3N2 Hong Kong flu, and 2009 H1N1 swine flu [6]. The most common cause of both seasonal and pandemic influenzas is influenza A virus [7]. Although symptoms of influenza mainly resolve within 2 weeks with no need for medical attention, some cases need hospitalization, and it may even rarely cause death [8]. Influenza complications are myriad and include pneumonia, bronchitis, sinus infections, ear infections, and exacerbation of many chronic conditions such as asthma, congestive heart failure, and chronic obstructive pulmonary disease. The best approach to prevent and control influenza outbreaks is vaccination; however, the emergence of antiviral drug resistance and antigenic drift in the virus and also a lack of long-lasting antibody titers do not provide complete protection against influenza infections [8].

1.3 Coronaviruses

Coronaviruses (CoVs) which are enveloped, positive-sense RNA viruses belonging to the *Coronaviridae* family infect human and a variety of vertebrates including birds, bats, snakes, mice, and other wild animals [9, 10]. Coronavirus infections caused 7–18% of adult's common cold, mainly during the winter and early spring [1, 3, 11]. Seven human coronaviruses (HCoVs) have now been identified: 229E, OC43, NL63, HKU1, severe acute respiratory syndrome (SARS)-COV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 [9]. SARS-CoV-2 (coronavirus disease (COVID-19)) was first recognized in Wuhan City, China, on 31 December 2019 and was responsible for the pandemic in humans via 45,968,799 people infections and 1,192,911 deaths (updated as of 01 November 2020) with case fatality rate of 3.4% (recently 7.8% estimated by Mahase [12]), but it has been mentioned that it is killing people in different countries at different rates [13, 14]. According to the World Health Organization (WHO), COVID-19 has spread to 219 countries (updated as of 01 November 2020) [9, 14]. COVID-19 has severity range of complications from flu-like symptoms to acute respiratory distress syndrome as well as gastrointestinal problems, which is more common in adults than in children [15]. Current incubation period of COVID-19 has been estimated to be 2–14 or –27 days (2 and 10 days (WHO) and 10 to 14 days China's National Health Commission (NHC)), and the mean incubation period was 5.2 days (95% confidence interval [CI], 4.1 to 7.0), with 95th percentile of the distribution at 12.5 days [16–18]. COVID-19 outbreaks have been perceived in the United States, India, Brazil, Russian Federation, France, Spain, Argentina, Colombia, the United Kingdom, and Mexico. In Iran, COVID-19 infected 612,772 people and caused 34,864 deaths (updated as of 01 November 2020) [19, 14]. Unfortunately, there is no specific recommended and unified approach (vaccines or antiviral treatments) to prevent and treat COVID-19 effectively [9, 20]. Lack of approved and effective treatment attracts medical healthcare practitioners to use complementary and alterna-

tive medicine to control the symptoms associated with the disease. Numerous traditional medicines especially Persian medicine have suggested herbs or supplements with low side effects to protect or prevent the complications of the disease.

In ancient Persia about 10,000 years ago, plants with various therapeutic actions were utilized to limit the complications of common cold [4]. In a chapter by Tayarani-Najaran et al. named "The history of Islamic medicine at a glance," the authors stated: "In the history of medicine, Islamic medicine or Arabic medicine refers to medicine developed in the medieval Islamic civilization and written in Arabic, the lingua franca of the Islamic civilization. Shaykh al-Ra'is (The Chief Principal) Abu Ali Hussain ibn Abdullah ibn Sina known as Avicenna (370-428 A.H./980-1037A.D.), the most prestigious scholar of Iran and the world of Islam. He wasn't only a physician and had a great dignity in philosophy as well. Avicenna's masterpiece is the book of "al-Qânūn fi al-Ṭibb" (The Canon of Medicine), which is the source book of medicine in the eastern and western worlds. Canon consists of 5 major books each divided into some arts, tuitions, sentences and chapters. There have been numerous expositions of whole Canon or its parts and it has been summarized many times. The book has been translated to European, Hebrew and Persian languages and it has been reprinted frequently" [21]. Common cold in Avicenna's book, *The Canon of Medicine* (*al-Qanūn fi al-Ṭibb*), is called catarrh or nazleh [5, 22]. Avicenna in his work classified catarrh as warm and cold. He determined that warm catarrh complications included redness of the eye and face, warmth, sharpness, dilution and yellowness of discharges, and burning sensation the in nose and throat, whereas cold catarrh appeared as tension and heaviness in the head, face, and forehead, thick whitish or livid discharge, roughness of the tongue, discharges with cold and unsavory nature, heaviness of senses, malaise, and losing the sense of smell. In *The Canon (Qânūn) of Medicine*, the potent therapeutic roles of a number of herbs have been discussed and their use implicated to prevent or diminish complications of cold, many of which are now recognized as antiviral agents in modern medicine [5]. Some important properties of medicinal herbs including availability, fewer side effects, and less toxicity of those components can result in their selection to treat infectious diseases [23].

Due to SARS-CoV-2 outbreak all over the world and lack of appropriate treatments, widespread interest in using herbal medicine, and appearance of drug-resistant viruses, we tried to gather, evaluate, and compare the potential pharmacological effects of medicinal herbs from Avicenna's perspective and modern medicine with antiviral effects which may lead to revival and introduction of appropriate traditional treatments to prevent or to reduce the symptoms of common cold. Data of modern studies were collected from several scientific databases including PubMed, Elsevier, Science Direct, Google Scholar, and Scopus. Meanwhile, the antiviral plants used mainly for common cold, and the related symptoms are introduced from Avicenna's *The Canon of Medicine*.

2 Antiviral Plants in Avicenna's *The Canon of Medicine* and Modern Medicine

This review addresses and evaluates the potential antiviral effects of medicinal herbs from Avicenna's work and modern medicine which are detailed in Tables 1 and 2.

2.1 *Glycyrrhiza* L.

The genus *Glycyrrhiza* (family Fabaceae) consists of about 30 species. It has been cultivated in several studies that the roots of *G. glabra* L. are worth considering in bioactivities like antiviral, anticancer, antiulcer, antidiabetic, anti-inflammatory, antioxidant, antithrombic, antimalarial, antifungal, antibacterial, etc. [24]. In *The Canon of Medicine*, Avicenna mentioned that *G. glabra* cleared the trachea and is useful for the treatment of lung diseases [25]. In recent literature, we found numerous evidence about the antiviral properties of *Glycyrrhiza.* Glycyrrhizic acid, the ingredient from the root of licorice (*G. glabra*), exerts antiviral activity against Kaposi's

Table 1 The antiviral herbs in Avicenna's *The Canon of Medicine*

	Latin name	Arabic name	Persian name	English name	Family
1	*Allium cepa* L.	Baṣal	Piaz	Onion	Amaryllidaceae
2	*Allium sativum* L.	Thawm	Sir	Garlic	Amaryllidaceae
3	*Cassia fistula* L.	Khiâr shanbar	Folus	Golden shower tree	Fabaceae
4	*Cinnamomum cassia* (L.) J. Presl	Salikhah	Darchin-e-chin	Chinese cassia	Lauraceae
5	*Cinnamomum verum* J.Presl	Darṣini	Darchin-e-sâygon	Cinnamome	Lauraceae
6	*Crocus sativus* L.	Zʿafarân	Zʿafarân	Saffron	Iridaceae
7	*Glycyrrhiza glabra* L.	Sus	Shirinbayân	Liquorice	Fabaceae
8	*Lavandula stoechas* L.	Oṣṭokhodus	Oṣṭokhodus	French lavender	Lamiaceae
9	*Mentha* spp.	Nʿonʿo	Nʿanʿa	Mint	Lamiaceae
10	*Zingiber officinale* Roscoe	Zanjabil	Zanjabil	Ginger	Zingiberaceae
11	*Ziziphus jujuba* Mill.	ʿOnâb	ʿOnâb	Jujube	Rhamnaceae

Informant agreement ratio for different uses category. n_t = number of taxa; n_{ur} = number of citation in each use category. *IAR* informant agreement ratio

Table 2 The potential antiviral effects of herbs in Avicenna's *The Canon of Medicine* and modern medicine

Species	Avicenna's *The Canon of Medicine*	Subject	Dose	Virus	Modern medicine	Ref.
Glycyrrhiza glabra L.	Clears the trachea – useful in the lung diseases	Mice	Not found	HSV	Induced apoptosis	[26]
		Vero cells	1000–4000 mg/L g	FFM-1 and FFM-2	Inhibited the replication of virus	[28]
		HEp-2 and A549 cells	300μg/mL (Radix Glycyrrhiza)	HRSV	Inhibition of HRSV-induced plaque formation and secretion of interferon beta (IFN-β) decreased the viral count within the cells	[30]
		Epithelial cells	1 and 2 mM (GRA)	HSV-1	Induced the production in higher amount of the autophagy activator Beclin 1	[31]
		Vero cells	50 mM or 1250μg/mL (LiCl and DG)	PRV	Inhibited the virus-induced cell apoptosis	[33]
		Hfl-1, A549, or MDCK cells	0.5–5 mM (GL)	IAV	Declined in the cytopathic effect, reduced viral RNA within the cells and in the cell supernatants, and reduced viral hemagglutination titers	[34]
Glycyrrhiza uralensis Fisch	Not stated	Colostrum s-deprived piglets	400 mg/mL	Rotavirus	Cured diarrhea	[29]
		MDCK cell	IC_{50} values of 48.0, 42.7, 39.6, and 49.1μM – 29.5 and 41.7μM (13 new oleanane-type triterpenoid saponins, uralsaponins M–Y (1–13) and 15 known analogues (14–28))	H1N1 and HIV	Showed antiviral effects	[32]

Zingiber officinale Roscoe	Removes the moistness in the head and throat	RC-37cells	5–10%	HSV-1	Disrupted the viral envelope via interfering with virion envelope structures	[38]
		HEp-2 and A549 cells	300 mg/mL	HRSV	Inhibited plaque formation via blocking viral attachment and internalization	[39]
		Sixty volunteer patients with proven HCV	500 mg	HCV	Decreased virus loads, the level of AFP, and markers relevant to liver function, such as AST and ALT	[40]
		102 HIV-positive patients	500 mg	HIV	Reduces the frequency of mild, moderate, and severe episodes of nausea	[41]
		MDCK cell	50–308μg/μl	H5N1	Inhibition plaque formation	[42]
		PBMCs	5% (HGG)	H1N2	Suppressed influenza virus replication	[43]
		Vero cells	10%	H9N2	Showed antiviral activity	[44]
		MDBK cells	55.84, 139.6, and 1396μg/ml	HSV-2	Disrupted herpesvirus envelope	[45]
		Nicotiana glutinosa leaves	100μg/mL	TMV	Exhibited high antiviral activities	[47]
		RC-37 cells	0.00001–0.1% drug-containing medium	HSV-2	Prevented the adsorption of virus via viral envelope interruption through reducing plaque formation	[48]
Curcuma longa L.	Not stated	HEp-2 cells	0.008, 0.015, 0.03, 0.06 nM	RSV	Inactivated the virus directly prior to entry into the host cells	[46]

(continued)

Table 2 (continued)

Cinnamomum cassia (L.) J. Presl	Showed cure effected in chest affections	HEp-2 and A549 cell lines	10, 30, 100, 300µg/ml	HRSV	Inhibited viral attachment, internalization, and syncytium formation	[51]
		Vero cells	50, 100, and 200µg/mL	H7N3A	Had antiviral potential in both pre-penetration and post-penetration infection.	[52]
		MDCK cell and A549 cell lines	25µg/mL (Procyanidin)	IAV	Inhibited the replication and induced apoptosis	[53]
Cinnamomum verum J. Presl	Had beneficial properties in coryza and cough and cleared the chest congestion	–	–	–	–	–
Cinnamomum zeylanicum Blume	Not stated	MDCK cell and A549 cell lines	3.1µL/mL (Eugenol)	H1N1	Displayed antiviral activity against	[54]
		MDCK and Vero cells	3.52%	H1N1 and HSV-1	Showed antiviral activity	[55]
Cassia fistula L.	Revealed therapeutic effects in the swelling of the throat when gargled with the juice of *Solanum nigrum* – cleansed the liver – useful in jaundice	HT1080 cells	80µg/mL (anthraquinone)	–	Induced human IFIT1 antiviral protein expression	[57]
Cassia sieberiana DC.	Not stated	GFP-reporter CD4+ T-cell line	84.8µg/mL	HIV-1	Inhibited wild-type (NL4.3) and antiretroviral (ARV)-resistant replication by 50%	[58]
Cassia siamea Lam.	Not stated	–	(Siameflavones A and B with five known flavones (3–7))	TMV	Showed anti-activity	[59]
Cassia javanica L.	not stated	Vero cell	250µM (EEE)	HSV-2	Inhibited virus replication and penetration to the host cell	[60]

Allium cepa L.	*Allium cepa* L. juice cleaned the head and the eardrop removed pus and excessive moisture	Embryonated chicken eggs	0.3 mL	H9N2	Increases the life of the embryos, decreased HA titers, destroyed the avian influenza virus subtype, decreased the propagation of the virus	[63]
Allium sativum L.	Exhibited cure effected in chronic and pleuralgia caused by cold	Vero cells	8.829μg/mL	MeV	Showed antiviral effects via blocking viral particles	[62]
		Embryonic SPF eggs	0.1 mL	IBV	Showed inhibitory effects in the chickens' embryo	[64]
Mentha piperita L.	*Mentha* spp. excreted beneficial effects in cases of the hepatitis	MT-4 cells	16–32μg/mL	HIV-1	Showed inhibitory activity against HIV-reverse transcriptase	[65]
		Vero cells	0.003 and 0.076μg	IBV	Revealed antiviral properties via inhibition of the viral replication and cessation of the IBV production	[66]
		RC-37 cells	0.001–0.1% drug-containing medium	HSV-1 and HSV-2	Reduced plaque formation and inhibited virus before penetration into the host cell	[67]
Crocus sativus L.	Displayed expectorant effects and strengthened the respiratory organs	Vero cells	(Crocin and picrocrocin)	HIV-1 and HSV-1	Crocin inhibited the replication and suppressed the penetration of virus Picrocrocin significantly inhibited the entry and replication of the virus	[70]
Ziziphus jujuba Mill	Was useful for the chest and lungs	A549 cells	50μM (BeA)	A/PR/8 virus	Showed anti-influenza activity via downregulating of IFN-γ level and reduced respiratory pathology such as increased necrosis, numbers of inflammatory cells, and pulmonary edema induced by virus	[73]
Lavandula stoechas L.	Showed anti-infection properties	–	–	–	–	–

(continued)

Table 2 (continued)

Lavandula latifolia Medik.	Not stated	Vero cells	0.05 and 0.005%	HSV-1	Possessed antiviral activity before or after infection	[76]
Thymus vulgaris L.	Not stated	RC-37cells	5–10%	HSV-1	Showed antiviral activity prior penetration	[38]
		Vero cells	0.003 and 0.076µg	IBV	Stopped avian infectious through inhibiting viral replication	[66]
		MDCK cell and A549 cell lines	3.1µL/mL	H1N1	Displayed 100% inhibitory activity	[54]
		RC-37 cells	25–100µg/mL	HSV-1	Reduced viral infectivity	[78]
		Embryonated eggs	50µg/mL	NDV	Exhibited the ability to reduce the viral potency by more than 56 folds (interfering with the cleavage of hemagglutinin-neuraminidase, the most important glycoproteins in NDV, and inhibiting virus attachment are the mechanisms of action)	[79]
Peganum harmala L.	Not stated	MDCK cells	100µg/mL (maximum nontoxic concentration)	H1N1	Showed antiviral activity which probably associated with preventing viral RNA replication and viral polymerase activity	[83]
		MDCK cells and mice	IC_{50} values of 15.7 (CI95%:11.7–21) µg/mL. 200 mg/kg/day of PHS extract or 20 mg/kg/day of oseltamivir	H1N1	Exhibited high activity against in MDCK cells. Increased the survival rate, reduce body weight loss, and decrease lung virus titer	[84]
		Vero cell	up to 667µg/mL	HSV-1	Reduced virus titer in the first passage and inhibited completely virus production in the third passage	[85]
Ocimum americanum L.	Not stated	GMK cell	9–78µg/mL	HSV-1F and HSV-2	Reduced the amount of plaques	[87]

Dracocephalum heterophyllum Benth. and *Dracocephalum tanguticum* Maxim.	Not stated	Vero cell mice	4 mg/mL^{-1} g/kg/day	HSV-2	Inhibited virus infection through diminishing the HSV-2 infectivity and inhibiting HSV DNA replication – increased the mean survival times and reduced the mortality	[91]
Dracocephalum canadense L.	Not stated	Vero cells	0.003 and 0.076μg	IBV	Possessed significant antivirus activities prior to/during infection	[66]
Ferula foetida L.	Not stated	HepG2, Hep3B, and MCF-7 cell lines	IC_{50} 0.26–0.86μg/mL	H1N1	Possessed significant antiviral activity	[93]
		Vero cell	10, 5, and 2.5μg/mL	HSV-1	Reduced the viral titer of the HSV-1 DNA viral strain KOS	[94]
Foeniculum vulgare Mill.	Not stated	Vero cell	0.8 and 0.025μg/mL–1.6 and 0.2μg mL	HSV-1-PI-3	Exhibited strong antivirus effects	[96]
Prunella vulgaris L.	Not stated	HeLa37 cell	Sub μg/mL	HIV-1	Displayed potent antiviral activity (inhibitory function is associated primarily with interference of early and post-virion binding events)	[98]
Valeriana wallichii DC.	Not stated	Huh-7.5 cell	12.5–300μg/mL (chloroform, water, and methanol extracts from roots of *Valeriana wallichii*) and 31.23–250μg/mL (methanolic subfractions F4)	HCV	Exhibited antivirus effects by binding with HCV nonstructural 5B (NS5B) protein	[102]
Eucalyptus oblique L'Hér	Not stated	RC-37 cells	IC_{50} values of 55μg/mL	HSV-1	Showed antiviral activity via disabling free virus particles and interfere with virion envelope	[78]

(continued)

sarcoma-associated virus by abolition of the inactive form of the virus via apoptosis. Glycyrrhizic acid is suggested to induce apoptosis by downregulation of the latency-associated nuclear antigen (LANA) which causes inhibition of p53-induced apoptosis in latent HSV infections [26]. Similarly, glycyrrhizic acid and its semisynthetic derivatives were reported to possess antiviral effects against DNA and RNA viruses, e.g., hepatitis A virus, hepatitis B virus, influenza virus, human immunodeficiency virus (HIV)-1, coronavirus, etc. [27]. Cinatl et al. assessed the effect of glycyrrhizin acid on two clinical isolates of coronavirus (FFM-1 and FFM-2) from patients with SARS. They found that glycyrrhizic acid inhibited the replication of SARS-associated coronavirus in Vero cells but the mechanism of glycyrrhizin's activity against SARS-CV is unclear [28]. In another study, the anti-rotaviral effects of *G. uralensis* Fisch extract was tested in colostrum-deprived piglets after induction of rotavirus diarrhea. Data showed extract (400 mg/mL) cured diarrhea and noticeably enhanced small intestinal lesion score and fecal virus shedding in piglets inoculated with porcine rotavirus K85 (G5P) strain [29]. Yeh et al. reported that hot water extracts of Radix Glycyrrhizae, Radix Glycyrrhizae Preparata, 18β-glycyrrhetinic acid (18β-GA), and ribavirin were dose-dependently effective against antihuman respiratory syncytial virus (HRSV) and inhibited the HRSV-induced plaque formation on both human epithelial type 2 (HEp-2) and low (A549) respiratory tract cell lines with better activity of Radix Glycyrrhizae Preparata on A549 cells and Radix Glycyrrhizae on HEp-2 cells. Overall, secretion of interferon beta (IFN-β) following treatment with 300μg/mL Radix Glycyrrhizae decreases the viral count within the cells and in the suspension in mucosal cells and counteract viral infection [30]. According to the study by Laconi et al. (2014), triterpene glycyrrhizic acid (GRA), the main product of the *G. glabra*, has strong anti-herpes simplex virus type 1 (HSV-1) activity. When GRA was added to the epithelial cells 24 h before the viruses, it triggered cellular autophagy process by producing the autophagy activator Beclin 1 and showed antiviral effects [31]. Song et al. tested 13 new oleanane-type triterpenoid saponins, uralsaponins M–Y (1–13), and 15 known analogues (14–28) isolated from the roots of *G. uralensis* against the influenza virus A/WSN/33 (H1N1) and HIV in Madin-Darby canine kidney (MDCK) cells. Compounds 1, 7, 8, and 24 exhibited anti-H1N1 activity in MDCK cells with IC_{50} values of 48.0, 42.7, 39.6, and 49.1 μM, respectively. In addition, compounds 24 and 28 demonstrated antihuman immunodeficiency virus (HIV) activities with the half maximal inhibitory concentration (IC_{50}) values of 29.5 and 41.7μM, respectively [32]. Interestingly, pretreatment of the Vero cells with both diammonium glycyrrhizin (DG), a salt from glycyrrhizinate (GL) that is a major active component of licorice root extract, and lithium chloride (LiCl) inhibited the virus-induced cell apoptosis and revealed anti-apoptotic effects during Piscine orthoreovirus (PRV) infection [33]. Wolkerstorfer et al. demonstrated that GL treatment resulted in a clear reduction in the number of IAV-infected human lung cells as well as a reduction in the cell culture infectious dose 50% (CCID50) titer by 90%. Data showed that pretreatment and treatment diminished the viral RNA within the cells and reduced the cell supernatants and also depleted the viral hemagglutination titers during and after virus adsorption. Generally, they suggested that antiviral activity of GL is interceded by an interaction with the cell membrane which leads to limitation of endocytotic activity and virtually virus uptake reduction [34].

2.2 *Zingiber* Mill.

The genus *Zingiber*, as a member of the Zingiberaceae family, with about 85 species, is distributed in tropical to warm-temperate Asia and is best known for the ginger of commerce, *Z. officinale* (L.) Roscoe (ginger) [35, 36]. For the last 2500 years, ginger has been widely used for its various medicinal properties all around the world. Numerous studies have carefully discussed the antiviral activity of ginger. According to *The Canon of Medicine*, *Z. officinale* removes

the moistness in the head and throat [25]. We realized that many studies have investigated the antiviral effects of the genus *Zingiber*. It was suggested that *Z. officinale* showed strong anti-hepatitis C virus (HCV) infection activity [37]. Schnitzler et al. indicated that the essential oil derived from ginger shows antiviral effects on HSV-1 before adsorption. Indeed, essential oil disrupted the viral envelope via interfering with virion envelope structures [38]. In another study, 300 mg/mL fresh ginger extract inhibited HRSV plaque formation in both HEp-2 and A549 cell lines via blocking viral attachment and internalization before viral inoculation [39]. A clinical trial showed that ethanolic extracts of *Z. officinale* decreased HCV loads, the level of α-fetoprotein (AFP), and markers relevant to liver function, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in 60 volunteer patients with proven HCV [40]. In another randomized clinical trial, the effects of ginger for prevention of antiretroviral-induced nausea and vomiting (N/V) were investigated in 102 HIV-positive patients who randomly received either 500 mg ginger or placebo two times per day, 30 min before each dose of antiretroviral regimen for 14 days. Results showed ginger significantly reduces the frequency of mild, moderate, and severe episodes of nausea than the control group [41]. In an in vitro study, the ethanol extracts of *Z. officinale* showed antiviral properties through plaque reduction against influenza virus H5N1 [42]. Additionally, in another in vitro study, a mixture of honey, ginger, and garlic (HGG) extracts showed antiviral activity through inhibiting H1N2 virus growth, and it also appeared to promote proliferation of human lymphocytes. Collectively, HGG 5% can suppress influenza virus replication in a dose-response manner in human peripheral blood mononuclear cells (PBMCs) in vitro [43]. Moreover, it was demonstrated that the aqueous extract of ginger (10%) processes anti-avian influenza virus H9N2 with minimal toxicity to Vero cells [44]. Camero et al. found that ginger essential oil (GEO) shows antiviral activity in Madin-Darby bovine kidney (MDBK) cells against Caprine herpesvirus 1 (CpHV-1) as a useful homologous animal model for the study of HSV-2 infection through disruption of herpesvirus envelope [45]. According to Yang et al. study, the modified citrate-coated silver nanoparticles (cAgNPs) of curcumin (*Curcuma longa* L.) revealed antiviral activity against RSV infection in HEp-2 cells by inactivating with the virus directly prior to entry into the host cells [46]. In addition, it has been reported that essential oils isolated from ginger (100μg/mL) exhibit high antiviral activity against tobacco mosaic virus (TMV) [47]. Koch et al. observed that ginger oil prevents the adsorption of HSV-2 via viral envelope interruption through reducing plaque formation on RC-37 cells [48].

2.3 *Cinnamomum* Schaeff.

The genus *Cinnamomum* contains about 250 species and belongs to the family Lauraceae which distributes mostly in Asia and in parts of South and Central America and Australia [49]. *Cinnamomum* species are used broadly in traditional and modern medicine and in food and pharmaceutical productions because of diverse phytochemically active compounds [50]. In Avicenna's *The Canon of Medicine*, it has been detected that *C. cassia* (L.) J. Presl showed curing effects in chest infections. In addition, *C. verum* J. Presl had beneficial properties in coryza and cough and cleared chest congestion [25].

We found five studies which discussed the antiviral activity of *Cinnamomum* species. It was shown that the hot water extract of *C. cassia* effectively prevented airway epithelia from HRSV infection via inhibiting viral attachment, internalization, and syncytium formation in both HEp-2 and A549 respiratory tract cell lines [51]. The *C. cassia* bark extract and its silver nanoparticles (50, 100, and 200μg/mL) have been reported to have antiviral potential against influenza H7N3A virus in Vero cells in both pre-penetration and post-penetration infection [52]. Dai et al. observed that procyanidin, common active compound of *C. cassia*, could inhibit the IAV replication at several stages of the life cycle. Indeed, procyanidin suppressed the accumulation of microtubule-associated protein 1A/1B light

chain 3 (LC3) II, and the dot-like aggregation of enhanced green fluorescent protein (EGFP)-LC3 also inhibited the formation of the Atg5–Atg12/Atg16 heterotrimer and the dissociation of the Beclin1/bcl2 heterodimer [53]. According to Vimalanathan and Hudson, eugenol of *C. zeylanicum* Blume essential oil (EO) has anti-influenza activity in both liquid and vapor phases. They confirmed that EO of *C. zeylanicum* (3.1μL/mL) exhibited antiviral activity against influenza virus A1/Denver/1/57 (H1N1) after 30-min exposure [54]. A blend composed of equal parts of *Eucalyptus globulus* Labill. cineol (leaves) and *C. zeylanicum* cinnamaldehyde (bark), *Rosmarinus officinalis* L. cineol (aerial parts), *Daucus carota* L. carotol (seed), and *Camelina sativa* (L.) Crantz oil (seed) significantly showed antiviral activity against viral units of H1N1 and HSV-1 [55].

2.4 *Cassia* L.

The genus *Cassia* originates from Southeast Asia and belongs to the subfamily Caesalpinioideae of the Fabaceae family and comprises around 600 species. Various medicinal properties of *Cassia* species have attracted the tendency to use this plant in traditional medicine [56]. Researchers have explained carefully the antiviral activity of *Cassia* species. In *The Canon of Medicine*, Avicenna showed *C. fistula* L. revealed therapeutic effects in the swelling of the throat when gargled with the juice of *Solanum nigrum* L. It cleansed the liver and is useful in jaundice [25].

We found some evidence about *Cassia* antiviral activity in modern studies. The study by Naresh et al. detected that anthraquinone-rich *C. fistula* pod extract significantly increased the expression of human interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) antiviral protein in HT1080 cells; thus, its modulation may be beneficial in the treatment of viral diseases. Due to the broad-spectrum antiviral activity of IFIT1 by blocking different stages of viral replication, translation and assembly of new viral proteins anthraquinones are suggested as potential agonistic compounds for tempting the innate immune system to treat viral infections [57]. Interestingly for the first time, Tietjen et al. reported that 84.8μg/mL *C. sieberiana* DC. root extracts inhibit the wild-type (NL4.3) and antiretroviral (ARV)-resistant HIV-1 replication by 50%. They recognized that this extract exhibits comparable efficacies against viruses harboring major resistance mutations to licensed protease, reverse transcriptase, or integrase inhibitors [58]. In another study, Zhou et al. evaluated the anti-TMV of two new flavones, siameflavones A and B (1 and 2), together with five known flavones (3–7) isolated from the stem of *C. siamea* Lam. Based on the results, compounds 1–5 showed anti-TMV activity with inhibition rates in the range of 11.6–18.5% [59]. Additionally, it has been proven that ent-epiafzelechin-(4α→8)-epiafzelechin (EEE) extracted from the fresh leaves of *C. javanica* L. inhibits HSV-2 replication in a dose-dependent manner. Results suggested that EEE could prevent penetration of HSV-2 to the cell and also inhibit HSV-2 replication at the late stage of its life cycle [60].

2.5 *Allium* L.

The genus *Allium* belongs to the monocot family Amaryllidaceae and includes approximately 850 species. Two main species of the genus garlic (*A. sativum* L.) and onion (*A. cepa* L.) have been widely used for their nutritional and medicinal properties from ancient time [61]. Avicenna in his work confirmed that nasal drop of *A. cepa* juice cleaned the head and the eardrop removed pus and excessive moisture. Also, he reported that the decoction of *A. sativum* exhibit cure effected in chronic and pleuralgia caused by cold [25].

Recently, Meléndez-Villanueva et al. assessed virucidal activity of gold nanoparticles (AuNPs-As) of garlic extract synthesized by green chemistry against measles virus (MeV). AuNPs-As showed antiviral effects against MeV replication in Vero cells at 50% effective concentration (EC_{50}) of 8.829μg/mL via blocking viral particles [62]. The evaluation of the antiviral effects of aqueous extract of red and yellow onion

against avian influenza virus subtype H9N2 demonstrated that the red onion extract decreases the mortality of the embryos and the yellow onion extract promotes the life of the embryos, and both of the extracts diminished hemagglutination (HA) titers. Overall, both extracts especially aqueous extract of the red onion annihilated the avian influenza virus subtype H9N2 and declined virus propagation in the embryonated chicken eggs [63]. The effects of *A. sativum* extract on infectious bronchitis virus in specific pathogen free embryonic egg have also been evaluated by Shojai et al. Based on the results, garlic extract possibly contributes to the inhibition of IBV in the chickens' embryo [64].

2.6 *Mentha* L.

Mentha species belong to the family Lamiaceae and are classified into 42 species. Mint extracts and their derived essential oils are highly valued because of their activities on a broad spectrum of microorganisms tested in vitro as well as various food matrices [56]. Some literature have observed antiviral activity of *Mentha* species. Avicenna did not mention the antiviral effects of *Mentha* spp. on common cold, while he confirmed its beneficial effects in cases of hepatitis [25].

In 1998, Yamasaki et al. reported that the aqueous extract of *M.* × *piperita* L. shows anti-HIV-1 effects in MT-4 cells via inhibitory activity against HIV reverse transcriptase [65]. Lelešius et al. determined that *M.* × *piperita* and *Dracocephalum canescens* L. extracts exert considerable antiviral properties via inhibition of the viral replication and cessation of the IBV production in Vero cells [66]. Schuhmacher et al. examined the effect of *M.* × *piperita* oil on herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) in vitroon RC-37 cells. They observed the high levels of virucidal activity of peppermint oil against HSV-1 and HSV-2. Its antiviral activity has been shown in noncytotoxic concentrations of the oil and significantly reduced plaque formation by 82% and 92% for HSV-1 and HSV-2, respectively, whereas higher concentrations of oil reduced viral titers of both herpesviruses by more than 90% and inhibited virus before penetration into the vitroon RC-37 cells [67].

2.7 *Crocus* L.

There are currently 88 recognized species of the *Crocus* genus that belongs to the Iridaceae family [68]. *C. sativus* L. (saffron) has been used in traditional and modern medicine because of biologically active components like crocin and crocetin for the treatment of a variety of diseases [69]. Ibn Sina (Avicenna) stated that *C. sativus* displays expectorant effects and strengthens the respiratory organs [25].

In a recent study, Soleymani et al. tested the anti-HSV-1 and anti-HIV-1 activity of Iranian saffron extract and its major ingredients including crocin and picrocrocin for the first time. They found no evidence of antiviral effects of aqueous saffron extract against the HIV-1 and HSV-1 virions, while crocin and picrocrocin showed significant antiviral activity against HSV-1 and HIV-1. Data showed that crocin inhibited the replication of HSV before and after entry of virions into Vero cells. Crocin suppressed the penetration of HSV into the target cells significantly as well as the replication of the virus after entry into the cells. Picrocrocin also significantly inhibited the entry and replication of the virus [70].

2.8 *Ziziphus* Mill.

The genus *Ziziphus* consists of about 170 species, belongs to the Rhamnaceae family, and the center of both distribution and differentiation of the genus is in South and Southeast Asia [71]. *Z. jujuba* Mill., known as *onnâb* in Iran and "the fruits of life" in China, has been widely used for centuries for its nutritional value and pharmacological properties [72]. It was stated that *Z. jujuba* fruit is useful for the chest and lungs in *The Canon of Medicine* [25].

Despite the therapeutic effects of *Ziziphus* species, there are few literatures on antiviral activity of these species. The betulinic acid (BeA)

of *Z. jujuba* (50 μM) has been confirmed to possess anti-influenza activity via downregulating of IFN-γ level in influenza A/PR/8 virus-infected A549 cells. Also, BeA significantly reduced respiratory pathology such as increased necrosis, numbers of inflammatory cells and pulmonary edema induced by influenza A/PR/8 virus infection [73].

2.9 *Lavandula* L.

The genus *Lavandula* (Lamiaceae), with more than 39 known species, is mostly distributed in Arabia, Mediterranean Coasts, Asia, Middle East, and Northern Africa [74]. This genus, as a medicinal plant, is widely used in treating a variety of diseases since ancient times [75]. Similarly, in *The Canon of Medicine*, it is stated that *L. stoechas* L. shows anti-infection properties [25]. Minami et al. in 2003 reported that the essential oil *L. latifolia* Medik. (Portuguese lavender) possessed antiviral activity against HSV-1 infection in Vero cells before or after infection [76].

3 Antiviral Plants in Modern Medicine

Avicenna's *The Canon of Medicine* has mentioned some plants as treatment for active viral infection and the related complications associated with respiratory viral infections. There are modern scientific evidences for those plants which are discussed above. In addition to the antiviral herbs considered in Avicenna's *The Canon of Medicine*, we reviewed other documents of antiviral effects of plants in modern medicine which are detailed in Table 2.

3.1 *Thymus* L.

The genus *Thymus* belongs to the Nepetoideae subfamily of Lamiaceae family which consists of about 215 species and originated from the Mediterranean region. This genus is one of the most popular plants all over the world because of its constituents and use in folk medicine, food preservatives, and pharmaceutical preparations [77]. There are numerous evidence about antiviral activity of *Thymus* species. Schnitzler et al. reported that *T. vulgaris* L. essential oil shows antiviral activity against HSV-1 before host cell penetration [38]. Similarly, Lelešius et al. found that *T. vulgaris* extracts inhibit avian infectious bronchitis virus (IBV) production in Vero cells via suppression of the viral replication [66]. *T. vulgaris* at 3.1μL/mL inhibited the activity of influenza virus A1/Denver/1/57 (H1N1) by 100% [54]. *Thyme* EO (with IC_{50} values of 11μg/mL) reduced viral infectivity against Herpes simplex (HSV-1, DNA virus) in RC-37 cells [78]. Rezatofighi et al. evaluated the effects of *T. vulgaris* extracts against Newcastle disease virus (NDV) in embryonated eggs. They reported that *T. vulgaris* reduce the viral potency by more than 56 folds. They suggested that interfering with the cleavage of hemagglutinin-neuraminidase, the most important glycoproteins in NDV, and inhibiting virus attachment is the mechanism of action [79]. In another study, Reichling et al. investigated the antiviral activity of thyme (*T. vulgaris*) oil, ginger (*Z. officinale*) oil, chamomile (*Matricaria recutita* L.) oil, and some other herb EO against HSV-1 and HSV-2 in Vero cells. Data showed a significant reduction of plaques of 95–99% for HSV-1 and of 70–98% for HSV-2, respectively, with pretreatment of viruses with essential oils for 1 h prior to cell infection. In fact, these findings suggested that essential oils can interfere with the virus envelope by masking viral components which are required for viral adsorption or entry [80].

3.2 *Peganum* L.

The genus *Peganum* (Nitrariaceae) has six species and one subspecies. *Peganum harmala* L. distributed from the Mediterranean Sea to Central Asia [81]. Its seeds, bark, and root have been widely used in folk medicine. Based on the evidence from most of the studies, beta-carboline alkaloids such as harmalol, harmaline, and harmine are responsible for most of its pharmaco-

logical effects [82]. In a study by Moradi et al., ethyl alcohol extract of *P. harmala* L. seeds with IC_{50} value of about 9.87 (CI95%: 7.3–11.3)μg/mL had inhibitory effect against influenza A/Puerto Rico/8/34 (H1N1, PR8) virus replication in MDCK cells. The antiviral activity is most probably associated with preventing viral RNA replication and viral polymerase activity [83]. Additionally, in another study, Moradi et al. assessed anti-influenza A (H1N1) virus activity of the *P. harmala* seed (PHS) extract in MDCK cells and evaluated the anti-influenza activity of PHS extract in vivo, BALB/c mice infected with 5LD50 of mouse-adapted influenza virus (H1N1, PR8). They demonstrated that the ethanolic extract of PHS exhibited antiviral activity against influenza virus with IC_{50} value of 15.7 (CI95%:11.7—21)μg/mL in MDCK cells. Also oral administration of PHS extract (200 mg/kg/day) or oseltamivir (20 mg/kg/day) promoted the survival rate, reduced body weight loss, and lung virus titer in infected mice [84]. Similarly, Kiani et al. showed that treating cells with *P. harmala* L. seed extract 1 h after HSV-1 infection in Vero cells can significantly reduce the virus titer in the first passage and inhibit the virus production in the third passage, which determine that the extract can prevent viral gene expression in transcription or translation level [85].

3.3 *Ocimum* L.

Ocimum is the most important genus of the subfamily Nepetoideae in the family Lamiaceae with more than 160 species [86]. *Ocimum* genus is considered as a best-known medicinal herb with historical reports of its antimicrobial, immunomodulatory, antistress, anti-inflammatory, antiulcer, antidiabetic, etc. [74].

Yucharoen et al. demonstrated anti-HSV activities of dichloromethane and methanol extracts of *Ocimum sanctum* L., *O. basilicum* L., and *O. americanum* L. in African green monkey (GMK) cells at various steps of the viral multiplication cycle. Overall, based on those study results, dichloromethane and methanol extracts of *O. americanum* could inhibit both of HSV-1F and HSV-2G by 100% plaque amount [87]. Chiang et al. studied the antiviral effects of the extracts and purified components of *O. basilicum* against DNA viruses (HSV, adenoviruses (ADV), and hepatitis B virus (HBV)) and RNA viruses (coxsackievirus B1 (CVB1) and enterovirus 71 (EV71)). They recognized that crude aqueous and ethanolic extracts of *O. basilicum* and the selected purified components, namely, apigenin, linalool, and ursolic acid, showed activity against viral infections [88].

3.4 *Dracocephalum* L.

The genus *Dracocephalum* a member of Lamiaceae family has about 60 species distributed in the temperate regions of the Northern Hemisphere [89]. *D. kotschyi* Boiss., as an endemic wild-flowering herb of Iran, has a number of pharmacological properties and active constituents [90]. We found few studies about antiviral activity of *Dracocephalum* species. In one report, the extracts from *D. heterophyllum* Benth. and *D. tanguticum* Maxim. showed anti-herpes simplex virus type 2 (HSV-2) activity in Vero cells. In fact, *D. heterophyllum* and *D. tanguticum* (4 mg/mL) fight against HSV-2 infection through diminishing the HSV-2 infectivity and inhibiting HSV DNA replication in early stages of HSV-2 multiplication. Also, *D. heterophyllum* and *D. tanguticum* (1 g/kg/day) increased the mean survival times and reduced the mortality of HSV-2-infected mice [91]. In another study, *D. canescens* L. possessed significant antiviral activity against IBV prior to/during infection [66].

3.5 *Ferula* L.

The genus *Ferula*, a member of family Apiaceae (Umbelliferae), consists of 180–185 species of flowering plants distributed in Central and Southwest Asia, Far East, and north India, and the Mediterranean basin *F. foetida* (Bunge) Regel (Asafetida) originated from Afghanistan and Iran (grows wildly in the southern and central mountains of Iran) and used in traditional medicine for

the treatment of various diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion, and influenza [92, 68]. Recent studies conducted on *F. foetida* L. reported various pharmacological actions including antiviral activity [92]. Lee *et al*. observed that the methanolic extract of *F. foetida* possessed significant antiviral activity against influenza A (H1N1) [93]. Ghannadi et al. measured the antiviral effects of three sesquiterpene coumarins badrakemin acetate, kellerin, and samarcandin diastereomer from *F. assa-foetida* L. against HSV-1. Data suggested that kellerin could significantly reduce the viral titer of the HSV-1 DNA viral strain KOS at concentrations of 10, 5, and 2.5μg/mL [94].

3.6 *Foeniculum* Mill.

Foeniculum is a member of Apiaceae family which comprises of two subspecies and three varieties according to the conventional system. Having multiple pharmaceutical activities made fennel (*F. vulgare* Mill.) one of the world's most important medicinal plants [95]. In a study, the antiviral activity of the essential oils obtained from *F. vulgare* collected at fully mature and flowering stages was investigated against the DNA virus HSV-1 and the RNA virus parainfluenza type 3 (PI-3). Results showed that oils and compounds exhibit strong anti-HSV-1 effects, ranging between 0.8 and 0.025μg/mL [96].

3.7 *Prunella* L.

The genus *Prunella* (Lamiaceae), with approximately 15 species, distributed widely in the temperate regions and tropical mountains of Europe and Asia, northwestern Africa, and North America. Based on recent studies, *Prunella* possesses antiviral, antibacterial, anti-inflammatory, immunoregulatory, anti-oxidative, antitumor, antihypertensive, and hypoglycemic functions [97]. Oh et al. characterized the anti-lentiviral activities of water and ethanol extracts *P. vulgaris* L. against HIV-1 infection. They reported that aqueous extracts showed activity against HIV-1 at subμg/mL concentrations with little to no cellular cytotoxicity at concentrations more than 100-fold higher. Also, they found that aqueous extracts were effective when added during the first 5 h following initiation of infection. Indeed, inhibitory function is associated primarily with interference in early and post-virion binding events [98].

3.8 *Valeriana* L.

The genus *Valeriana* (Caprifoliaceae) comprises more than 350 species [99]. *V. officinalis* L., commonly called valerian, is a perennial flowering plant native to Europe and Asia and naturalized in North America and used both as a mild sedative and sleep-promoting aid in Western Europe [100]. *Valeriana wallichii* DC. is widely identified as a medicine for various ailments and disorders from centuries in some part of the world especially Ayurveda [101]. Ganta et al. have evaluated the anti-HCV potential of water, chloroform, and methanol extracts from the roots of *V. wallichii*, in Huh-7.5 cells infected with J6/JFH chimeric HCV strain. Methanol extract of *V. wallichii* inhibited HCV by binding with HCV nonstructural 5B (NS5B) protein [102].

3.9 *Eucalyptus* L'Hér

Eucalyptus with about 700 species is a genus in the Myrtaceae family. Many literatures determine various effects of *Eucalyptus* species such as antiviral, antitumor, antihistaminic, etc. [103]. We found just one study about antiviral effects of *Eucalyptus* species. In this study, Astani et al. reported that EO of *Eucalyptus*, rich in 1,8-cineole (88%), shows its antiviral activity against HSV-1 with IC_{50} values of 55μg/mL (RC-37 cells) via disabling free virus particles and interfering with virion envelope [78].

4 Conclusion

The outbreaks of viral infections in the last hundred years have killed millions of people and have done irreparable damage to various aspects of human life, including the economic implications. The world is currently counteracting the effects of the prevalence of new virus infections (COVID-19). Due to the rapid spread of COVID-19 and lack of definitive treatment for this disease, it seems investigation on the potential antiviral effects of medicinal herbs in the treatment COVID-19 as one of the new convenient approaches is necessary. Medicinal plants, as valuable resources, have a positive effect on inhibiting several viral infections. In the studies we reviewed, potential antiviral properties of some plant genera including *Glycyrrhiza*, *Zingiber*, *Cinnamomum*, *Cassia*, *Allium*, *Mentha*, *Crocus*, *Ziziphus*, and *Lavandula* have been widely considered in traditional and modern medicine against diverse viral infections. We found more details concerning the viral inhibitory activity of *G. glabra*, *Z. officinale*, *C. cassia*, and *M.* × *piperita*. Interestingly, it has been found that *G. glabra* possessed antiviral effects against replication of SARS-associated coronavirus in vivo. From the data in the literatures, the recommended effective doses for some of these plants in in vivo and in vitro studies were 300–500 mg/mL and 5–300μg/mL, respectively. Studies on the antiviral mechanisms have shown that induction of apoptosis by downregulation of the LANA, suppression of plaque formation, stimulation of the production of higher amounts of the autophagy activator Beclin 1, decline in viral RNA within the cells and in the cell supernatants, viral hemagglutination titers, and enhanced human IFIT1 antiviral protein expression are some of the important mechanisms of the mentioned medicinal herbs for inhibiting virus entry and also its replication. In addition, disruption of the viral envelope via interfering with virion envelope structures or dissoluting the envelope and interacting with the viral envelope through reducing plaque formation are among the main functions of medicinal plants against viral infections. Accordingly, medicinal plants reserve as important treatment options to fight against some viral infections. Hence, it is worthy to consider effective components of these antiviral herbs in in vivo, in vitro, and in clinical trials on humans to achieve new beneficial remedies to cure or reduce the harmful effects of viral infections as well as COVID-19 on human health.

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Antifungal Activity of Curcuminoids and Difluorinated Curcumin Against Clinical Isolates of *Candida* Species

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Abstract

Background: Acquired resistance to antifungals is rising particularly among *Candida* species. Herbal ingredients have biological and pharmacological activities, which make them potential fungicidal agents. The present study investigated the effects of curcumin (CUR) and difluorinated curcumin (CDF) on *Candida* species.

Material and Method: CUR and CDF were examined against *Candida* isolates obtained from patients candidemia due to *C. albicans* (n = 13), *C. dubliniensis* (n = 2), *C. parapsilosis* (n = 2), and *C. tropicalis* (n = 1); and laboratory strains of *C. albicans* (TIMML 1292 and TIMML 183), *C. krusei* (TIMML 1321), *C. parapsilosis* (TIMML 2201), and *C. tropicalis* (TIMML 731) based on the M27-A3 guideline.

Results: At the concentrations of 1–512μg/mL, none of the CDF and CUR showed a significant minimum inhibitory concentration (MIC) range against *Candida* isolates. There

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was no significant difference between the effects of CUR and CDF against *Candida* species.

Conclusion: The CUR and CDF did not exert any inhibitory effect on the growth of *Candida* strains. Any possible effect on other yeast and filamentous fungi needs to be further investigated.

Keywords

Curcumin · Difluorinated-Curcumin · *Candida* · Antifungal

1 Introduction

Nowadays, *Candida* species have become more frequent and common because of different factors such as the increase in the use of systemic antibiotics, chemotherapy, corticosteroids, etc. [1–3]. As reports show, in the United States, *Candida* species are the fourth leading cause of hospital-acquired bloodstream infections [4]. More than 90% of invasive candidiasis are caused by *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei* [1]. However, recently non-*albicans Candida* species have emerged as important opportunistic pathogens in humans [5]. The quick rise of multidrug-resistant *Candida* and the slow pace of novel antifungals development has become a serious concern [6]. Thus, various studies express more interest in natural products such as medicinal plants or essential oils, and testing for their antifungal activities [7, 8]. Recently, many studies have determined the efficiency of herbal extracts and their derivatives in treating bacterial and fungal infections [9, 10]. Often these medicinal plants have chemicals or metabolites that can be effective against human pathogens; nevertheless, their antimicrobial susceptibility should be tested on clinical isolates [7, 11]. However, there is not enough evidence about the *in vitro* activity of herbal plants against clinically significant *Candida* species. As a result, it is necessary to determine the antifungal susceptibility of these plants on common invasive *Candida* species. Curcumin (CUR) or diferuloylmethane is the main polyphenolic compound that can be found in the rhizome of Curcuma longa (turmeric) [12]. Turmeric is a well-known member of the Zingiberaceae family, which is used in South Asian traditional medicine to heal fresh wounds, and as a counterirritant for insect bites [13]. CUR has shown an acceptable safety plus numerous biological activities such as antioxidant, anti-inflammatory, antimutagenic, anti-tumor, antimicrobial, immunomodulatory, and anti-proliferative effects which can be effective against a wide variety of diseases [11, 14–24]. Owing to its relatively low bioavailability, several structural analogs of CUR have been developed. 3,4-difluorobenzylidene curcumin, or difluorinated curcumin (CDF), is one of the analogs that has been shown to have improved bioavailability and metabolic stability compared with CUR [25, 26]. Some reports show that CUR has an effective fungicidal activity against a limited number of fungi [27]. Nonetheless, there is not enough evidence about the antifungal effect of these compounds against various *Candida* species. The main focus of this study is to find out the impact of CUR and CDF against clinical isolates of *Candida* species obtained from patients with candidemia, along with *Candida* laboratory strains.

2 Materials and Methods

In this study, the antifungal effect of CUR and CDF was evaluated on 18 *Candida* clinical isolates collected from blood specimens of patients with candidemia (specialized pediatric Hospital, Mashhad, Iran), and 4 *Candida* laboratory strains. All of the clinical isolates were identified using the Vitek MS instrument (bioMérieux, Marcy-L'Etoile, France). The laboratory strains included *C. albicans* (TIMML 1292, and TIMML 183), *C. krusei* (TIMML 1321), *C. parapsilosis* (TIMML 2201), and *C. tropicalis* (TIMML 731). Moreover, the identified clinical isolates included *C. albicans* (n = 13), *C. dubliniensis* (n = 2), *C. parapsilosis* (n = 2), and *C. tropicalis* (n = 1). The

antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-A3 guidelines [28].

Curcuminoids were obtained from Sami Labs Ltd. (C3 Complex®, Bangalore, India). Synthesis of CDF was performed on the basis of a previously published method [29]. In brief, the mixture of curcumin (1 mmol) and piperidine (0.05 mmol) was added to difluorobenzaldehyde (1 mmol) in methanol. The reaction mixture was stirred for 48 h under N2 stream at room temperature. Synthesis of CDF was confirmed by the validation of its chemical structure using nuclear magnetic resonance spectroscopy.

Briefly, all isolates were sub-cultured on sabouraud dextrose agar (SDA, Sigma, Germany) and incubated at 35 °C for two days. To prepare inoculum suspensions, yeasts were dissolved in a sterile saline solution. The transmittance rate of these yeast suspensions was set to 75–77% at a wavelength of 530 nm using a spectrophotometer. Subsequently, suspensions were diluted 1:1000 in RPMI 1640 medium to reach the final concentration of 1–3 × 10^3 CFU/ml. Moreover, 3-N-morpholinepropanesulfonic acid (MOPS) (Bio basic, Canada) was used as a buffer for RPMI 1640 medium. First, all of the 96-well plates were filled with 0.1 ml of RPMI 1640 medium; then, the indicated concentrations of CUR and CDF (previously dissolved in dimethyl sulfoxide (DMSO) 1%) along with the fungal suspensions were added to them, and then incubated at 35 °C for two days. The final concentrations of CUR and CDF were 1–512 (1, 2, 4, 8, 16, 32, 64, 128, 256, and 512) μg/ml. Eventually, the minimum inhibitory concentration (MIC) ranges were evaluated visually as the lowest concentration of CUR or CDF, which inhibited at least 80% of the fungal growth, in comparison to positive control well.

3 Results

Based on the results, neither CUR nor CDF could inhibit the fungal growth compared to the control. Therefore, CUR and CDF could not exert a significant MIC range on clinical isolates and laboratory strains of *Candida*. Moreover, there was no significant difference between CUR and CDF against *Candida* species. On the other hand, *Candida* clinical isolates did not show a different susceptibility compared with laboratory strains.

Table 1 summarizes information about the efficacy of CUR and CDF as antifungal agents used in this study.

4 Discussion

The development of new resistance mechanisms against antifungal agents, especially azoles, in *Candida* species is a critical issue for public health worldwide [30]. Azole resistance among *Candida* species can happen owing to cellular changes induced by stress responses or upregulation of drug transporters [31]. Moreover, some *Candida* species such as *C. glabrata* and *C. auris* are described to be multidrug-resistant [32]. This study aimed to evaluate the antifungal activity of CUR and CDF against the clinical isolates and laboratory strains of *Candida*. In general, none of these compounds showed admissible antifungal activity against the tested isolates. Though some studies found the formulation of curcumin and its analogs can be developed against fungal pathogens like *Candida* species [27, 33]. Various studies show that some natural products can have antifungal activities. Thus, they are valuable as the potential source to develop novel antifungal agents [10, 34]. In traditional medicine, some plants or herbal extracts are described to be effective in preventing or curing infectious diseases [35]. This, mainly the aromatic compounds and secondary metabolites, as a line of defense, can act against microbial invasions [11]. Polyphenols are a great example of such products, can be found in a wide variety of edible plants [9]. Turmeric is a well-known medicinal plant that comes from the Zingiberaceae family [36]. The most active component of turmeric is a lipophilic polyphenol called curcumin [37]. Many factors, such as geographical conditions, can impact the growth and nutrition composition of turmeric. Therefore, 100 grams of turmeric powder may

Table 1 The antifungal susceptibility profiles for curcuminoids and *difluorinated curcumin* among clinical isolates and laboratory strains of *Candida*

Candida species (clinical isolates and laboratory strains)	No. (%)	Antifungal compounds (CUR/CDF)	MIC (μg/ml)	Negative control	Positive control
C. albicans	15 (65.21%)	CUR	Not achieved	–	G
		CDF	Not achieved	–	G
C. parapsilosis	3 (13.04%)	CUR	Not achieved	–	G
		CDF	Not achieved	–	G
C. dubliniensis	2 (8.69%)	CUR	Not achieved	–	G
		CDF	Not achieved	–	G
C. tropicalis	2 (8.69%)	CUR	Not achieved	–	G
		CDF	Not achieved	–	G
C. krusei	1 (4.34%)	CUR	Not achieved	–	G
		CDF	Not achieved	–	G
Candida isolates	23 (100%)				

MIC Minimal inhibitory concentration, *G* Indicates the yeast growth in positive control wells, *CUR* Curcuminoids, *CDF Difluorinated curcumin*

contain around two to five grams of curcumin [27, 38]. Some researches show that this polyphenolic substance has antioxidant, antimicrobial, and anti-inflammatory activities; therefore, it is useful against bacterial and fungal pathogens [39–42]. Nonetheless, it can decrease the adhesion and biofilm growth of some fungi and bacteria, leading to less severe symptoms in patients [43, 44]. Studies suggest that curcumin can directly affect cell wall permeability by inhibiting or activating pathways such as MAP-kinase and calcineurin-mediated signaling pathways, which play an influential role in the maintenance of cell wall integrity [45]. Moreover, some studies show that curcumin can decrease the amount of aflatoxin B1 produced by *Aspergillus flavus* too [46].

However, there are limited data about the antifungal activity of curcumin as a natural compound against human fungal pathogens. Besides, there is limited evidence about the biological and pharmacological effects of difluorinated curcumin, as an analog for curcumin, and its antifungal properties. Altogether, most of the studies on curcumin centered on the effect of this compound against *Aspergillus* and *Candida* species [9, 39]. In 2015, Zhang *et al.* conducted an *in vitro* study about the inhibitory effects of curcumin against non-*C. albicans* species, and concluded that curcumin effectively prevents the biofilm formation and hyphal extension of *Candida* spp. [47]. In another study, Tsao *et al.* evaluated the effects of curcumin combined with amphotericin B or fluconazole against *Candida* isolates, and showed that curcumin, at concentrations of 32 to 128μg/ml, can increase the antifungal potential in treating Candidiasis [48]. In 2015, Carmello *et al.* investigated the effects of photodynamic therapy mediated by curcumin, which achieved the increase of reactive oxygen species (ROS) and the DNA damage of *C. albicans*. Moreover, a study by Kumar *et al.* confirmed that curcumin can damage the cell wall of *C. albicans* [43]. In 2020, Zarrinfar *et al.* studied on the

effects of curcuminoids and difluorinated curcumin against dermatophyte isolates such as *Trichophyton tonsurans, T. interdigitale, T. mentagrophtes, Microsporum canis,* etc., and concluded that this natural compound and its analog could be effective in preventing and treating dermatophytosis [49]. Interestingly, other researchers described curcumin and its analogs as effective antifungal agents against the genera of *Alternaria*, *Aspergillus*, and *Penicillium* too. Thus, it can be helpful to analyze the effect of these analogs against clinical isolates. However, there are limited data about the possible antifungal effects of difluorinated curcumin on *Candida* species [27]. In the current study, the effect of these compounds was not significant and acceptable against the clinical isolates and laboratory strains of *Candida*. These findings contradict the results obtained by other studies, which therefore requires further investigation using different designs and tested strains to explore the possible reasons underlying discrepant findings.

5 Conclusion

The results of the present study showed that neither CUR nor CDF had any significant inhibitory effect against both clinical isolates and laboratory strains of *Candida*. Thus, further investigations are required to find out whether these compounds have any other effects on *Candida* spp. or other fungal pathogens.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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Investigation of the Effects of Difluorinated Curcumin on Glycemic Indices in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: Curcumin is an antioxidant agent that improves glycemia in animal models of diabetes. Clinically curcumin use is limited due to poor solubility, weak absorption, and low bioavailability; therefore, this study to investigate the effects of curcumin's analog, difluorinated curcumin (CDF), on fasting blood glucose (FBG), oral glucose tolerance test (OGTT), and insulin tolerance test (ITT), in streptozotocin (STZ)-induced diabetic rats was undertaken.

Methods: STZ-induced diabetes rats were randomly assigned to six groups (7 rats per group). They were treated daily by oral gavage with curcumin (200 and 100 mg/kg/day), CDF (200 and 100 mg/kg/day), and metformin (200 mg/kg/day) as a positive control group,

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for 4 weeks. Two diabetic control (DC) and normal control (NC) groups (non-diabetic rats) received normal saline and citrate buffer, respectively. FBG was measured at the beginning and end of the treatment (Day 0 and week 4) and OGTT and ITT were performed to determine glucose tolerance and insulin sensitivity.

Results: Cur100, CDF 100, and CDF200 significantly decreased FBG levels after 4 weeks oral administration by −34% (−150 mg/dL ± 70, $p = 0.02$), −36% (123 mg/dL ±67, $p < 0.04$), and − 40% (−189 mg/dL ± 91, $p = 0.03$), respectively. Glucose sensitivity by OGTT showed a significant improvement in glucose tolerance ability in all treated groups compared with DC group. ITT demonstrated that insulin response improved significantly in Cur100 and CDF 200 groups.

Conclusion: Overall, CDF improved glucose tolerance and insulin sensitivity, while reducing FBG compared to curcumin, suggesting that curcumin analogs may have therapeutic utility in diabetes.

Keywords

Diabetes · Curcumin · Difluorinated curcumin · Glucose tolerance · Insulin response · Streptozotocin

1 Introduction

Diabetes mellitus (DM), a chronic disorder with an increasing prevalence [1], is characterized by insulin deficiency and hyperglycemia, which in turn leads to microvascular and macrovascular diabetic complications [2–5]. DM is a leading cause of disability, morbidity, and mortality, which places a substantial economic burden on society [6, 7]. Hence, several therapeutic compounds and therapeutic strategies have been developed to improve overall glycemic control.

Curcumin is the major polyphenolic active compound in rhizomes of turmeric. A wide range of biological activities and therapeutic effects on diabetes, cancer, cardiovascular diseases, and other human disorders have been reported for curcumin [8–29]. Several experimental and clinical studies have confirmed the beneficial effects of curcumin supplementation on glycemia, but the effects are too modest for a therapeutic agent.

Low therapeutic potency of curcumin is due to poor water solubility, low bioavailability, and extensive first-pass intestinal and hepatic metabolism followed by rapid excretion through the gallbladder after oral intake [30]. Low bioavailability of curcumin results from the symmetric structure in which two aromatic phenolic groups are linked by two α, β-unsaturated carbonyl groups (Fig. 1). The carbonyl groups compose a diketone structure with both keto- and enol-tautomeric forms [31]. The enol form is rapidly metabolized, causing low bioavailability of curcumin [32–34]; therefore, blocking this isoform by modifying the active methylene group can improve curcumin's metabolism and bioavailability [35]. Intact, conjugated, and reduced states are the main forms of curcumin present in the body, though the latter two forms show markedly less potency than the intact compound [30, 36–38]. Following oral dosing, elimination of curcumin is via the fecal route [30, 38] and the remaining compound is conjugated during enterohepatic recirculation and intestinal absorption processes in hepatocytes and enterocytes [39, 40]. Consequently, minor free and intact curcumin can reach an effective therapeutic window in plasma after administration [41, 42].

3, 4-difluorobenzylidene curcumin [CDF] (Fig. 1) is an active curcumin analog in which instead of C-H or C-OH bonds, C-F bond with higher metabolic stability results improve the pharmacokinetic properties of curcumin through retardation of the metabolic breakdown of the compound [43, 44]. Since CDF shows similar steric conformation to curcumin, it has the same biological activities [45], with greater pharmacological potency compared to the original molecule. This study was undertaken to evaluate CDF

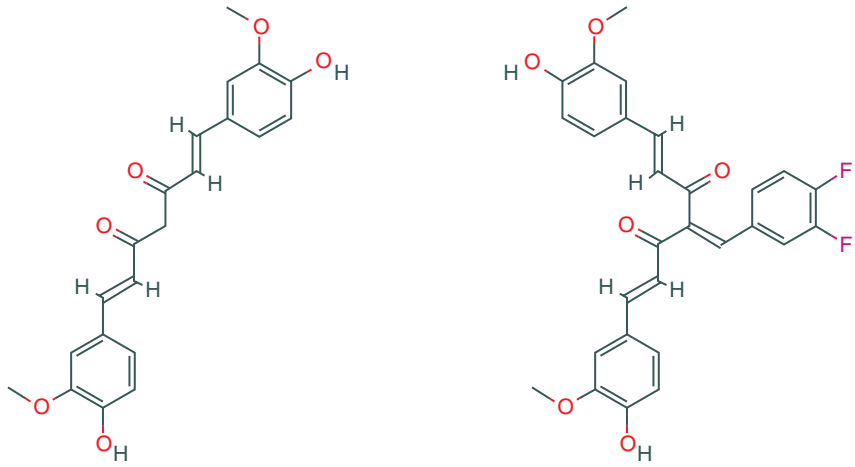

Fig. 1 Chemical structures of curcumin (left) and difluorobenzylidene curcumin (right)

effects on glucose tolerance and insulin response in diabetic rats compared with the anti-diabetic effects of curcumin.

2 Materials and Methods

2.1 Preparation of Curcumin and CDF

The synthesis of CDF was based on a previously described method [46]. Briefly, difluorobenzaldehyde (1 mmol) was added to a solution of curcumin (368 mg, 1 mmol) and piperidine (50μl, 0.05 mmol) in methanol. The reaction mixture was stirred at room temperature under nitrogen stream for 48 h. After completion of the reaction (determined using HPLC), the solvent was removed by rotary evaporator and the unreacted regent was washed with chloroform:hexane (9:1) and dried to yield CDF as yellow solid. Synthesis of CDF was confirmed using melting point, 1HNMR, 13CNMR, 19FNMR, and mass spectrometry.

2.2 Animals

A total of 35 male Wistar-Albino rats (179 ± 5.5 g) were purchased from the laboratory animal research center of medicine faculty, Mashhad University of Medical Sciences, Mashhad, Iran. All animal handling procedures were carried out in strict accordance with the Animal Welfare guidelines approved by the Institutional Ethics Committee and Research Advisory Committee of the Mashhad University of Medical Sciences. The animals were housed in an air-conditioned room at a constant temperature of 22 ± 2 °C with a 12:12 h light/dark cycle and fed a standard rodent diet and water ad libitum. At the end of the study, all animals were euthanized by intraperitoneal injection (ip.) (30 mg/kg) of thiopental sodium.

2.3 Developing Streptozotocin-Induced Diabetes in Rat

Diabetes condition was induced in the overnight fasted (12 h) rats by intraperitoneal injection of a single dose (60 mg/kg) of streptozotocin (STZ; Sigma–Aldrich) freshly dissolved in citrate-buffered saline (0.1 M, pH 4.5). On the third and seventh days after STZ injection, FBG levels were measured and rats with blood glucose levels >180 mg/dL were included in the study. Diabetic rats were randomly divided into six groups (7 rats per group). Four treatment groups, including (Cur100 and Cur200) and (CDF100 and

CDF200), received daily oral gavage of curcumin and CDF at the dosages of 100 and 200 mg/kg/day, respectively, for 4 weeks. The positive control group received metformin (200 mg/kg/day), and the diabetic control (DC) group received saline buffer by oral gavage. Non-diabetes rats (n = 7) were included as a normal control (NC) group that received citrate buffer intraperitoneally. Before (week 0) and after 4 weeks of treatment, fasting blood glucose (FBG) was measured from the tail vein.

2.4 Oral Glucose Tolerance Test (OGTT)

To assess glucose tolerance, an OGTT was conducted on overnight fasted rats gavaged with glucose at a dose of 2 g/kg after 4 weeks of treatment. Blood glucose levels were measured by a glucometer (EasyGluco, South Korea) at time point 0 min (prior to glucose load), 30, 60, 90, 120, 150, and 180 min after oral glucose load [47]. The results were analyzed as the integrated area under the curve for glucose ($AUC_{glucose}$) calculated by trapezoid rule using GraphPad Prism version 7.04.

2.5 Insulin Tolerance Test (ITT)

Insulin tolerance test was performed to determine the insulin response indicating the measure of peripheral utilization of glucose. Insulin (0.8 U/kg) was intraperitoneally (i.p.) injected into overnight fasted rats. Blood glucose was measured at 0 min (prior to insulin injection), 15, 30, 45, 75, 105, 135, and 165 min after insulin injection [48] and the results were expressed as $AUC_{glucose}$.

2.6 Statistical Analysis

Statistical analysis was performed by SPSS Statistics version 20 software and GraphPad Prism version 7.04 software. The results were analyzed using one-way ANOVA and Dunnett's post-hoc multiple comparison tests to evaluate the significance of differences between animal groups. Values were expressed as mean ± SD and lower-upper 95% confidence interval of the mean. Results with $p < 0.05$ were regarded as statistically significant.

3 Results

3.1 Fasting Blood Glucose Levels

Comparing FBG levels at pre−/post-treatments revealed that Cur100, CDF100, and CDF200 significantly decreased FBG levels after 4 weeks oral administration by −34% (−150 mg/dL ± 70, $p = 0.02$), −36% (123 mg/dL ±67, $p < 0.04$), and − 40% (−189 mg/dL ± 91, $p = 0.03$), respectively, while Cur200 decreased FBG levels but not significantly by −19% (65 mg/dL ± 91, $p = 0.2$). Metformin as the positive control decreased FBG level by −72% (−191 ± 68 mg/dL, $p = 0.01$) after 4 weeks treatment, whereas no significant changes were seen in FBG levels for the NC and DC groups (Fig. 2).

3.2 Glucose Tolerance

Following the OGTT, blood glucose levels showed significant elevation at 60 min after oral gavage of glucose (2 g/kg) in the DC rats indicating significantly impaired glucose tolerance compared to the NC rats. Glucose tolerance was significantly improved in the treated diabetic rats compared with the DC rats. In treated diabetic rats, glucose levels at 30 min started to decrease markedly reaching baseline levels at 180 min (Fig. 3a). The $AUC_{glucose}$ values over 180 min in the treated diabetic rats were significantly ($p < 0.0001$) higher than the NC rats. Analyzing AUC values demonstrated that blood glucose levels in Cur100, Cur200, CDF100, and CDF200 groups were significantly ($p < 0.001$) decreased by −23%, −10%, −17%, and −39%, respectively, compared to the DC group. As a positive control, metformin was found to decrease AUC values by −49% (Fig. 3b).

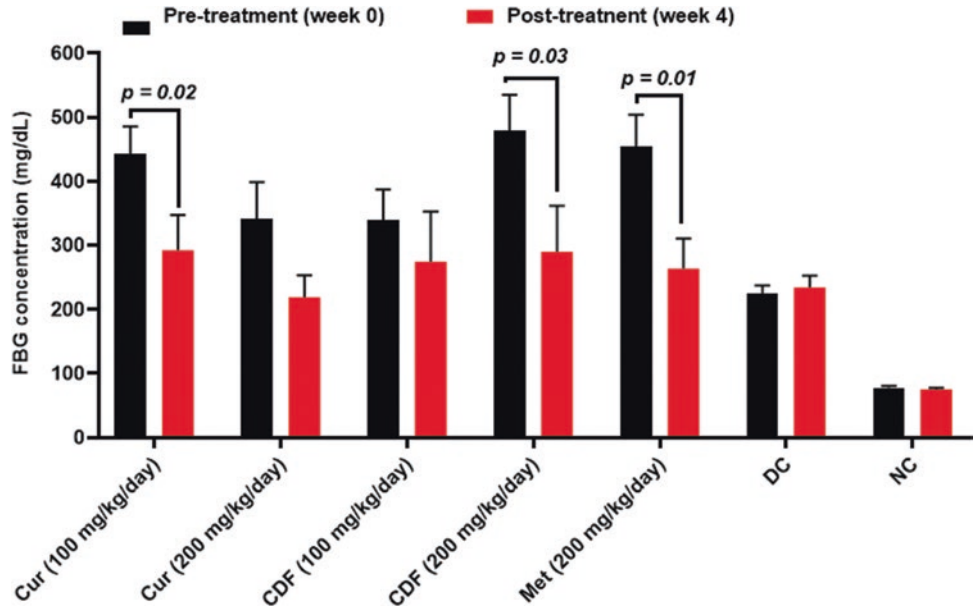

Fig. 2 Analysis of the FBG levels before (week 0) and after (week 4) treatment with curcumin and CDF in diabetic rats. Values are expressed as mean ± SD. The results were analyzed using the paired two-tailed t-test to evaluate the significance of the differences. p-values <0.05 were statistically considered significant. Curcumin; Cur, difluorinated curcumin; CDF, diabetes control; DC, fasting blood glucose; FBG, normal control; NC

3.3 Insulin Tolerance Test (ITT)

The insulin challenge (0.8 U/kg, i.p.) was performed to measure insulin response 4 days after the OGTT. The levels of blood glucose and $AUC_{glucose}$ in the DC group were significantly ($p < 0.0001$) higher at different time points after insulin administration than the NC rats. The blood glucose levels and $AUC_{glucose}$ in the treated rats were lower during ITT than the DC rats. The blood glucose levels in treated diabetic rats were not significantly higher at 75 to 165 min post insulin administration compared to the NC rats (Fig. 4a). When compared to the DC group, AUC values showed significant ($p < 0.001$) reductions by −23% and −49% in Cur100 and CDF200 groups, respectively, while non-significant reductions by −14% and −20% were found in Cur200 and CDF100 groups. A decrease of 51% was also indicated on AUC values in the metformin group compared to the DC group (Fig. 4b).

3.4 Body Weight Changes

Measuring bodyweight at pre- and post-treatment time points revealed no significant change in bodyweights in Cur, CDF, metformin, and NC groups after 4 weeks' treatment. However, bodyweight was significantly decreased in the DC group by −20% ($p < 0.01$) (Fig. 5).

4 Discussion

These data show that 4 weeks' daily oral gavage of CDF decreased the FBG level and improved glucose tolerance and insulin response compared to intact curcumin in STZ-induced diabetic rats. Biodistribution assays show that CDF is preferentially accumulated in the pancreas, and its tissue concentration reaches twofold higher than curcumin [49]. Reduction of the blood glucose levels seen in the OGTT in curcumin- and CDF-

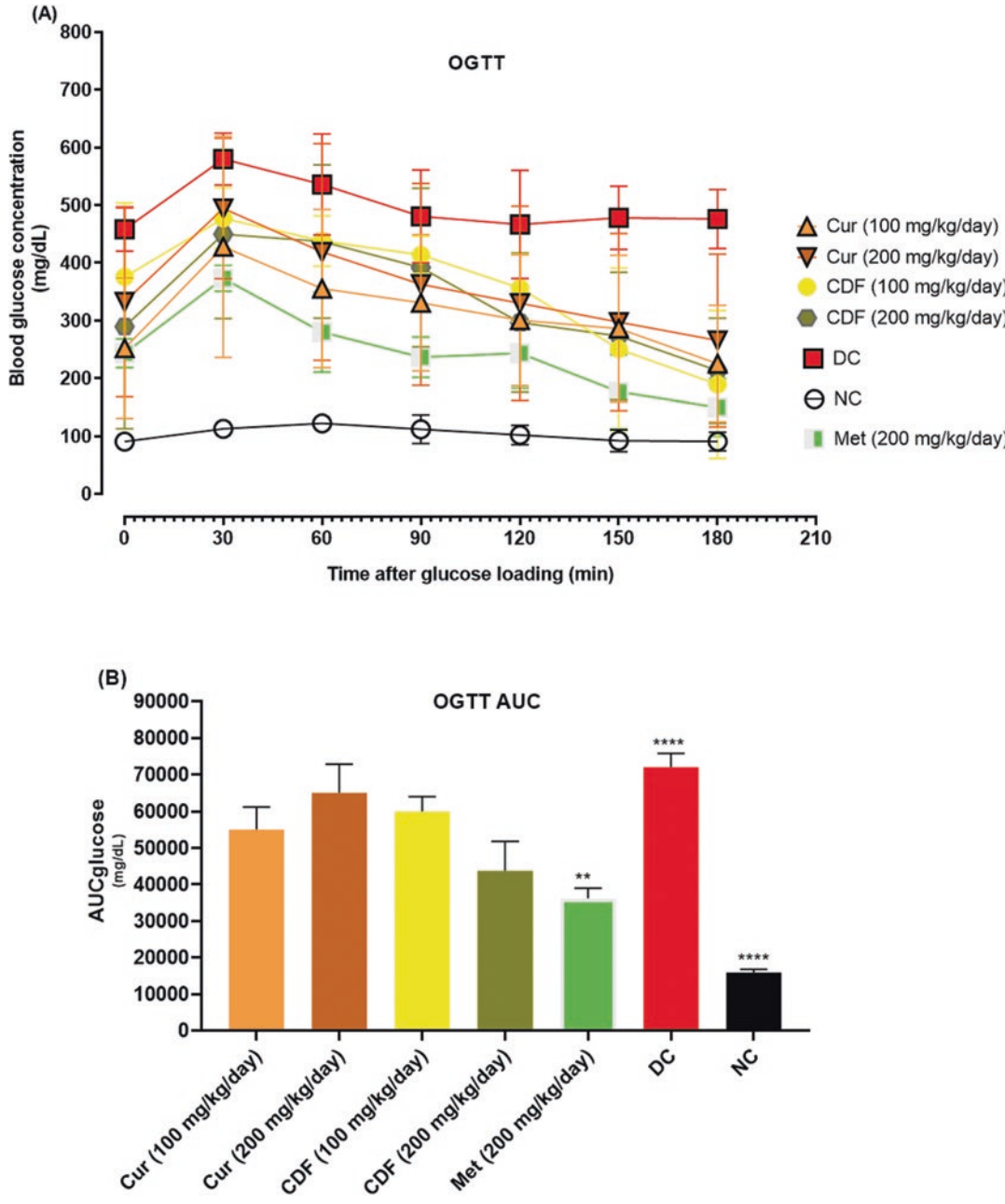

Fig. 3 Evaluating glucose tolerance via (a) oral glucose tolerance test (OGTT) and (b) analysis of corresponding areas under the glucose curve ($AUC_{glucose}$). Values are expressed as mean ± SD. The results were analyzed using one-way ANOVA, followed by Dunnett's *post-hoc* multiple comparison tests to evaluate the significance of the differences between groups. *p*-values <0.05 were statistically considered significant. **** and ** signs show $p < 0.0001$ and $p < 0.001$. Curcumin; Cur, difluorinated curcumin; CDF, diabetes control; DC, fasting blood glucose; FBG, normal control; NC

treated diabetic rats indicates increasing glucose tolerance that may result due to elevated peripheral utilization of glucose. From a mechanistic point of view, curcumin and its analogs can

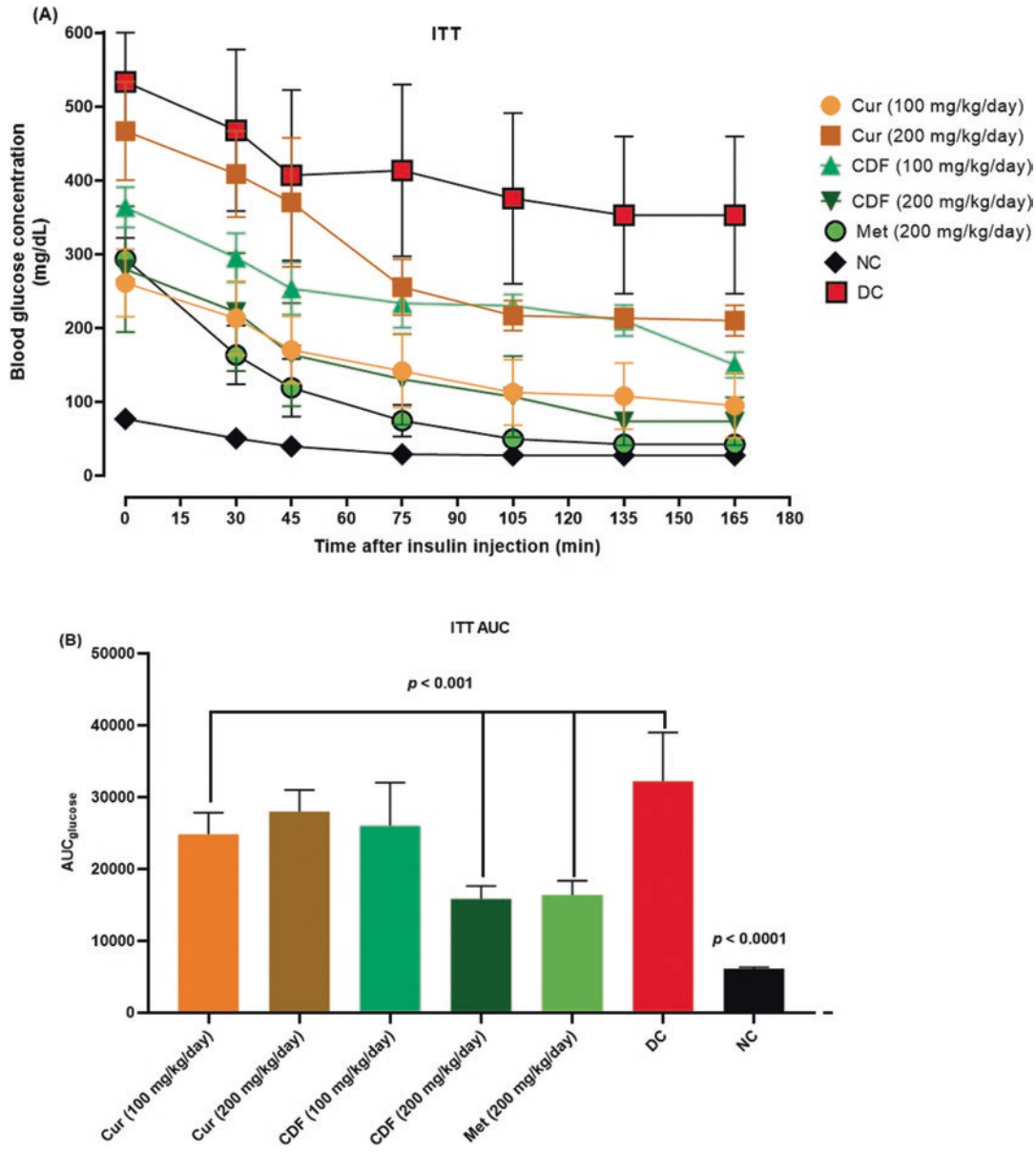

Fig. 4 Evaluating insulin response via (a) insulin tolerance test (ITT) and (b) analysis of corresponding $AUC_{glucose}$. Values are expressed as mean ± SD. The results were analyzed using one-way ANOVA, followed by Dunnett's *post-hoc* multiple comparison tests to evaluate the significance of the differences between groups. *p*-values <0.05 were statistically considered significant. Curcumin; Cur, difluorinated curcumin; CDF, diabetes control; DC, fasting blood glucose; FBG, normal control; NC

improve peripheral glucose uptake, partly, through insulin secretion by pancreatic cells via several mechanisms. It was shown that curcumin elevates recovery of damaged islets through promoting expression and activity of heat shock response proteins, Hsp70, and heme oxygenase-1 (HO-1), in pancreatic β-cells [50–52]. Curcumin can also activate the volume-regulated anion channels in β-cells associated with the depolarization of the cell membrane potential and the generation of electrical activity, whereby enhancing insulin secretion [53]. Additionally, curcumin

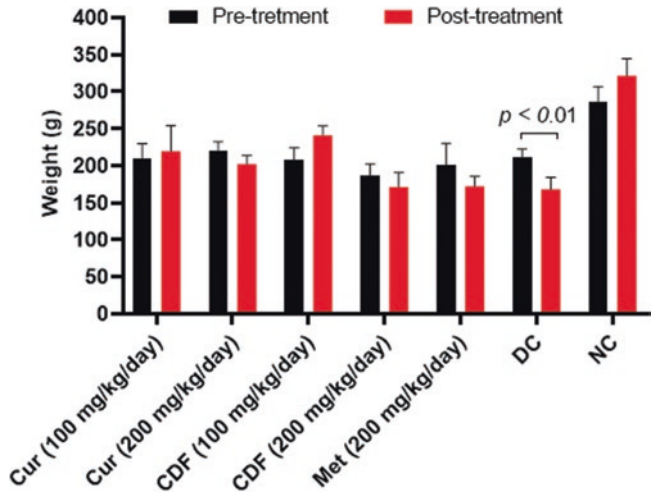

Fig. 5 Bodyweight changes. Bars show bodyweight mean of rats (n = 7) in the different study groups at pre- and post-treatment time points. Error bars show ±SD. The results were analyzed using the paired two-tailed t-test to evaluate the significance of the differences. *p*-values <0.05 were statistically considered significant. Curcumin; Cur, difluorinated curcumin; CDF, diabetes control; DC, fasting blood glucose; FBG, normal control; NC

was found to affect insulin secretion through independent pathways via increasing the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) [54, 55] to elevate peripheral glucose uptake.

Several pre-clinical and clinical trials have reported the effect of curcumin supplementation on glycemic control in diabetes [56, 57]. The anti-diabetic activity of curcumin may be due to its potent ability to repress oxidative stress [58]; however, this is the first report showing the anti-diabetic effect of CDF as a potent curcumin analog.

Curcumin is a small natural molecule that has been used over many years medicinally because of pharmacological properties, including antidiabetic, antioxidant, anti-inflammatory, anticancer, cardioprotective, and neuroprotective activities [59]. However, insolubility and low bioavailability have restricted its clinical applications and led to new formulations of curcumin including multiple analogs and derivatives [60]. CDF, a fluorinated analog of curcumin, displayed three times greater bioavailability than curcumin. Higher bioavailability of CDF has been found to correspond with higher pharmaceutical activities compared to curcumin, such as greater inhibitory effects on tumor cells, particularly those that were chemo-resistant [49]. The present study revealed that oral administration of CDF exerted greater anti-diabetic effects than intact curcumin in diabetic rat models.

The ITT challenge in curcumin- and CDF-treated diabetes rats showed that curcumin treatment could increase peripheral glucose uptake, perhaps through effects on the molecular targets that enhance insulin sensitivity. Curcumin can affect the insulin pathway by activating the insulin receptor and insulin receptor substrate-1 (IRS1) in the liver, muscle, and adipose tissue, thereby increasing insulin sensitivity and glucose uptake [61–63]. In addition, modulating the activity of glucose homeostasis-related enzymes may allow curcumin to improve insulin sensitivity: curcumin through protein kinase A (PKA) inhibition and inducing AMP-activated protein kinase (AMPK) can elevate the activity of hepatic glucokinase (GK) and glycogen content, which in turn increases insulin sensitivity and attenuates blood glucose [64–66]. Downregulation of the gluconeogenic enzymes such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) are other enzymes whose activity may be moderated by curcumin [64, 67]. Moreover, curcumin can increase glucose uptake mediated skeletal muscle cells by up-

regulating glucose transporter-4 (GLUT4) in the cell membrane [68]. Dysregulation of adipokines, such as adiponectin, leptin, resistin, and visfatin, is implicated in insulin resistance, and curcumin has been shown to modulate these cytokines [69].

Weight loss is a hallmark of poorly controlled diabetes and in the present study both curcumin and CDF did not affect bodyweight in diabetic rats over 4 weeks' treatment likely due to the reduction in hyperglycemia.

In conclusion, CDF improved glucose tolerance and insulin sensitivity, while reducing FBG compared to curcumin, suggesting that curcumin analogs may have therapeutic utility in diabetes.

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Evaluation of the Effect of Crocin on Doxorubicin-Induced Cardiotoxicity

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Abstract

Despite newer advances in cancer treatment, chemotherapy is still one of the most widely used treatment strategies in this field. However, this treatment strategy faces major challenges. Doxorubicin (Dox) is an effective chemotherapeutic agent used to treat various cancers. However, several studies have shown that the use of Dox in therapeutic concentrations is

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associated with serious side effects, such as cardiac toxicity. The use of natural products in combination with chemotherapeutic agents to reduce side effects is a novel approach, and several studies have shown promising results. In this regard, we examined the effect of Crocin on doxorubicin-induced cardiotoxicity in rat and H9c2 cell line. The in vitro model on H9C2 cells and the in vivo models on rats were treated with doxorubicin. Cell viability, DNA damage, and apoptosis were measured in H9C2 cell line in the presence and absence of Crocin. Oxidative stress and various inflammatory parameters, as well as cardiac function tests, also were assessed in doxorubicin-induced cardiotoxicity animal model in the presence and absence of Crocin. Our results showed that Crocin can significantly decrease apoptosis in H9C2 cell line through a reduction in ROS production and DNA damages. Moreover, evaluation of the effect of Crocin on doxorubicin-induced cardiotoxicity animal model showed that Crocin also can significantly reduce oxidative stress and inflammatory parameters in the serum of the animals. Assessment of cardiac function revealed that Crocin has a significant protective effect against doxorubicin-induced cardiotoxicity in the animal model. Our data indicate that Crocin significantly attenuated doxorubicin-induced cardiotoxicity. Hence, Crocin could be potentially used as an adjuvant treatment in combination with Dox to reduce cardiotoxicity.

Keywords

Doxorubicin · Cardiotoxicity · DNA damage · Oxidative stress · Inflammation

1 Introduction

Despite newer advances in cancer treatment, chemotherapy is still one of the most widely used treatment strategies in this area [1–3]. However, chemotherapy has several major challenges [4]. Doxorubicin (Dox) is a chemotherapeutic agent that is commonly used for the treatment of several cancers, and it has proven to be highly effective in the treatment of various cancers [5]. Dox inhibits cancer cell growth and division by inhibiting the enzyme topoisomerase II (topo II) [6]. Despite its high effectiveness, currently, its dose-dependent harmful side effects such as cardiotoxicity have seriously questioned the widespread clinical use of Dox [7]. Studies have also shown that in patients who take Dox as a chemotherapeutic agent, the deaths due to heart failure are around five times greater than in patients who undergo treatment with other chemotherapeutic agents [8]. Various studies have also confirmed that the main cause of doxorubicin-induced cardiotoxicity is due to increased production of free radicals that induce oxidative stress [9, 10]. There is another hypothesis that attributes the main cause of doxorubicin-induced cardiotoxicity to damages of DNA in cardiac cells [11]. However, other factors such as inflammation, apoptosis, and lipid peroxidation are also involved in Dox-associated cardiotoxicity [12].

Considering that oxidative stress is the main cause of doxorubicin-induced cardiotoxicity, it would appear that the use of antioxidant compounds in combination with Dox could reduce the level of doxorubicin-induced cardiotoxicity [13, 14]. Researchers are therefore trying to identify novel antioxidant compounds that can be used in combination with Dox to reduce the therapeutic dose and adverse side effects and to improve the safety of doxorubicin [14, 15]. Recently there has been a great deal of focus on herbal medicine and natural products. We have selected Crocin in this study as a natural antioxidant and anticancer compound to be combined with Dox [16]. Crocin is obtained from *Crocus sativus L.* (saffron), which is widely cultivated in the eastern and northeastern parts of Iran.

It has been established that Crocin as an antioxidant can defend the cells and tissues against oxidation by neutralizing free radicals [16–20]. However, there is little knowledge of the impact

of Crocin on doxorubicin-induced cardiotoxicity. To evaluate the ameliorative effect of Crocin on doxorubicin-induced cardiotoxicity, the H9C2 cell line from rat heart myoblast was cultivated and treated with doxorubicin in the presence and absence of Crocin, and cell toxicity was assessed. The effects of Crocin on doxorubicin-induced cardiac toxicity in rats were also evaluated.

2 Materials and Methods

2.1 Chemicals

Chemical reagents, including doxorubicin (Dox) hydrochloride, dichloro-dihydro-fluorescein diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide, Triton X-100, and DMSO were provided (Sigma-Aldrich, St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM), penicillin-streptomycin, and fetal bovine serum were purchased (Gibco, Germany).

2.2 Cell Culture

The cells were obtained from the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran. DMEM media with high glucose was used to culture H9C2 cells. These media supplemented with heat-inactivated FBS 10%, L-glutamine 2 mM, antibiotics (100 unit/ml of penicillin and 100μg/ml of streptomycin), and incubation at 37 °C and 5% CO_2, in a humidified atmosphere, were done. Every 2–3 days, the culture medium was changed and Trypsin-EDTA 0.05% used to cells expanded to the new flask at 80% of confluence.

2.3 Cell Viability Assay

To use a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay [21], the viability of cell was evaluated. In a flat-bottom 96-well plate, cells were cultured at 7000 per well to assess the impact of drugs after 24- and 48-h treatment. To determine the cell viability, 10μL of MTT stock solution at 12 mM concentration was added to wells, and then incubation was done for 4 h at 37 °C. In a humidified chamber, after adding 100μL of SDS-HCl solution to each well, the plate was incubated for 4–18 h at 37 °C. Before final evaluation at 570 nm by ELISA reader (StatFAX303), each well was precisely mixed using a pipette.

2.4 Measurement of Radical Oxygen Species (ROS)

To determine the levels of intracellular ROS following treatment with Dox in the presence and absence of Crocin, the cell line was seeded at 105 cells per well in a 24-well plate. After treatment with above drug insult with a time exposure of 24 and 48 h located in a dark place with the presence of 10μM H2DCF-DA for 30 min at 4 °C, the incubation of cells was performed. Based on excitation/emission at 485/530 nm, the intensity of fluorescence was measured.

2.5 Reverse Transcription-Quantitative PCR (RT-PCR)

To conduct RT-PCR, RNA extraction was performed using TRIzol (Sigma, USA). To purify total RNA, the sample was treated with DNAse (Fermentas, Germany) based on the manufacturer's instructions. RNA quality and concentration were evaluated by agarose gel electrophoresis and spectrophotometry at 260 nm, respectively. By random hexamer primers, total RNA (1μg) was reverse transcribed using reverse transcriptase enzyme (Takara). Following cDNA synthesis, real-time PCR was done. *BAX* and *BCL2* expression levels were measured by specific primers. According to GAPDH transcript as an internal control, the data were normalized. Primer3 online software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design the primers that have been shown in Table 1.

Table 1 Sequences of primers used for RT-qPCR

Bax	**Forward primer**	CCCGAGAGGTCTTCTTCCGTG
	Reverse primer	CCGGAGGAAGTCCAGTGTCC
Bcl2	**Forward primer**	CTGGTGGACAACATCGCTCT
	Reverse primer	GCATGCTGGGGCCATATAGT
Actb	**Forward primer**	TTCTTGCAGCTCCTCCGTCG
	Reverse primer	AGTCCTTCTGACCCATACCCA

2.6 Assessing DNA Damage

The alkaline SCGE (comet) assay was conducted to evaluate DNA damages following treatment with Dox in the presence and absence of Crocin in the cell line. This technique was performed according to the method described by Sadeghnia et al. with some modifications [22].

In brief, the cells were divided into three groups including one for Dox and one for Dox in combination with Crocin and control. After 24 h of incubation, trypsinization (Trypsin-0.25% EDTA) was performed to detach the cells; next, 20,000 cells were prepared to examine with single-cell gel electrophoresis. Alkaline lysis was performed per the manufacturer's instructions. The slides were electrophoresed at 0 °C in the dark for 30 min at 25 V and approximately 300 mA. Then, the slides washed three times and stained with 50μl of 20 mg/mL ethidium bromide. Finally, a fluorescence microscope (Nikon, Kyoto, Japan) at 400X magnification was applied to observe the slides. And computerized image analysis software (CASP software) was used to calculate the percentage of DNA in the comet tail (% tail DNA), which is an estimate of the DNA damage [23].

2.7 Determine the Apoptosis Condition

To determine apoptosis condition in the cell line, following treatment with Dox in the presence or absence of Crocin, the cells were lysed, and caspase 3 activity was assessed by using commercial rat ELISA kit obtained from BioVision (Cat# E4592).

2.8 Animals

Twenty-one Wistar albino male rats (weighing 230–250 g) were used in this study. Animals were housed in standard conditions in terms of humidity (45 ± 5%), temperature (24 °C), and a light/dark cycle (12 h/12 h). Standard rat diet and water ad libitum were used to feed the rats during this study.

The study protocol was approved by the Animal Care Committee of Kerman University of Medical Sciences, Iran.

2.9 Experimental Design

Twenty-one Wistar albino male rats were randomly categorized into three groups of seven rats in each group. The Dox cardiotoxicity animal model was established according to a previous study conducted by Benzer et al. [24].

1. Control group: Normal saline orally for 14 days.
2. Doxo group: A single dose of Dox (40 mg/kg) was injected intraperitoneally into the rats on the 7th day
3. Doxo + Crocin group: A single dose of Doxo (40 mg/kg) on the 7th day of treatment schedule along with daily administration of Crocin (100 mg/kg b.w./day) for 14 days. Finally, on 15th day, blood samples were obtained from the rats to evaluate various cardiac function parameters.

2.10 Measurement of Oxidative Stress and Inflammation Parameters

Commercially available colorimetric kits were applied to measure quantitatively the serum levels of MDA (Nalondi, Iran, Cat# NS-15022), TAC (Naxifer, Iran, Cat# NS-15012), TOS (Natos, Iran, Cat# NS-15016), and catalase (Nactaz, Iran, Cat# NS-15054) following the manufacturer's protocols. Tnf-α (Diaclone, Cat# 872.010.001) and Il-1β (Diaclone, Cat# 670.040.096) levels as two main inflammation factors were measured by using rat ELISA kits.

2.11 Examination of Cardiac Function Parameters

Centrifugation (3500 rpm, 5 min) was used to separate the serums. Cardiac lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activities were measured with their specific Pars Azmoon kits (Pars Azmoon Tehran, Iran, Cat# 9565516 and Cat# 29K1C9, respectively) by using an autoanalyzer.

2.12 Statistical Analysis

All statistical analyses were performed using the SPSS for version 16 software package (SPSS, IL, USA). Data were expressed as the mean ± SEM. Comparisons were performed using student's *t*-test (between two groups) and ANOVA (for ≥ 3 groups). Bonferroni correction was used for multiple comparisons. A p-value < 0.05 was considered as statistically significant.

3 Results

3.1 Determine the Optimal Dox Concentration

Concentrations ranging from 0.5, 1.0, 2.0, 3.0, and 4.0μM of Dox were used to determine the IC50 concentration of Dox in H9C2 cells. MTT assay results revealed that the IC50 concentration of Dox was about 2μM for the cell line (Fig. 1a). Hence, this concentration (2μM) was used in the study.

3.2 Determine the Effect of Crocin on Cell Viability

Effect of Crocin was evaluated in combination with Dox in H9C2 cell line. H9C2 cells were cultured in 96-well plates with 2μM Dox. Afterward, Crocin at 0, 25, 50, 75, and 100μM were added. However, data analyses have shown that adding Crocin to the cell line, that is, before treated with Dox, increases cell viability in a dose-dependent manner (Fig. 1b).

3.3 Determine the Effect of Crocin on ROS Production

The fluorometric method was used to measure the intracellular ROS levels of the cells that were treated with Dox in the presence/absence of Crocin (100μM). Significant differences were found between intracellular ROS levels in the presence/absence of Crocin in the cell lines following 24 and 48 h (Fig. 2).

3.4 Effect of Crocin on DNA Damage

As shown in Fig. 3, a significant decrease in the %tail DNA was observed in H9C2 nuclei when treated with Dox in the presence of Crocin (100μM), as compared to those of that treated with Dox alone ($P < 0.001$).

3.5 Determine the Effect of Crocin on Cell Death

To assess the effect of Dox in combination with Crocin (100μM) on cell death in H9C2 cell line, Bax and *Bcl2* mRNA expressions, as two main

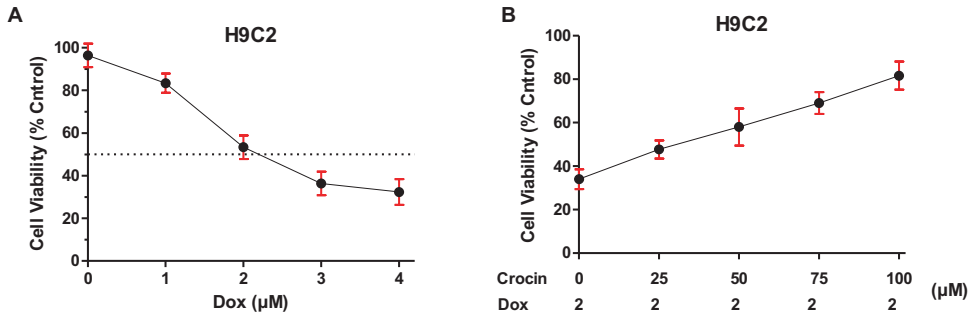

Fig. 1 Detection of optimal concentration of Dox and Crocin. (**a**) Cell survival curve of H9C2 cells was assessed when the cells are treated with Dox in different doses from 0μM to 4μM for 24 h by using MTT assay. IC50 were determined as the Dox concentrations that caused inhibition of 50% cell viability. 2μM were determined as IC50 for H9C2. (**b**) To detect optimal concentration of Crocin, the cells were treated with 2μM of Dox in the presence of different doses of Crocin (0, 25, 50, 75, and 100μM). Bars represent the means ± SD of duplicate determinations of triplicate measurements

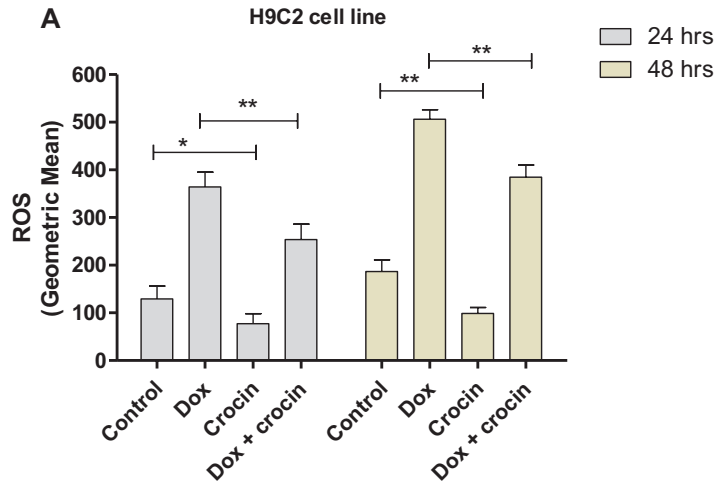

Fig. 2 Effects of Crocin on reactive oxygen species (ROS) production H9C2 cell lines at 24 and 48 h. Bar graph shows the different ROS productions in the presence and absence of Crocin (100μM) in H9C2 cell line. Bars represent the means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$

regulators of apoptosis pathway, were measured by RT-qPCR method. H9C2 cells were treated with Dox in the presence and absence of Crocin (100μM) for 24 h, and afterward, mRNA expression analysis was performed. As shown in Fig. 3, Bcl2 mRNA expression was significantly increased in the cell line when they were treated with Dox in combination with Crocin in comparison to the cells that treated with Dox alone (Fig. 4a). A nonsignificant reduction of mRNA expression of *Bax* was observed in H9C2 cells that were treated with Dox in the presence of Crocin compared to the cells that were treated with Dox alone (Fig. 4b).

Furthermore, to investigate the effect of Crocin on Dox-induced cytotoxicity H9C2 cell line, the protein activity of caspase 3 was assessed by rat ELISA kits after lysis of the cells. The results showed that caspase 3 levels significantly decreased when a combination treatment of Dox and Crocin (100μM) was performed on H9C2 cells in comparison to Dox treatment alone (Fig. 4c).

3.6 Effect of Crocin on Oxidative Stress

To determine the impact of Crocin on oxidative stresses and inflammation, the rats were treated with Dox in the presence (100μM) and absence of Crocin, and various parameters in the rat serum were assessed.

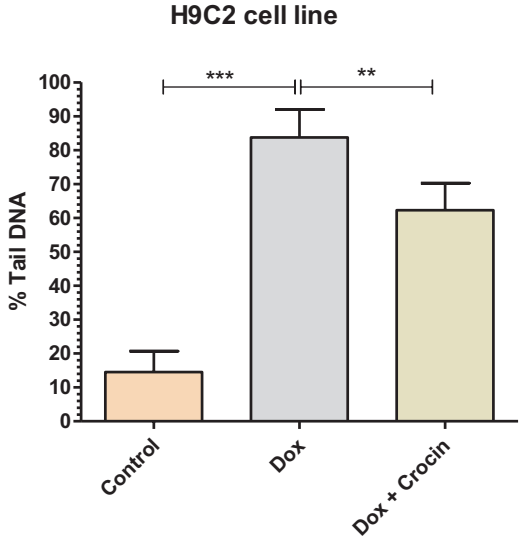

Fig. 3 Evaluation of DNA damage (%tail DNA) when the cell is treated with Dox in the presence/absence of Crocin (100μM). (%tail: percent smear of DNA in the comet tail). Bars represent the means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$

Data are shown in Table 2. These comparisons reveal that MDA levels were significantly higher in the Dox group compared to those treated with Dox in combination with Crocin group ($p < 0.01$). Likewise, both the Dox group and Dox in combination with Crocin group had significantly higher MDA levels compared to the control group ($p < 0.001$ and $p < 0.01$, respectively).

The serum TAC levels of the group that was treated with Dox in combination with Crocin were significantly higher in comparison with those that treated with Dox alone ($p < 0.01$). And control group also had significantly higher serum TAC level rather than the Dox and Dox + Crocin groups ($p < 0.001$ and $p < 0.01$, respectively). Interestingly, data confirmed that the rat treated with Crocin had significantly higher TAC serum levels in comparison to the control group ($p < 0.01$).

Furthermore, the serum TOS level was statistically lower in the Dox in combination with Crocin group in comparison to the rat group that was treated with Dox alone ($p < 0.01$). The serum TOS level was also significantly lower in the control rat group rather than the Dox and Dox + Crocin groups ($p < 0.001$ and $p < 0.01$, respectively).

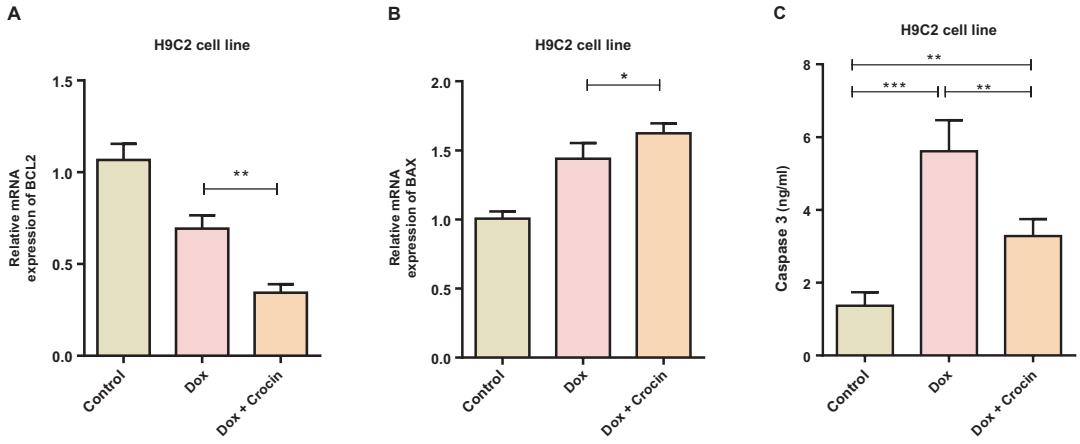

Fig. 4 BCL2 and BAX mRNA expression levels and cleaved caspase 3 protein expression in H9C2 cell lines when the cells were treated with Dox in presence and absence of Crocin. (**a**) BCL2 relative mRNA expression with and without Crocin in H9C2 cell line. (**b**) BAX relative mRNA expression levels with and without Crocin in H9C2 cell line. (**c**) Caspase 3 activity in the presence and absence of Crocin in H9C2 cell line. Bars represent the means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Table 2 Effect of Crocin treatment on oxidative stress and inflammation parameters in Dox-treated rats

	Control	Dox	Crocin	Dox + Crocin
MDA (nmol/ml)	1.43 ± 0.57	2.89 ± 0.85[c]	1.37 ± 0.87[f]	2.23 ± 0.14[c,e,i]
TAC (nmol/Fe(II))	291.86 ± 95.85	179.69 ± 98.76[c]	338.66 ± 101.01[b]	205.18 ± 86.58[e,b,i]
TOS (μmol H_2O_2 Equiv/L)	10.36 ± 2.65	16.22 ± 3.81[c]	10.44 ± 3.05	14.06 ± 3.59[e,b,h]
Tnf-α (pg/mL)	3.11 ± 0.58	6.12 ± 0.86	2.69 ± 0.57	4.48 ± 0.65[d,b,h]
IL-1β (pg/mL)	16.25 ± 1.21	44.22 ± 1.81	14.02 ± 0.85	33.14 ± 1.32[e,c,i]

Values are presented as mean ± SD
MDA malondialdehyde, *TAC* total antioxidant capacity, *TOS* total oxidant statues
Compared with the control group: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$. Compared with the Dox group: [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$. Compared with the Crocin group: [g]$p < 0.05$, [h]$p < 0.01$, [i]$p < 0.001$

3.7 Effect of Crocin on Inflammatory Parameters

The serum levels of Tnf-α and Il-1β as inflammation markers were found to be decreased significantly in the group treated with Dox in the presence of Crocin in comparison with the group treated with Dox alone ($p < 0.05$ and $p < 0.01$, respectively). However, the serum levels of Tnf-α and Il-1β also were significantly lower in the control group compared with the Dox in combination with Crocin and Dox alone groups ($p < 0.01$ and $p < 0.001$, respectively). Besides, a nonsignificant reduction in the Il-1β and Tnf-α serum levels was found in the rats treated with Crocin in comparison to the control group. Data are presented in Table 2.

3.8 Effects of Crocin on Rat Cardiac Function Tests

As shown in Fig. 5, it was observed that CK-MB and LDH activities were significantly decreased in the rat group treated with Dox in the presence of Crocin in comparison with the rat group treated with Dox alone ($P < 0.01$, in both tests). Moreover, CK-MB and LDH activities in the control group were statistically lower than the other groups.

4 Discussion

Cardiotoxicity is one of the main challenges during the clinical use of Dox, a chemotherapeutic agent [25]. Previous studies have reported that in combination with Dox, the use of a natural product with antioxidant properties can be used to minimize its side effects, such as cardiotoxicity [7, 13, 14, 24, 26, 27]. The mechanism of doxorubicin-induced cardiotoxicity is not well understood. However, several studies have shown that increasing the production of free radicals [10, 27] and DNA damages [11, 28] are the main causes of Dox cardiotoxicity. Here we examined the effect of Crocin on the in the cell line and animal model of doxorubicin-induced cardiotoxicity.

Results have shown that cell viability increases significantly in the presence of Crocin in a dose-dependent manner (Fig. 1b). This finding is consistent with the observations of past studies that reported an increase in the cell viability of Dox-treated H9C2 cells in the presence of Crocin.

To find the cause of increased cell viability of Dox-treated H9C2 cells in the presence of Crocin, ROS production, DNA damage, and apoptosis condition were assessed. Surprisingly, the production of ROS in cells treated with Crocin alone (Crocin group) was significantly lower than the control cells at 24- and 48-h posttreatment (Fig. 2). Also, data showed a statistical decrease in ROS production in the presence of Crocin in Dox-treated H9C2 cells at 24 and 48 h after treatment (Fig. 2). The present results appear to be consistent with other studies that have found that

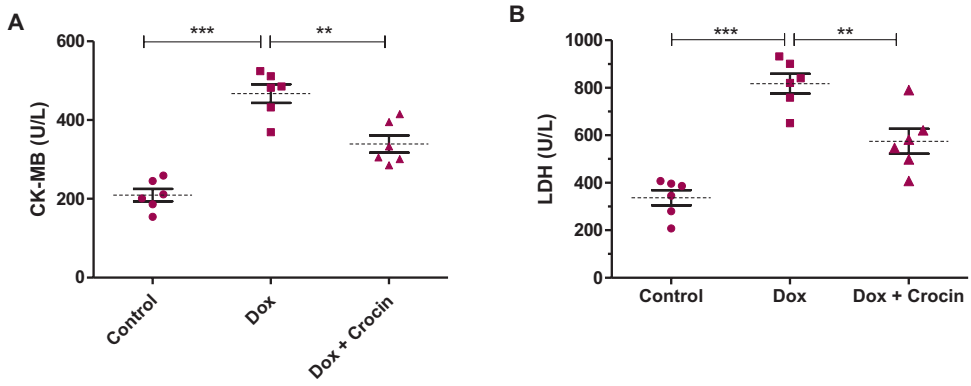

Fig. 5 Cardiac function tests were measured in rat treated with Dox in the presence and absence of Crocin. (**a**) Creatine kinase-MB (CK-MB). (**b**) Cardiac lactate dehydrogenase (LDH). Bars represent the means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$

Crocin can reduce the production of ROS [29, 30].

Results showed that the DNA damage (one of the main causes of doxorubicin-induced cardiotoxicity) increases about 69.35% (%tail DNA) when the cells are treated with Dox (2μM) in comparison to control group, while the cells treated with Dox (2μM) in combination with Crocin (100μM) the DNA damage (%tail DNA) showed 22.81% decrease in comparison to those treated only with Dox (Fig. 3). These findings support Sadeghnia et al.'s study which reported that administration of saffron (Crocin is obtained from saffron) could reduce DNA damage in the rat hippocampus [22].

Due to the reduction in DNA damage and ROS production, the results of the apoptosis study also showed that Crocin reduced the apoptosis of Dox-treated H9C2 cells (cardiomyoblast cells) (Fig. 4). However, several studies have reported the anticancer effect of Crocin and have shown that by increasing the production of ROS, Crocin induces apoptosis in cancer cells [31, 32]. This discrepancy may be due to the cancerous and normal nature of the model under study. However, these results need further investigation.

Data indicated that Dox treatment resulted in a reduction in total antioxidant capacity, while it increased the serum levels of MDA and TOS in the animal model. The results also showed that these oxidative and antioxidant criteria are effectively altered by Crocin (Table 2). Moreover, the results showed that Crocin can reduce inflammatory markers (TNF-α and Il-1β) caused by Dox in the animal model (Table 2). This finding is in agreement with several previous studies that confirmed the antioxidant and anti-inflammatory properties of Crocin [33–35].

Previous studies have shown that the release of LDH and CK-MB from heart cells into the serum results from heart injury. Therefore, measurement of the serum levels of these two markers to assess heart damage is widely accepted [36]. The results of this study have shown that the use of Crocin could significantly reduce the serum levels of LDH and CK-MB in doxorubicin-treated rats (Fig. 5). These findings have therefore confirmed the cardiac protective effects of Crocin. Likewise, other studies also have been shown the cardioprotective effects of Crocin [37, 38].

5 Conclusion

This study showed that crocin can ameliorate doxorubicin-induced cardiac toxicity through reducing the ROS production, DNA damage, and apoptosis, as well as promoting antioxidant status. And all of these factors, in addition to the

anti-inflammatory properties of crocin, result in its cardiac protective effects.

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Conflict of Interest The authors declare that there is no conflict of interest.

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The Role of Chemokines in Cardiovascular Diseases and the Therapeutic Effect of Curcumin on CXCL8 and CCL2 as Pathological Chemokines in Atherosclerosis

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Abstract

Curcumin, as a vegetative flavonoid, has a protective and therapeutic role in various adverse states such as oxidative stress and inflammation. Remedial properties of this component have been reported in the different chronic diseases including cancers (myeloma, pancreatic, breast, colorectal), vitiligo, psoriasis, neuropathic pains, inflammatory disorders (osteoarthritis, uveitis, ulcerative colitis, Alzheimer), cardiovascular conditions, and diabetes.

Cardiovascular disorders include atherosclerosis and various manifestations of athero-

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sclerosis such as stroke, and myocardial infarction (MI) is the leading cause of mortality globally. Studies have shown varying expressions of inflammatory and non-inflammatory chemokines and chemokine receptors in cardiovascular disease, which have been highlighted first in this review. The alteration in chemokines secretion and chemokine receptors has an essential role in the pathophysiology of cardiovascular disease. Chemokines as cytokines with low molecular weight (8–12 kDa) mediate white blood cell (WBC) chemotactic reactions, vascular cell migration, and proliferation that induce endothelial dysfunction, atherogenesis, and cardiac hypertrophy.

Several studies reported that curcumin could be advantageous in the attenuation of cardiovascular diseases via anti-inflammatory effects and redress of chemokine secretion and chemokine receptors. We present these studies with a focus on two chemokines: CXCL8 (IL-8) and CCL2 (chemoattractant protein 1 or MCP-1). Future research will further elucidate the precise potential of curcumin on chemokines in the adjustment of cardiovascular system activity or curcumin chemokine-based therapies.

Keywords

Curcumin · Cardiovascular disorders · CCL2 · CXCL8 · Angiotensin

1 Introduction

1.1 Cardiovascular Disease

Several studies showed that cardiovascular diseases contribute to the mortality of more than 15 million people annually [1]. Nowadays, it is well established that oxidative stress alongside inflammatory responses is a risk factor for cardiovascular disorders [2]. Inflammation and chemokines play a pivotal role in the initiation and progression of all types of cardiovascular disturbances including heart failure [3], MI and ischemia-reperfusion [4, 5], left ventricular (LV) systolic dysfunction [6], and atherosclerosis [7]. Accordingly, blood levels of inflammatory markers such as C-reactive protein (CRP) [8] and cytokine TNF-α [3] are high in patients with cardiovascular disease, and anti-inflammatory drugs have shown to attenuate cardiovascular complications [9]. On the other hand, chronic inflammatory constitutions, including diabetes, smoke [10], and virus infection [11], are risk factors for atherosclerosis and myocarditis. For example, research on the post-infarct remodeling has shown that inflammatory agents accelerated the progression of left ventricular systolic dysfunction [12, 13]. Also, it was reported that triggering the inflammatory immune responses and chemokine secretion is responsible for the worsening heart failure [14] and myocardial injury [15, 16].

2 Role of Chemokines in Cardiovascular Disease

Chronic inflammatory responses activate the responsible blood cells such as monocytes and macrophages that initiate atherosclerosis, ischemia-reperfusion eventuated myocardium injuries [4, 5]. Aggregation of macrophages is also noted in the formation of atherosclerotic plaques and the progression of atherosclerosis [10] which is the leading cause of the cardiovascular disease [17]. Activation of accumulated monocytes and macrophages in the atherosclerotic plaque produces cytokines such as inflammatory molecules [8]. The largest family of cytokines are small molecular weight molecules (8–12 kDa) known as chemokines [18]. Chemokines based on the position of the N-terminal cysteine residues are divided into four canonical subclasses, being C, CC, CXC, and CX3C [19]. Induction of cellular migration or chemotaxis is a common characteristic of all the chemokines and a group of cytokines that function like chemokines [20]. In collaborative group work, chemokines activate integrins on the endothelial cells, which leads to the capture of leukocytes at the sites of activated endothelium. On the other hand, they induce leukocyte migration to

the subendothelial site through the gradient concentration. Thus, chemokines can contribute to the development of atherosclerosis by regulating the number of circulating leukocytes. Besides, chemokines can influence monocyte survival, leukocyte activation, development of foam cells, proliferation of smooth muscle cells, cell egress from lesions, and (lymph-)angiogenesis, along with the formation of thrombosis, each with significant impact on cardiovascular disease (Fig. 1). It has been shown that CCL2 (MCP-1), CCL5 (RANTES), CX3CL1 (fractalkine), CXCR2, and CXCR3, as well as CXCL12/CXCR4 axis, have distinct functions in atherosclerosis [21, 22].

Various studies showed that CCL2 and CCL5, belonging to C-C motif ligand family, play a crucial role in the pathogenesis of cardiovascular disorders [22]. Furthermore, growth-regulated oncogene-alpha (GRO-α), CXCL1 [22]; interleukin-8 (IL-8), CXCL8 [23, 24]; and interferon-inducible protein-10 (IP-10), CXCL10 [25], which are connected to the family of CXC chemokine, possess a pivotal role in the pathogenesis of endothelial dysfunction, atherosclerosis, hypertension, stroke, and coronary heart disease via LDL accumulation in the subendothelial layer, increase of proliferation of smooth muscle cell, WBC adhesion to the endothelial layer, and promoting WBC infiltration to the subendothelial space. Endothelium with the production of vasorelaxant factors such as NO has a vital role in the dilation of blood vessels. High chemokine secretion due to the endothelial dysfunction reduces the synthesis of NO [22, 26].

Also, the regulation of chemokine secretion and chemokine receptor expression has a vital role after the development of cardiovascular disorders [27]. Several cardiovascular drugs, including calcium channel blockers, beta-blockers, and angiotensin-converting enzyme inhibitors, which are widely used these days can alter the chemokine-/receptor-related mechanisms [27]. Due to

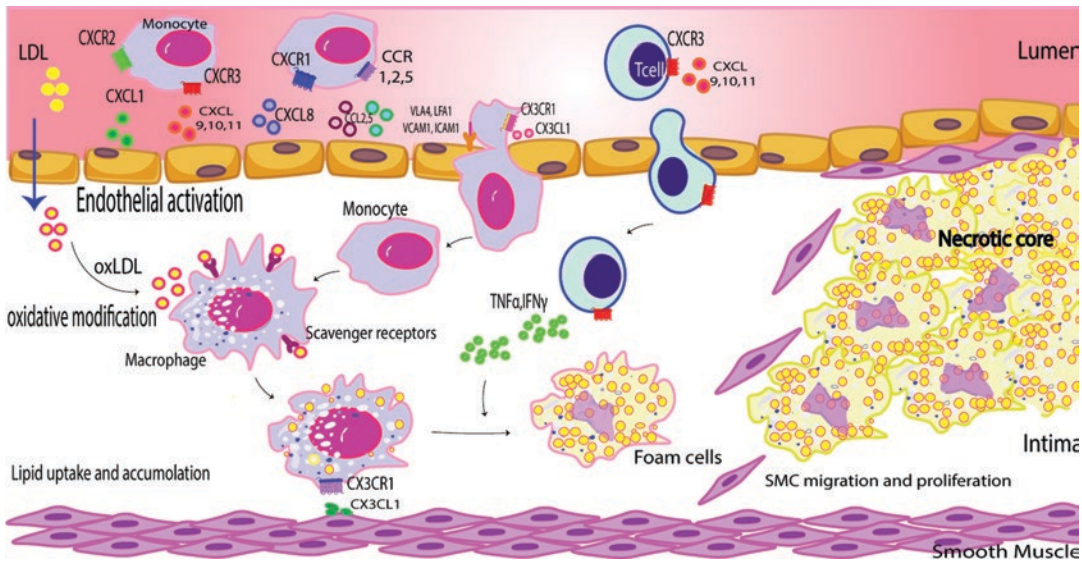

Fig. 1 The pathogenic role of chemokines in atherosclerosis. Chronic endothelial damage, such as hyperlipidemia, can stimulate monocyte adhesion to the endothelium, leading to their migration to the intima. LDL is transported to the intima due to endothelial cell damage, where it is oxidized and then subsequently taken up by macrophages to form foam cells. A component of LDL called lysophosphatidic acid stimulates endothelium that results in CXCL1 secretion, which interacts with CXCR2 to recruit monocytes to the endothelium. Also, the interaction of CCL1/CCL2 with CCR1, CCR2, and CCR5, as critical chemokine receptors for leukocyte uptake, is useful in the recruitment of monocytes, after passing through the endothelium, is finally differentiated into macrophages, and harvests modified modifiers through scavenger receptors. Chemokines also affect vascular smooth muscle cells (SMCs) and cause them to migrate to the lesion. Migration and proliferation of these cells in the lesion can be seen as a hallmark of the vascular remodeling characterizing atherosclerosis

the importance of chemokines in cardiovascular diseases, the role of these immune system mediators is discussed below.

2.1 CCL5-CCR5

The chemokine CCL5 and its primary receptor CCR5 have widely established roles in the development of atherosclerosis [28, 29]. Several studies have demonstrated the potential use of CCL5 levels as a biomarker for cardiovascular disease. Hence, the manipulation or deletion of CCR5 and CCL5 has been shown to have beneficial effects on the outcome of the disease in animal models [30, 31].

In addition to binding to specific cell surface receptors (CCR1 and CCR5), CCL5 also binds to other inflammatory soluble factors including CXCL4, CCL17, and CXCL12 that influence their activities. Laboratory studies (in vitro and mouse models) showed that binding of CCL5 to CXCL4 increases the monocyte recruitment to the activated endothelium. The interaction of CCL5 with CCL17 facilitates the formation of CCR5 and CCR4 heterodimers on dendritic cells, leading to long-term receptor expression at the cell surface and further chemotaxis of dendritic cells [32, 33].

2.2 CCL2-CCR2

The CCL2 and its receptors (CCR2) regulate the recruitment of monocytes in atherosclerosis and myocardial infarction, as well as the bone marrow egress of monocytes [29, 34]. The use of lipid nanoparticles delivering a short interfering RNA against CCR2 decreased recruitment of monocytes and atherosclerosis [35]. Elevated plasma level of CCL2 was associated with an increased risk of death in acute coronary syndrome. Recent research applying a proteomic approach identified CCL2 as an inflammatory biomarker related to severity and outcome of heart failure [36]. Recent studies have also identified circadian rhythmicity in CCL2-/CCR2-mediated chemotaxis, with chronopharmacological targeting of CCR2 reducing atherosclerosis without disrupting recruitment of microvascular leukocyte [37].

2.3 CXCR2/CXCR3

CXCL1, CXCL2, CXCL5, and CXCL8 can bind to CXCR1 and CXCR2, which are essential receptors for neutrophil recruitment. Animal experiments have identified the functions of CXCR2 and CXCR3 and their ligands in atherosclerosis so that CXCL1 is involved in the recruitment of monocytes and the accumulation of macrophages during atherosclerosis [38, 39]. The function of CXCR3 in atherosclerosis is well known and involves the recruiting of T cells, as demonstrated by the deletion of CXCL10 in mice, which resulted in a decline in the number of T cells in plaques. In contrast, the number of Treg cells increase, and this may be an explanation for the reduction in atherosclerosis in mice [40, 41].

2.4 CXCL12-CXCR4

The CXCL12 chemokine and its CXCR4 receptor play an intricate role in cardiovascular disease [42]. CXCL12 is a homeostatic chemokine produced in multiple tissues and various types of cells, which regulates the homing of stem cells (CXCR4-positive progenitor cells), and leukocytes in the bone marrow and manages their release into peripheral blood and tissues on injury or stress [43]. The cardiac protective role of the CXCL12/CXCR4 axis in myocardial ischemia can be attributed to the increased progenitor cell migration in myocardial ischemia and elevated levels of neoangiogenesis [44–46]. In atherosclerosis induced by diet, vascular protection by endothelial apoptotic bodies was related to protective signals CXCL12/CXCR4 in the endothelium and the recruitment of progenitor cells [47]. On the other hand, some studies demonstrated the dual role of the CXCL12/CXCR4 axis in cardiovascular diseases, which may be related to a subset of the disease or a specific type of affected

cells. The negative effect of CXCR4 on MI possibly correlated with the recruitment of pro-inflammatory cells into the ischemic heart [48]. The CXCL12/CXCR4 signaling caused an increased proliferation of fibroblasts and collagen synthesis in cardiac fibroblasts [49].

2.5 CXCL1-CXCR2

CXCL1, also known as growth-regulated oncogene or GRO-α, can bind to CXCR2 [24]. Stimulation of endothelial cells by lysophosphatidic acid that is located in LDL structure initiates the release of CXCL1 [50]. CXCL1 leads to the regeneration process of endothelial cells such as inhibition of neointima formation [24].

2.6 CXC3L1-CX3CR1

CXC3L1 (fractalkine) plays an important role in the pathogenesis of hypertension and atherosclerosis [21] via induction of smooth muscle cell proliferation [24], WBC adhesion to the endothelium, and migration of T lymphocytes, monocytes, and NK cells toward the peripheral tissues [51] and via restriction of cell apoptosis [52] and endothelium NO synthase activity [53]. It was reported fractalkine and elevated fractalkine receptors are associated with endothelial dysfunction through inhibition of endothelium NO synthase [53].

Vascular WBC adhesion is accompanied by cell membrane adjoin form of fractalkine, while WBC chemotaxis is the duty of this chemokine-soluble form [51]. Expression of fractalkine is present in the atherosclerotic lesions of people with diabetes and also in posttransplantation vasculopathy [54].

Apoptosis of monocytes decreased the inflammatory damage result in WBC migration. Fractalkine restricts monocyte apoptosis. Studies showed fractalkine and glomeruli endothelium receptors play a crucial role in interstitial fibrosis [52], hypertension via CXC3L1/CX3CR1, an increase of expression of TGF-α [55], and collagen type 1 [56] mediated renal fibrosis. It was reported that angiotensin II increased the numbers of fractalkine receptors such as CX3CR1 on arteries [57] by stimulation of expression of fractalkine receptors or induction of expression of activating factors such as NF-κB [53].

2.7 CXCL10-CXCR4

CXCL10 also known as interferon gamma-induced protein 10 (IP-10) is a chemokine that induces migration of smooth muscle cells and is able to increase endothelium permeability in the vascular wall [25]. Past reports showed the levels of CXCL10 [58] and CXCR4 [59] in patients with essential hypertension were higher than normotensive people. Treatment of human monocytes and TH-1 cells by inhibitors of angiotensin-converting enzyme (ACE) including perindopril and imidapril decreased the levels of CXCL10 and CCL1 [60]

3 Role of Curcumin in the Attenuation of Cardiovascular Disorders

Nowadays, there is an increase in the use of herbal products for various disorders due to fewer adverse effects and safety compared to different pharmaceutical agents [61, 62]. Several plants and natural ingredients possess protective effects on the cardiovascular system [63, 64]. Curcumin (diferuloylmethane, C21H20O6) is a yellowish polyphenolic material from curcuminoid compounds that are derived from the roots *of Curcuma longa* (turmeric) of the ginger family. Regions of Southern and East Asia are where *Curcuma longa* is mainly cultivated [65]. Potential beneficial effects of curcumin in almost all body systems, including cardiovascular, respiratory, urinary, gastrointestinal, and endocrine systems, are reported [66–71]. Numerous studies have reported that curcumin usage is useful in the management of cancer, depression, arthritis, metabolic syndrome, inflammatory bowel disease, premenstrual syndrome, and nonalcoholic fatty liver disease [66]. Oral administration of cur-

cumin is relatively safe apart from some mild adverse effects such as mild gastrointestinal intolerance [66].

There is growing evidence on the potential beneficial cardiovascular effects of curcumin in various disease states including hypertension, thrombosis, aortic aneurysm, cardiac arrhythmia, myocardial infarction, and stroke [72–76].

Furthermore, studies show that various biological properties of curcumin could be advantageous in the attenuation of cardiovascular diseases [77–79]. Part of remedial effects of curcumin on cardiovascular disease may have been due to its anti-inflammatory property and its ability to suppress chemokines [80, 81] that are discussed in the section below. Some research on the anti-inflammatory effects of curcumin on chemokines influencing cardiovascular disease is restricted to CCL2 and CXCL8 and is discussed in depth below.

4 Curcumin Effects on CCL2 in Cardiovascular Disorders

There is a relationship between CCL2 chemokine and cardiovascular disorders. Some studies demonstrated that the expression and production of CCL2 chemokine are elevated in atherosclerosis, angina pectoris, unstable angina [82], and hypertension [27]. Studies showed that curcumin downregulates CCL2 hyperproduction found in cardiovascular disorders [83–85]. CCL2 can be synthesized by the blood vessel cells, myocytes, and kidney cells in response to oxidative stress, cytokines, growth factors, hormones (endothelin-1, angiotensin), or hemodynamic stimuli (shear stress, blood flow) [82]. CCL2 connects to CCR2 receptors that are present on dendritic cells, microglia [82], and leukocytes (T cells, macrophages, monocyte), resulting in the increased migration of leukocytes to inflammation sites [86] which aggravates the progression of the disease.

Study of Mettimano showed CCR2 gene is essential in the regulation of blood pressure [87]. Accordingly, the lack of CCR2 [88] or increase of CCR2 [27] is associated with hypertension. Therefore, reducing the production of CCL2 production could potentially attenuate cardiovascular disorders.

Hypertension is one of the significant risk factors for cardiovascular disease [27]. A study showed that CCR2 and CCR7 expression in patients with hypertensive heart disease is higher than normotensive people [59]. Several animal studies showed hypertension is associated with an increase of CCL2/CCR2 expression [89]. Diet of 1% *Vigna angularis* beans that contains significant content of polyphenol reduced oxidative stress and expression of CCL2/CCR2 in the renal tissue, thereby reducing blood pressure in spontaneously hypertensive rats [90]. It was possible that since polyphenol content of curcumin is similar to that of *Vigna angularis* beans thereby could potentially reduce blood pressure.

Curcumin raised the life span of *Trypanosoma cruzi*-induced myocardial mice demonstrating the anti-inflammatory property of curcumin [91]. Infiltration of activated CCR2+ macrophages leads to tissue injury [92], whereas resident CCR2− macrophages regulate angiogenesis, cardiac regeneration [93, 94], and electrical conduction facilitation within the atrioventricular node [6, 95]. Curcumin possibly by inhibition of infiltration of CCR2+ macrophages and decreasing the inflammatory cytokines improves the life span of *Trypanosoma cruzi*-induced myocardial mice.

Curcumin not only changes the CCL2 response pathway in CCR2 + cells but also alters CCL2 production. Karimian reported that curcumin decreased CCL2 generation in different tissues by downregulating the MAPK and NF-κB signaling pathway [85]. N-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a cytoplasmic protein complex that acts as a transcription factor for the control of chemokine expression in endothelial, vascular smooth muscle cells [86]. NF-κB also controls the expression of adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells and vascular smooth muscle cells [27]. Curcumin decreased the expression of IL-1β, IL-6, and TNF-α by

repressing the signaling pathway of PI3 kinase-Akt-NF- κB in myocarditis model induced by coxsackievirus B3 [96]. Prevention of activity NF-κB is associated with a reduction in blood pressure and tachycardia in spontaneously hypertensive rats [97]. Intragastric infusion of curcumin (75 mg/kg/day) to rats prevented the expression of TNF-α, IL-12, CCL2, MIP-2, COX-2, iNOS, and COX-2 by inhibition of endotoxin-mediated activation of NF-κB in hepatic Kupffer cells [98].

Early growth response 1 plays a vital role in the pathophysiology of acute and chronic cardiovascular disease and is associated with induction of TNF-alpha and IL-6 expression. Wang et al. studied a rat model of myocardial ischemia-reperfusion injury and found that prior administration of curcumin inhibited early growth response 1 expression and reduced the infarct size [99]. Low expression of inflammatory cytokines such as IL-6 and TNF-α and hyperexpression of STAT-6 and anti-inflammatory cytokines such as IL-13 and IL-4 have been postulated as the mechanisms by which curcumin inhibited myocardial progress in cardiac myosin-induced myocarditis [100]. Another study showed curcumin increased expression of IL-10, which is an anti-inflammatory cytokine [98, 101, 102].

The migration of monocytes to endothelial cells is activated by inflammatory cytokines such as TNF-α. TNF-α stimulates NF-κB and increases CCL2 production and endothelial VCAM-1 expression [86]. It was reported that curcumin mixture, but not curcumin alone, has an inhibitory effect in the vessel-WBC adhesion. An in vitro study showed that the use of a mixture of curcumin and luteolin (1 μM + 0.5 μM) or a mixture of curcumin and resveratrol (5 μM + 5 μM) inhibited the monocyte TNF-α-induced migration process to human vascular endothelial cells (EA. hy926). Consumption of curcumin and luteolin (500 mg/kg + 500 mg/kg) combination for 2 weeks restricted TNF-α-stimulated monocyte adhesion to C57BL/6 mice aortic endothelium [27].

It was presumed, thereby raising the levels of CCL2 and VCAM-1 due to TNF-α reduction in both in vivo and in vitro studies [27]. Study of Shimizu showed curcumin (150 mg/kg) inhibited the expression of NF-κB and increased the expression of PPAR-gamma resulting in a reduction of infarct size in rats with MI resulting from left anterior descending artery occlusion [10].

Also, the inhibitory effect of curcumin on the neuroinflammation resulting from CCL2 secretion in brain tissues is noted. Saika et al. reported that intraperitoneal injection of curcumin (50 mg/kg) inhibited CCL2 expression in the ventral tegmental area of mice administered with methamphetamine [103].

Furthermore, there are reports on the relationship between the presence of hypertension and CCR2 level. In one study, on a larger group of patients with hypertension, no evidence of this association was found [104]. Animal studies have shown that CCL2 chemokine activity in the brain can have a significant impact on neurogenic hypertension. Inhibition of CCL2 expression and its receptors CCR1 and CCR2 in the brain stem unexpectedly resulted in increased blood pressure in spontaneously hypertensive rats. In animals injected with CCL5 in the vicinity of the nucleus tractus solitarius of the brain, a decrease in blood pressure was observed. Moreover, this effect was much less evident in control rats not affected by hypertension [105].

Accumulation of WBC, lipids, and cell debris in the vessel wall leads to the production of atherosclerotic lesions [17]. After sometimes, lesions grow and can occlude an artery or rupture and form thrombus [17] resulting in myocardial infarction. The size of the atherosclerotic lesions was decreased by curcumin treatment in mice with high-fat diet-induced atherosclerosis [106]. Um et al. reported that curcumin supplementation exerts anti-atherosclerotic activity in hypercholesterolemia rabbits [107]. Supplementation with curcumin was associated with a reduction in serum levels of total cholesterol, LDL, and triglycerides [84]. Curcumin and curcuminoids decrease lipid peroxidation, plasma LDL, TG, and platelet aggregation and increase plasma HDL preventing the progression of atherosclerosis [108]. Furthermore, curcuminoids possess the anti-atherosclerotic property and can alter the secretion of chemokines. Zhang et al. reported

that the use of curcumin decreased atherosclerotic size, blood cytokine level, and macrophage aggression in apoE knockout mice with high-fat diet [109]. Oxidized LDL in the inner layer of arteries resulted in a reaction of ROS with LDL cholesterol. Curcumin reduced the generation of CCL2 and ox-LDL accumulation in the inner layer of arteries and macrophage by balancing JNK and NF-kB function [110]. Foam cells are macrophages that store oxidized LDL and able to secrete ROS, matrix metalloproteinase (MMP), and TNF-α [111]. TNF-α initiates the migration of monocytes toward endothelium. TNF-α-induced migration stimulates the secretion of endothelial CCL2 and soluble adhesion molecule including VCAM-1 and ICAM-1 to recruit more monocytes [111]. The use of diet with 0.2% curcumin for 8 weeks reduced the serum lipids and CCL2, VCAM-1, ICAM-1, and MMP levels in New Zealand white rabbits fed with a high cholesterol diet [107].

CCL2 is widely expressed in the atherosclerotic lesions including vascular endothelial cells, smooth muscle cells, and monocytes/macrophages in the atherosclerotic lesions [112, 113]. Curcumin inhibited the increase of PMA-induced CCL2 expression through blockading AP-1 binding to CCL2 promoter and by inhibiting NF-kB activity in U937 cells [114].

Abnormal proliferation and synthesis of collagen and elastin in vascular smooth muscle cells contribute to the occurrence and progression of vascular remodeling [115]. The proliferation and migration of vascular smooth muscle cells to intima play a pivotal role in the pathogenesis of restenosis post-angioplasty, hypertension, and atherosclerosis [116]. There are no studies about the effect of curcumin on vascular remodeling. Still, Zhang et al. reported that curcumin inhibited the proliferation and epithelial-mesenchymal transition of human colon cancer-derived metastatic SW620 cells [117]. Wnt signaling cascade arrest, a downturn of expression of CCR4 and vimentin, and upregulation of expression of E-cadherin could be the potential explanations for suppressed proliferation and differentiation of curcumin-treated colon cancer-derived metastatic SW620 cells [117]. Curcumin prevented the proliferation and activation of lymphocyte, resulting in reduced lymphocyte productions of IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [98, 101, 102]. Inhibition of CCL2 secretion also could restrict the proliferation. According to the study by Yao et al. CCL2 mediate the angiotensin II-induced proliferation of rat aortic smooth muscle cells. Yao et al. demonstrated that angiotensin II stimulates the expression and secretion of CCL2 in rat aortic smooth muscle cells via intracellular ERK and JNK signaling molecules. CCL2 contributed to the angiotensin II-induced cell proliferation by CCR2.

Obesity is a low-grade metabolic inflammation and is a risk factor for many common chronic diseases including heart disease, stroke, hypertension, and diabetes mellitus. Activation of macrophages within the adipose tissue leads to the production of pro-inflammatory mediators that are responsible for obesity-related cardiovascular disorders [118]. There are studies about the potential role of curcumin on the prevention of chronic conditions due to its anti-inflammatory and antioxidants effects on adipose tissue.

Curcumin reduced the migration of macrophages induced by inflammatory mediators secreted by adipocytes. One in vitro study showed that curcumin inhibited TNF-a, NO, and CCL2 secretion from adipose cells which were isolated from obese mice mesenteric adipose tissue and were treated with mouse Raw 264.7 macrophages [119]. Obesity-induced inflammatory responses were suppressed by curcumin via downregulation of DNA-binding and transcriptional activities of NF-kB, AP-1, down the production of antioxidants, MAPK [120], TNF-a, IL-1b, and IL-6 [121].

Cell deaths were observed in all of the animal models, including syngeneic heart transplantation, myocardial infarction, reperfused myocardial infarction, and diphtheria toxin cardiomyocyte ablation [122]. In an MI animal model, tissue failure and collateral tissue damage were observed due to cardiomyocyte death and then infiltration and shift of CCR2+ monocytes [123]. This mobilization and recruitment of monocytes were stimulated by CCL2 release

from CCR2+ macrophages [122]. Infiltration of mononuclear cells in the myocardial tissue by CCL2-dependent of IL-17 is essential in the pathogenesis of viral myocarditis [124]. IL-17 upregulates CCL2 by activation of TRAF6, p38MAPK, and c-Jun/AP-1 pathways [124]. According to the ability of curcumin in interference of signaling molecule of AP-1 [120], we could conclude that curcumin may improve viral myocarditis by reduction of IL-17-induced CCL2 generation and consequently inhibition of CCL2-induced migration of monocytes via blocking c-Jun/AP-1 signaling cascade.

Angiotensin II can stimulate secretion of inflammatory markers like CCL2 of vascular cells [125]. Angiotensin II, along with hypertrophy-induced left ventricular dysfunction and remodeling, leads to the development of heart failure [75]. Curcumin inhibited the activity of p300 histone acetyltransferase as a regulator of angiotensin-induced transcriptional factors in rats with moderate-sized myocardial infarction after left coronary artery ligation [75].

5 The Effect of Curcumin on CXCL8 in Cardiovascular Disorders

Alteration in tissue CXCL8 content has a vital role in the pathogenesis of hypertension [126] and atherosclerosis [127]. One of the results of CXCL-8 effect on endothelial cells is the generation of endothelial products such as plasminogen activation inhibitor 1 (PAI-1) and ET-1 [128] that disrupt the homeostasis of endothelium causing endothelial dysfunction. Disruption of homeostasis of endothelium is the result of disequilibrium between the production of vasoconstrictors and vasodilators. CL8 increases the risk to coronary heart disease due to hypertension resulting in a rise of cell division and reduction in cell apoptosis [126]. It also stimulates the migration of leukocytes into the subendothelial vascular wall [127]. Increase in endothelial cell proliferation and decrease of apoptotic events have been demonstrated in multiple hypertension models [126]. CXCL8 induced 12-lipoxygenase production in porcine smooth muscle cells and in this way has a positive effect on blood pressure [129].

CXCL8 has an attraction to CXCR1 and CXCR2 receptors. Activation of these receptors that are located on endothelial cells is associated with stimulation of signaling pathways of Rho and Rac.

Atherosclerosis is a risk factor for the progression of various disorders, including hypertension, type 2 diabetes, and dyslipidemia that could result in coronary conditions [83, 130]. Alteration in tissue CXCL8 content has an essential role in the pathogenesis of hypertension [126]. Several animal studies have shown that hypertension is associated with an increased expression of chemokines such as CCL2, CCL7, CXCL8, and CCL12 [89].

CXCL8 and CCL2 are recognized as inflammatory markers [131] that are elevated in the blood of many patients with diabetes. They are responsible for the development of vascular disease with an increase of insulin resistance, glycosylation of enzymes, and inflammatory response [132]. Jain et al. reported oral gavage of curcumin (100 mg/kg) for 7 weeks decreased the blood levels of TNF-α, CXCL8, IL-6, CCL2, and hyperglycemia in streptozotocin-treated diabetic rats [133]. In vitro part of this study also showed concentrations of 0.01–1 μM of curcumin were able to decrease TNF-α, IL-6, CCL2 secretion, and lipid peroxidation in U937 monocytes exposed to high glucose levels (35 mM) [133]. Suppression of COX-2, iNOS, and p38 MAPK/JNK signaling pathway is part of the protective mechanism of curcumin in acute colonic inflammation [102].

There are several studies demonstrating the relationship between oxidative stress and chemokines in the initiation and progression of cardiovascular disorders [134]. Reactive oxygen species (ROS) induces instability and rupture of atherosclerotic plaques [135]. Stimulation of ROS has been observed in vitro after chemokine stimulation [136]. Cross-sectional and longitudinal studies showed that an increase in serum levels of CXCL8 and CCL2 in unstable angina is significantly correlated with decreased plasma levels of antioxidants and increased lipid peroxidation [86].

Namdari et al. reported that treatment with curcumin-loaded magnetic hydrogel nanocomposite increased the levels of GPX and SOD that are antioxidant enzymes and reduced the elevated level of MDA as a product of peroxidation of lipids, in cardiac tissue of rats induced with heart failure using doxorubicin [137]. Similarly, Swamy et al. also concluded that curcumin increased the levels of GSH, SOD, and CAT and decreased levels of MDA in cardiac tissue of doxorubicin-induced myocardial toxicity [138]. Consequently, usage of curcumin reduced the chemokine production and improved oxidation status in the cardiac tissue. Furthermore, administration of curcumin resulted in a reduction of chemokine secretion by oxidative stress [82] in cardiomyocyte.

There were no changes in TNF-α, interleukins, lymphotoxins, oligosaccharides, LDL, ROS, CCL2, and CXCL8 after administration of resveratrol (200 mg) in combination with curcumin (100 mg) in subjects with abdominal obesity after consumption of a high-fat meal [139]. Result of randomized crossover trial showed that administration of 1 g/day curcumin for 4 weeks in obese individuals reduced IL-1α, IL-1β, IL-4, IL-6, VEGF, and TNFα serum levels, but there were no significant changes in IL-2, IL-6, CXCL8, IL-10, IFN-γ, EGF, and CCL2 levels [83].

6 Conclusion

There is an extensive body of evidence to support the use of chemokine-targeted therapy in the treatment of cardiovascular diseases. CXCL8 and CCL2 are inflammatory factors, known to play an essential role in the development of atherosclerosis. Increased concentrations of CXCL8 and CCL2 have been confirmed in cardiovascular patients. Various studies have shown that curcumin can play an important role in reducing the blood concentration of these chemokines. In conclusion, a significant amount of evidence can be found supporting the therapeutic potential of specific chemokine/chemokine receptor blockade. In vivo use of curcumin may be beneficial for patients with cardiovascular diseases who are affected by the enhanced production of various proinflammatory cytokines.

Competing Interests The authors declare that there are no competing interests regarding the publication of this paper.

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Health Benefits of Turmeric and Curcumin Against Food Contaminants

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Abstract

Food contaminants are one of the most important and concerning issues worldwide. Protecting the public from the harm of contaminated foods has become a daunting task. On the other hand, the elimination of these contaminants from food seems impossible. Therefore, one of the best solutions is to recommend inexpensive and publicly available food additives like many spices used in food as flavoring and coloring. *Curcuma longa* or turmeric is one of the well-known spice, which confers many medicinal properties. Curcumin is the main active ingredient in turmeric, which has many health benefits. Recent research has revealed that turmeric/curcumin has protective effects against toxicants, mostly natural and chemical toxins. In this review article, we reviewed studies related to the protective effects of turmeric and its active ingredient against food contaminants.

Keywords

Curcumin · Food contaminant · Toxicity

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1 Introduction

Food contaminants are among the most important causes of various diseases worldwide and have many serious consequences for human health [1]. The symptoms of the foodborne illness due to contaminations range from mild gastroenteritis to chronic complicated and fatal cases or different types of cancers [2]. These contaminants may be potentially harmful or enter the body at higher doses than the standard dose. There are several sources of contamination from the field to the plate. Contaminations may occur through the soil, water, collection, storage, pack-

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aging, disinfectants, personal care products, and so on [3]. Environmental pollution due to industry development in recent years makes food contamination a severe health problem worldwide. For example, consuming contaminated food with heavy metals and different types of metals cause many complications [4]. In 2013, the US Centre for Disease Control and Prevention reported more than 11,000 foodborne infections, with several contaminant sources including metals and chemicals [5]. In 2010 in Nigeria, about 400 to 500 children died of acute lead poisoning due to the consumption of food contaminated with lead-contained soil and dust [6]. Furthermore, between 2009 and 2010 in the United States, 1527 prevalence of foodborne diseases resulted in 29,444 sickness cases and 23 deaths [5]. Considering such incidents and general health consequences in the past, complete elimination of these contaminants seems impossible despite efforts and monitoring. Therefore, it seems necessary to find a way to fight these contaminants or reduce their effects and complications.

Curcuma longa, which is known as turmeric, is a herb, which belongs to the Zingiberaceae (ginger) family [7, 8, 9]. Turmeric has beneficial effects for many therapeutic applications such as bacterial and fungal infections, neurological disorders like Alzheimer's disease and depression, cardiovascular disorders, hepatic damages, hyperlipidemia, diabetes, and inflammatory states such as arthritis [7, 10–15]. Many of these properties of turmeric are attributed to curcumin. Curcumin, a polyphenolic compound derived from the dried rhizomes of *Curcuma longa* is known for its pharmacological properties like neuroprotective, pulmonoprotective, chemopreventive, hepatoprotective, wound healing, anti-ischemic, immunomodulatory, and anti-inflammatory activities [16–25]. Furthermore, turmeric and its active ingredient, curcumin, has an antidotal effect against natural and chemical toxins [26]. Two similar aromatic rings where O-methoxy phenolic groups are located and linked to α, β-unsaturated β-diketone moiety constitutes the molecular structure of curcumin. Curcumin is lipophilic so it can easily cross the molecular membrane of cells [27]. Curcumin is an electron donor due to the presence of conjugated double bonds in redox reactions. It is a powerful antioxidant, which significantly reduces lipid peroxidation, regulates antioxidant enzymes, and scavenges reactive oxygen species. Many therapeutic uses of curcumin are due to its anti-inflammatory and antioxidant effects [28]. In the present review, we discuss the potential impact of turmeric/curcumin as a food additive against food contaminants. For this purpose, we studied three essential groups of food contaminants, and the beneficial effects of turmeric/curcumin (Fig. 1).

2 Heavy Metals

Heavy metals are generally referred to those metallic elements with relatively high densities and atomic weights [29, 30]. Some of these metals such as copper [Cu], iron [Fe], magnesium [Mg], manganese [Mn], selenium [Se], and zinc [Zn] are considered as essential nutrients which play a significant role in several biochemical and physiological functions in human body [29, 31]. Both the insufficient supply of these micronutrients and their excess amounts can lead to various adverse health effects and deficiency diseases [29]. Among heavy metals lead [Pb], arsenic [As], chromium [Cr], cadmium [Cd], and mercury [Hg] constitute inorganic chemical hazards to humans and the ecosystem. They have also been identified as priority contaminants due to their persistence and irreversible toxic properties. Nowadays, heavy metal contamination is the main threat to human health and is of great public health significance. The possible contamination sources include direct ingestion or contact with contaminated soil. The food chain and drinking of contaminated groundwater effects of these metals on human health have been extensively reviewed. All of them are systemic toxins and induce multiple organ damage, even at low contact levels. Moreover, these metals or their related compounds have revealed heavy metal-induced toxicity and carcinogenicity [29–33].

Heavy metal toxicity causes several adverse health problems in humans and animals and is

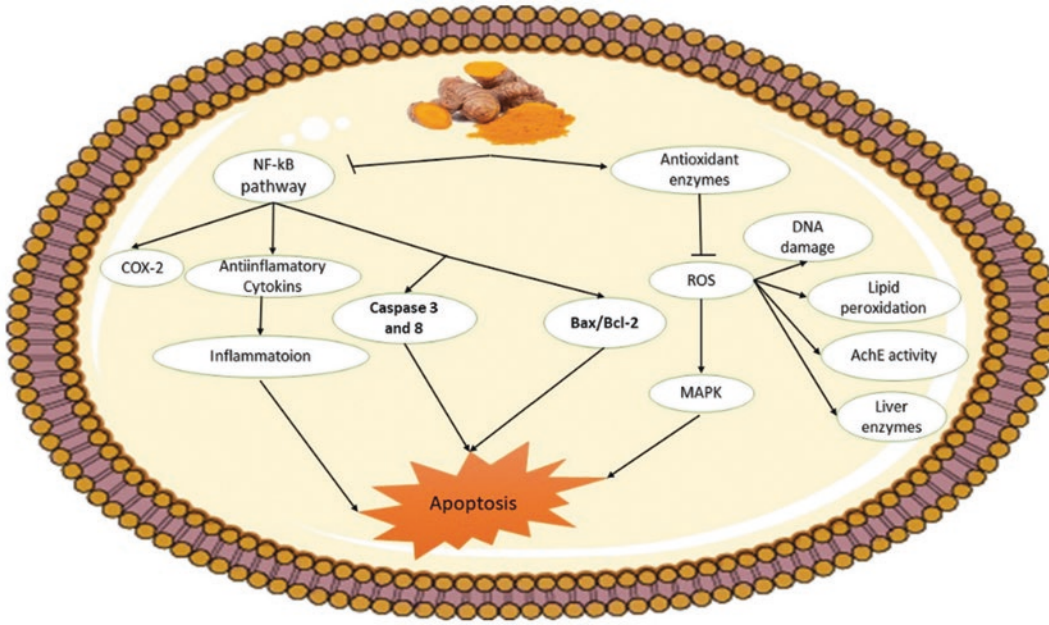

Fig. 1 Curcumin showed strong protective effects against several natural and chemical food toxicants, which attributed to its antioxidative, anti-inflammatory, and anti-apoptotic properties

still a significant health concern today [34]. Chelation has been considered one of the most usual treatment strategies for metal poisoning to stimulate metal excretion [31, 34].

Dietary strategies have several safety and efficacy issues in alleviating Pb and Cd toxicity [34]. Vitamins, edible plants, phytochemicals, and probiotics revealed protective effects against Pb and Cd toxicity and very few side effects [34]. At this time, medicinal plants have been considered as the possible treatments for the heavy metals poisoning in the scientific communities. Recent studies demonstrated that some medicinal herbs such as *Curcuma longa* (turmeric), *Allium sativum* (garlic), *Ginkgo biloba* (ginkgo), and *Coriandrum sativum* (cilantro) have potential to treat heavy metal poisoning [31].

Some edible plants like turmeric have ameliorative effects against oxidative stress induced by heavy metal toxicity [35]. It is believed that the preventive effect of curcumin on heavy metals-induced toxicity is related to its free-radical-scavenging and metal-binding properties [30].

Curcumin is a polyphenol compound which isolated from the rhizome of this herb [7, 30, 36]. It has several anti-inflammatory, antioxidant, and anticancer properties [7, 30, 35, 36]. Curcumin has a detoxifying effect on heavy metal poisoning [31]. Curcumin has significant protective effects against lipid peroxidation induced by lead and cadmium and through chelation of both these toxic metals and can reduce their neurotoxicity and tissue damage [37]. In addition, curcumin treatment can diminish arsenic-induced cholinergic dysfunctions and neurotoxicity induced by copper and some drugs like cisplatin and bupivacaine [38]. In some animal models, curcumin showed different aspects of nephron-protective effect in preventing drug-induced nephrotoxicity [39] (Table 1).

3 Cadmium (Cd)

Cadmium (Cd) is a toxic metal with the extended environmental and occupational distribution. Using rechargeable nickel-cadmium batteries and cigarette smoking are the primary sources of cadmium poisoning in the general population. Moreover, in non-smokers, the consumption of

Table 1 Protective effects of turmeric/curcumin against heavy metal toxins in vivo and in vitro studies

Substance	Model	Dosage and duration	Key effects	Ref
Heavy metals				
Cadmium				
Cell culture studies				
Curcumin	The human bronchial epithelial (HBE)	10–30 μM for 24 h	↓secretion of IL-6 and IL-8	[45]
Animal studies				
Curcumin	Rats/oral	50 mg/kg, for 7 days	↓ BUN, ↓ Cr, Improved renal histopathology	[41]
Curcumin	Rats	250 mg/kg, for 7 days	↓ BUN, ↓ Cr, normalized tissue SOD and CAT	[43]
Curcumin	Rats/oral	250 mg/kg, for 5 days	↓ MDA, ↑GSH Improvement of Cd-induced morphological changes	[44]
Curcumin	Albino mice/oral	100 mg/kg, for 15 and 45 days	↓MDA, ↑ SOD, CAT and GSH	[46]
C. longa	Rats/oral	200 mg/kg, for 6 weeks	↓HSC activity, ↓liver fibrosis, ↓ liver enzymes	[47]
Curcumin + Vitamin C	Rats/oral	200–400 mg/kg + 100 mg/kg, for 27 days	↑GSH, scavenging of free radicals	[48]
Curcumin	Rats/oral	250 mg/kg for 7 days	↓ALT, ↓AST, ↑SOD, ↑CAT	[49]
Curcumin	Mice/oral	300 mg/kg for 2 weeks	Improved social behavior, ↑AChE, ↑testosterone and progesterone	[50]
Curcumin	Mice/oral	300 mg/kg for 2 weeks	↑serotonin, ↑dopamine, ↑GSH Improved anxiety, neuromuscular, and cognitive problems	[51]
Curcumin	Rats/oral	100 mg/kg for 3 days	Partially reversed oxidative stress, edema, necrosis, and spermatological damage induced by Cd-chloride	[52]
Arsenic				
Cell culture studies				
Curcumin	Neural stem/progenitor cells (NSPCs) derived from hADSCs	Different concentrations of curcumin for 24 h	↓As-induced apoptosis, ↓As-associated ROS generation	[64]
Animal studies				
Curcumin	Mice/oral	200 mg/kg for 6 weeks	↓ALT, ↓AST, ↑As-methylation, accelerate As excretion	[53]
Curcumin	Rats/oral	15 mg/kg	↓ BS, urea, cr, total lipid, chol, TG, LDL-c, and transaminases	[58]
Tetrahydrocurcumin (THC)	Rats/oral	80 mg kg/day for 28 days	↓ALT, ↓AST, ALP, bilirubin, improved serum lipid profile, ↑GSH, SOD, CAT	[59]
Curcumin	Rats/oral	100 mg/kg for 14 days	↓BUN, ↓Cr, ↑GSH, SOD, CAT, Ameliorated brain and kidney histopathological injuries	[61]

(continued)

Table 1 (continued)

Substance	Model	Dosage and duration	Key effects	Ref
Curcumin	Rats/oral	100 mg/kg for 28 days	Increased memory performance and learning and memory ↑AChE in frontal cortex and hippocampus	[62]
Curcumin	Rats/oral	100 mg/kg for 28 days	Protection of cholinergic deficits, modulating expression of pro-, anti-apoptotic proteins in brain	[63]
Chromium				
Animal studies				
Curcumin	Rats/oral	400 mg/kg for 10 days	Inhibit the increase of AST, ALT, and LDH, Ameliorated liver histological and oxidative damage	[65]
Curcumin	Mice/oral	5 and 7.5 mg/kg for 5 weeks	↓ Sperm head abnormalities	[66]
Curcumin	Rats/oral	400 mg/kg for 10 days	↑ renal CAT and SOD, ↓ oxidant stress	[67]
Lead				
Animal studies				
Curcumin	Rats/oral	100 mg/kg for 45 days	↓ LPO, ↑GSH, SOD and CAT	[68]
C. longa	Rats/oral	500 mg/kg for 28 days	↓ ALT, AST, ALP, LPO, ↑GSH	[69]
Nanocurcumin	Mice/oral	15 mg/kg for 2 weeks	↓ ROS, chelating of Pb from blood and soft tissues, ↑ antioxidant enzymes	[72]
Curcumin	Rats/oral	200 mg/kg for 4 weeks	↓AST, ALT, BUN and Cr, ↑Alb, normalized immunoglobulin levels	[74]
Curcumin	Rats/oral	100 mg/kg for 3 times a week	↓Urea and Cr, ↑CAT and SOD, ↓MDA, ↓ Pb concentration	[75]
Mercury				
Animal studies				
Curcumin	Mice/oral	150 and 300 ppm, from first day of pregnancy until postnatal day 15	↑dopamine, serotonin, AChE, GSH, CAT and SOD	[76]
Curcumin	Rats/oral	50 mg/kg for 21 days	↓AST, ALT and Cr, inhibit Hg accumulation in liver and kidney	[78]
Curcumin	Rats/oral	80 mg/kg for 3 days	↓ Urea, Cr, Uric acid and BUN, recovered histopathological changes of kidney	[80]

some specific foods increases the risk of cadmium exposure. Its chronic exposure has been associated with damage to vital organs. It leads to heart, brain, liver and renal dysfunction, reproductive system defects, lung damage, bone fractures, and an increased risk of cancers and cardiovascular disease mortality among the male gender [29, 32–35, 40].

There have been numerous in vitro and animal studies investigating the protective effects of curcumin on Cd-induced toxicity [40].

Kidneys are susceptible to target organ of Cd accumulation, and its chronic exposure causes Cd-mediated nephrotoxicity. Based on the findings of several animal studies, curcumin has a reno-protective effect. In male rat with Cd-induced nephrotoxicity, which was treated with 50 mg/kg of curcumin, renal biomarkers were considerably reduced. Furthermore, those histopathological changes induced by Cd in the renal cortex ameliorated after curcumin treatment [41]. Deevika et al. examined the antioxidant activity of curcumin against the nephrotoxic effects of Cd in rats. This study revealed that administration of curcumin in adult male rats reduced serum levels of BUN and Cr and showed a significant protective role against Cd-induced nephrotoxicity via normalizing the antioxidant

enzymes such as superoxide dismutase (SOD) and catalase [42] in tissue samples [43]. Moreover, in another study, a reduction of malondialdehyde (MDA) content as a lipid peroxidation marker in renal tissue was observed in curcumin-treated rats. Moreover, curcumin administration could have prevented further reductions in glutathione (GSH) levels. Therefore, curcumin has the potential to improve the biochemical and histological changes caused by Cd [44].

Cadmium inhalation promotes the secretion of IL-6 and IL-8 from human airway epithelial cells. It can cause chronic pulmonary inflammation, which plays a role in the pathogenesis of lung cancer and chronic obstructive pulmonary disease. Curcumin as a natural antioxidant could regulate the secretion of these pro-inflammatory cytokines and prevent cadmium-induced airway inflammation [45]. Treatment with oral curcumin showed a significant reduction in MDA level and improved antioxidant enzyme activity in albino mice's lung tissue [46].

Acute Cd intoxication can induce liver damage and is associated with an elevation in serum liver enzyme levels, and activation of hepatic stellate cells (HSC) into myofibroblast-like cells leads to liver fibrosis [26]. Several animal studies have shown turmeric/curcumin's hepatoprotective activities on cadmium acetate-induced liver injury [47–49]. Consumption of *C. longa* in rat attenuated Cd′s toxic effects through reduction of HSC activity and liver fibrosis. Turmeric showed a protective effect on Cd-induced hepatocytes degenerative changes [47]. In another study, co-administration of curcumin with vitamin C revealed ameliorative effects against Cd-induced hepatotoxicity through increasing the levels of antioxidant enzymes like GSH and scavenging of ROS [48]. Also, curcumin in adult male albino rats showed similar hepatoprotective effects, including reducing liver transaminase levels and an elevation in serum protein concentration and antioxidant enzymes (SOD and CAT) [49]. Exposure to cadmium can cause some neurotoxic effects, including behavioral disorders and neuronal dysfunction. In Cd-intoxicated mice, when curcumin is administered orally significant ameliorating effects in the social behavior, acetylcholinesterase (AChE) enzyme activity, and hormonal dysfunction (testosterone and progesterone) were reported [50].

Another study was conducted to evaluate the beneficial effects of curcumin against Cd-induced toxicity in the brain of male mice. According to its findings, curcumin consumption significantly improved dopamine and serotonin levels in the forebrain tissue of these animals. The authors reported that curcumin provides beneficial effects for cognitive and neuromuscular problems in male mice [51]. Moreover, curcumin treatment has a protective effect against Cd-induced immunotoxicity, reproductive toxicity, and colon toxicity [40]. Acute Cd-chloride exposure produced primary reproductive damage and led to infertility through increased oxidative stress (decreased SOD, CAT, and GSH levels), edema, necrosis, and spermatological damage. Curcumin treatment could prevent Cd-induced reproductive damage in male rats [52].

4 Arsenic (As)

Humans and animals are exposed to arsenic (As) through food, drinking water, environment, drugs, and chemical warfare. Several disorders have been linked to chronic exposure to As dust, including dermal lesions (e.g. hyperkeratosis and pigmentation changes), increased risk of skin, lung and other cancers, peripheral neuropathy, and peripheral vascular disease [26, 29, 33, 53]. The results of a case-control study show that maternal occupational arsenic exposure has a positive association with cleft palate occurrence and maternal dietary arsenic exposure may lead to cleft lip and palate [54]. In vitro study with human lymphocytes showed that curcumin modulated arsenic-induced genotoxicity and played a role in the prevention and repair of the DNA damage and a reduction in ROS generation [55]. Arsenic poisoning leads to liver diseases like hepatomegaly, ascites, liver fibrosis, and cirrhosis [53, 56, 57].

Administration of curcumin in a mouse model for 6 weeks diminished the elevation of liver

enzymes in arsenic intoxicated mice and demonstrated protective effects against hepatic injuries and oxidative stress [53]. In As-intoxicated rats, using curcumin reduced hepatic damage by scavenging free radicals, reducing lipid peroxidation and chelating arsenicals compounds. Curcumin improved biochemical alterations induced by As intoxication such as transaminases, AChE, total protein and albumin, blood glucose, BUN, and Cr levels [58]. Administration of tetrahydrocurcumin (THC), a major metabolite of curcumin, significantly improved dyslipidemia, oxidative damage, and hepatic mitochondrial toxicity and its ultrastructural alterations in arsenic intoxicated rats [59]. DNA damage with lipid peroxidation and increased levels of ROS have been reported in the chronically arsenic-exposed population. The results of a clinical trial showed beneficial effects of 3 months of curcumin administration against As-induced DNA damage [60].

Arsenic belongs to neurotoxic heavy metals [26]. Curcumin and its nanoparticle have shown protective effects against As-induced toxicity by reducing lipid peroxidation and ROS production in the brain and kidney of rats [61]. In another study, As-intoxicated mice were treated with 100 mg/kg of curcumin for 28 days. The results exhibit the neuroprotective effect of curcumin by increasing learning and memory performance and activity of AChE in the hippocampus and frontal cortex [62]. Curcumin has a protective impact on functional and ultrastructural changes in the brain mitochondria in As-intoxicated rats. The neuroprotective effect of curcumin in As-induced cholinergic deficits is associated with modulating the expression of pro- and anti-apoptotic proteins in the hippocampus and frontal cortex [63].

Moreover, curcumin showed preventive outcome on the harmful effects of As on neurogenesis such as the viability and apoptosis of neural stem and progenitor cells [64].

5 Chromium (Cr)

Chromium (Cr) is widely used in different industries and medical and dental implants. Its exposure occurs through inhalation and ingestion of chromium-contaminated foods and water [26, 29]. Chromium is a hepatotoxic heavy metal which can cause parenchymal necrosis and hepatic steatosis. Cr-induced liver injury is accompanied by increased ROS levels, mitochondrial destruction, and apoptosis [26]. In several experimental studies, the hepatoprotective effects of curcumin are well-documented. Curcumin pretreatment at a 400 mg/kg dose reduced Cr damages to the liver via improving histopathological changes and antioxidant enzymes content. The beneficial effects of curcumin against Cr-induced hepatotoxicity were related to the prevention of mitochondrial dysfunction [65]. Chromium can cause toxicity in the male reproductive system through the production of free radicals and DNA damage. It is shown that curcumin can prevent Cr-induced genotoxicity [66]. Curcumin has a preventive effect against Cr-induced nephrotoxicity through a mitochondrial pathway. Curcumin treatment in male rats attenuated Cd-related renal dysfunction, histopathological damage, and oxidant stress also ameliorated the antioxidant enzyme activities [67].

6 Lead (Pb)

Lead exposure generally results from inhalation of lead-contaminated particles, and ingestion of food, water, and household paints [29, 32]. Humans are more prone to lead poisoning mostly through Pb-related industries. Lead poisoning affects almost all functions in the human body [32–34]. The mechanisms involved in lead poisoning include oxidative stress and ROS production, reduction in liver content of cytochrome P450, heme synthesis inhibition, suppression of antioxidant enzymes, and elevation of liver enzymes [26, 30]. Curcumin has the potential to prevent against Pb-induced neurotoxicity. Treatment with 100 mg/kg of curcumin in rodents triggered a significant reduction in lipid peroxidation and lead levels in all the brain areas [68]. In another study, treatment with curcumin revealed ameliorative effects against Pb-induced memory deficits in male Wistar rats [69]. The potential cardioprotective effects of curcumin on

lead poisoning have been considered. The results of a study in rats suggested that administration of exercise and curcumin had ameliorative effects on lead-induced cardiotoxicity through a decrease in the amount of biochemical markers of myocardial damage [70].

Using *Curcuma longa* in rats revealed a hepatoprotective effect by lowering liver enzymes and elevating antioxidant content [71]. Also, the administration of 15 mg/kg of nano-curcumin was effective against lead-induced toxicity in mice. This treatment attenuated ROS and increased antioxidant enzymes [72]. Other study determined the probable protective effects of exercise training and curcumin against oxidative liver damage by lead acetate in mice. The authors suggested that a combination of curcumin and exercise training may have beneficial hepatoprotective effects [73].

Lead acetate considerably increased the serum levels of liver and kidney biomarkers and decreased albumin. Moreover, serum immunoglobulins were significantly decreased. Using of curcumin in Pb-intoxicated rats decreased fibrous and necrosis of hepatocytes. In addition, this treatment could ameliorate changes in serum biomarkers of liver and kidney and immunoglobulins levels [74]. Exposure to Pb increased serum levels of BUN, Cr, and renal MDA but reduced renal antioxidant enzymes. Co-treatment with 100 mg/kg curcumin revealed a significant amelioration in nephrotoxic, oxidative, and histopathological alterations induced after Pb exposure. Curcumin may have a renoprotective effect against Pb exposure due to its effective antioxidant property [75].

7 Mercury (Hg)

Mercury is a toxic pollutant in the environment capable of causing a wide variety of health side effects in humans and animals [29, 32]. Food is the most common source of mercury exposure to humans. High fish consumption and using dental amalgam increases the risk of exposure to mercury [32, 33, 76].

The prophylactic and therapeutic role of curcumin against mercury toxicity has been investigated experimentally in rats. Curcumin at a dose of 80 mg/kg had a protective effect on Hg-induced oxidative stress parameters in the liver, kidney, and brain. This treatment effectively reversed liver and kidney biomarkers and reduced Hg concentration in the tissues [77].

In another study, the protective effect of curcumin on mice's development and behaviors was evaluated after a perinatal exposure of Hg. This study showed that curcumin treatment could increase cognition and had protective effects against anxiety behaviors. Therefore, curcumin has demonstrated a protective neurobehavioral impact against Hg-induced neurotoxicity [76]. In experimental rats, curcumin administration considerably reduced Hg accumulation up to 60% in the liver and kidneys. Furthermore, it attenuated the levels of their serum biomarkers and improved the functions of these organs [78]. The liver is a significant organ involved in the deposition and excretion of Hg. Exposure to Hg remarkably elevated ROS formation, apoptosis, serum LDH, and ALT activities. Treatment with curcumin in Hg-poisoned mice counteracted Hg-induced hepatic injuries through the involvement of oxidative stress antagonism [79]. Mercury is a nephrotoxic metal and can cause impaired renal functions and some histopathological alterations such as swelling of the glomerulus and degeneration of renal tubules with the obstructed lumen. Curcumin treatment was effective in improving all renal function variables and protect against Hg-induced nephrotoxicity [80].

8 Chemicals

Food chemical contamination indicates the presence of chemicals, which should not be present in the food. Alternatively, chemicals present in an amount that is at a higher concentration than the amount, which is attributed as safe. The chemical contaminants are one of the leading causes of food contamination associated with foodborne disease prevalence. Chemical contaminants are

present in almost all everyday products [81]. Turmeric and its main constituent, curcumin, as a spice and coloring food additive, can prevent many of these chemical contaminants-induced complications (Table 2).

9 Acrylamide

Acrylamide (C_3H_5NO), one of the most important food contaminants, is a Maillard reaction when reducing sugars and asparagine are heated. Acrylamide forms in starch-rich foods such as bakery products, potato chips, breakfast cereal, and even coffee. Acrylamide is a 2A human carcinogen that causes toxicity in different body organs such as kidney, liver, and nervous system. It metabolizes in the body and generates reactive oxygen species, which causes cellular and subcellular complications such as oxidative stress, mitochondrial loss, lipid peroxidation, and DNA damages [82]. Previous studies demonstrated the protective effects of turmeric and its main constituent, curcumin on acrylamide-induced toxicity. Turmeric and its derivatives can prevent the formation of acrylamide in food and protect the body from its harmful effects. For example, turmeric in freeze-dried extracts of mint, fennel, and turmeric to pita bread inhibited the acrylamide formation [83]. An in vivo study by Morsy et al. showed that in the rats fed on the acrylamide diet, damages in the tissues of the brain, kidney, and lungs have occurred. On the other hand, the administration of turmeric had ameliorated the antioxidant status in these organs [84]. Curcumin has a protective effect against acrylamide-induced damages such as increasing cell mortality, lipid peroxidation, reactive oxygen species (hydroxyl radical and hydrogen peroxide), and MAPKs signaling pathways decreasing antioxidant levels [85]. Curcumin as a neuroprotective and cognitive-enhancing substance prevented acrylamide-induced spatial memory impairment by reversing tau abnormalities and phosphorylated cAMP response element-binding protein reduction hippocampus [86]. Curcumin as a chemopreventive agent is a natural antagonist of acrylamide, which can avoid the side effects caused by acrylamide exposure such as hepatocellular carcinoma by preventing acrylamide-induced proliferation, as well as inhibiting protein expression of cytochrome P450 2E1 (CYP 2E1), epidermal growth factor receptor (EGFR), cyclin D1, and nuclear factor-kB (NF-kB) [87]. Furthermore, C-150, a Mannich-type curcumin derivative has potent anticancer effects by inhibiting cell proliferation by reducing NF-kB transcription and PKC-alpha [88]. In addition, curcumin effectively prevented acrylamide-induced cell proliferation and apoptosis through the expression of miR-21, which could be a promising target for the prevention and treatment of different cancers [89, 90]. Curcumin as an antioxidant prevents acrylamide-mediated genotoxicity due to reduced acrylamide-induced ROS production, DNA fragments, micronuclei formation, and cytotoxicity [67, 91, 92]. A study by Senthilkumar et al. on *Drosophila melanogaster* demonstrated that curcumin prevented developmental and behavioral toxicity through acrylamide exposure [93].

10 Bisphenol A

Bisphenol A (BPA) has been used mainly in many consumer products, for example, food packaging, plastics, PVC, dental sealants, and thermal receipts. It is commonly found in canned vegetables and packaged foods [94, 95]. It causes damages to the endocrine by binding to estrogen receptors, which leads to estrogenic effects [96, 97]. There are many reports on the protective effects of turmeric and its main constituent, curcumin on BPA-induced toxicity. BPA consumption in rats caused testicular toxicity by oxidative damages. Administration of curcumin protects testicular tissues by reducing MDA levels and increasing antioxidant enzyme activities in testes of rats [98]. Akintunde et al. evaluated the effect of curcumin on neuro-testicular dysfunction. They concluded that curcumin prevented BPA-induced HPGH linked with Parkinson's disease through modulating AChE and locomotive activities, decreasing the intracellular $NO^{\bullet}$ level, preventing striatum-endocrine injury and oxida-

Table 2 Protective effects of turmeric/curcumin against chemicals toxins in in vivo and in vitro studies

Substance	Model	Dosage and duration	Key effects	Ref.
Chemicals				
Acrylamide				
Cell culture studies				
Curcumin	Mouse Leydig (TM3) cell lines	2.5 µM for 24 h	↑cell viability, antioxidant levels ↓lipid peroxidation, ROS, MAPKs	[85]
Curcumin	HepG2, A549, HeLa and PC-3 cells	50 mM for 48 h	↓Cell proliferation, CYP 2E1, EGFR, cyclin D1 and NF- kB	[87]
C-150 (a Mannich-type curcumin derivative)	Glioblastoma cells	1.0 µM for 24 h	↓Cell proliferation, NF-kB, PKC-α	[88]
Curcumin	HepG2 cells	50 mM for 24 h	↓Cell proliferation, ↑apoptosis in HepG2 cells through inhibiting miR-21	[89]
Curcumin	HepG2 cells	0.63, 1.25 and 2.50 microg/ml for 24 h	↓ DNA damage ↓ ROS	[90]
Curcumin	HepG2 cells	2.5 microg/mL for 24 h	DNA damage and ROS, ↓cytotoxicity and genotoxicity	[91, 92]
Animal studies				
Turmeric	Male albino rats Sprague-Dawley strain, Oral with their food	Turmeric was added (0.5%) on diet for 11 days.	↓Tissue damage to the kidneys, brain, and lungs	[84]
Curcumin	Male SPF class Sprague-Dawley rats, oral gavage	90 mg/kg curcumin for 49 days	↑P-CREB and BDNF ↓Tau abnormalities	[86]
C-150 (a Mannich-type curcumin derivative)	Female nude rats, intravenously into the tail veins	3 mg/kg, 28 days	↓ Cell proliferation ↑ median survival time	[88]
Curcumin	Wild type (Oregon – K) *Drosophila melanogaster*	10 µM, for 7 days	↓ ROS ↑ AchE activity	[93]
Bisphenol A				
Animal studies				
Curcumin	Male Wistar rats, oral gavage	100 mg/kg bw dissolved in olive oil	↓MDA ↑GPx, GST, SOD, CAT ↓ Histopathological abnormalities	[98]
Curcumin	Male Wistar rats, oral gavage	100 mg kg − 1 bw per day in olive oil for 28 days	↓MDA ↑GPx, GST, SOD, CAT ↓ Histopathological abnormalities	[100]
Curcumin	Male Wistar rats, oral gavage	50 mg/kg bw dissolved in olive oil for 14 days	↓ locomotive alterations ↑ AChE ↓ NO ↑ LH, FSH, testosterone ↑ sperm motility, sperm count daily sperm production ↓ total sperm deformity ↓ MDA ↑ SOD, CAT ↓ Histopathological alterations	[99]

Table 2 (continued)

Substance	Model	Dosage and duration	Key effects	Ref.
Curcumin	Adult female Wistar rats and Pups	Pups received 20 mg/kg, *i.p.* from PND7 to PND28	↑ Learning and Memory and NSC Proliferation and Differentiation in the Hippocampus	[101]
			↓ Apoptosis and Neurodegeneration	
Perfluorooctane Sulfonate				
Animal studies				
Curcumin	Female Swiss albino rats (6–8 weeks), oral gavage	80 mg/kg bw, one dose per 48 h for 4 weeks	↓ DNA damage in bone marrow	[107]
Curcumin	Male Swiss albino rats (6–8 weeks), oral gavage	80 mg/kg bw, one dose per 48 h for 4 weeks	↓ DNA damage in peripheral blood	[108]
Curcumin	Male Swiss albino rats (6–8 weeks), oral gavage	80 mg/kg bw, one dose per 48 h for 4 weeks	↓ micronucleus frequency ↓ expression levels of caspase 3 and 8	[42]
Nitrosamines				
Animal studies				
Curcumin	Adult male Sprague-Dawley rats, oral gavage	50 and 100 mg/kg, oral gavage daily for 4 weeks	↑ body weights	[115]
			↑ liver function (↓ GOT, GPT, TG, T-chol)	
			↓ liver fibrosis	
			↓ TGF-β1/Smad signaling pathway	
Curcumin	Male C3H/HeN, Oral with diet	0.2% curcumin-containing diet 4 days before DEN	↑ body weights	[116]
			↑ liver weights	
			↓ p21(ras), PCNA and CDC2 proteins	
			↓ HCC	
Curcumin	Adult male Wistar rats, oral gavage	100 mg/kg bw (5 days a week) for 15 weeks	↓ ERα and AR gene expression levels in the liver tissue	[117]
			↓ HCC	
Curcumin	Male Wistar rats, oral gavage	200 mg/kg and 600 mg/kg, 1 day before DEN treatment	↓ $p21^{ras}$ and p53	[118]
			↓ PCNA, p34cdc2 and cyclin E	
			↓NF-kB	
Curcumin	Male Albino mice, 6–8 weeks old, *i.p.*	100 mg/kg 3 days a week 2 weeks	↓ Tumor incidence	[119]
			↓ HIF-1α	
Curcumin nanoparticles	Male Kunming mice (18–22 g). *i.v.*	2 mg/kg, once a week for 36 weeks	↓C-myc, VEGF, PCNA	[120]
			↑ Bax/Bcl-2	
Curcumin	Male albino rats, oral gavage	100 mg/kg after 3 months of DENA administration, for 15 successive days	↓ TGF-β and Akt	[121]
			↑ caspase-3	
			↓ ALT, AST	
			↓ lipid peroxidation	
			↓ Histopathological abnormalities	

(continued)

Table 2 (continued)

Substance	Model	Dosage and duration	Key effects	Ref.
Curcumin	Male Albino rats Wistar strain, oral gavage	100 mg/kg and 200 mg/kg dissolved in corn oil, for 4 days	↑ HO-1	[126]
			↑ NF-E2	
			↓ AST, ALT and c-GT	
			↓ hepatotoxicity	
			↓ oxidative damage	
Benzo[a]pyrene				
Cell culture studies				
Turmeric, Curcumins	Mouse liver S9/microsomes	Turmeric powder (0.1–1.0 mg), curcumin (0.06–0.36 mg), for 30 min	↓ dose-dependent in the levels of [^{3}H]B(a)P-derived DNA adducts	[152]
Animal studies				
Curcumin	Male Sprague-Dawley rats	50, 100, or 200 mg/kg daily by oral gavage, for 30 days	↓CYP 1A1	[144]
			↓CYP 1B1	
			↓ plasma levels of BPDE	
Curcumin	Male Swiss albino mice	Curcumin in standard laboratory diet, for 16 days	↓CYP 1A1/1A2 in lung and liver	[146]
			↑binding of Nrf2 to antioxidant response element occurred in nuclear extracts from lungs and liver	
			↓BAP-induced AhR protein levels, phosphorylation, nuclear translocation, and subsequent binding to DNA	
			↑Nrf2 protein levels	
Turmeric	Male Swiss albino mice	1% turmeric in standard laboratory diet, for 30 days	↓CYP 1A1	[147]
			↓CYP 1A2	
Turmeric, curcumin	Male albino mice	0.1, 0.5 and 3% in the diet, for 4 weeks	↓ DNA adducts	[149]
Turmeric	Male albino mice	0.1% in the diet, for 4 weeks	↓ DNA adducts	[150]
Curcumin	Female A/J mice (4–5 weeks old)	0.5–2.0% dietary commercial grade curcumin:	↓percentage of mice with tumors	[151]
		(a) 2 weeks before, during, and for 1 week after carcinogen administration;	↓number of tumors per mouse	
		(b) 1 week after carcinogen treatment until the end of the experiment;	↓tumor size	
		(c) during both the initiation and postinitiation periods.	↓number of adenomas and adenocarcinomas of the duodenum and colon	
			↓number of papillomas and squamous cell carcinomas of the forestomach	

Table 2 (continued)

Substance	Model	Dosage and duration	Key effects	Ref.
Curcumin	Male albino rats aged 4 weeks	Orally administered 100 mg of curcumin/kg body weight, three times weekly until 2 weeks	↓liver microsomal MDA concentration	[153]
			↓ DNA fragmentation percentages	
			↓8-OHdG	
			↓mutations	
			↓oxidative stress	
Curcumin	Female A/J mice	2% curcumin in the diet, for 14 days	↓forestomach tumorigenesis	[154]
			↓hepatic EROD activity	
			↓CYP1A1	
Curcumin-free aqueous turmeric extract (CFATE), ethanolic turmeric extract, and turmeric (T)	Swiss female albino mice	Oral administration of CFATE as sole source of drinking water, twice a week for 4 weeks	↓forestomach tumorigenesis	[155]
Curcumin	Adult male Wistar rats (isolated testicular germ cell population)	Oral administration of 50 mg/kg, for 60 days	↓Bax/Bcl2 ratio	[156]
			↓expression of pro-apoptotic proteins	
			↓expression of p53 dependent apoptotic genes	
			↓mitochondria to cytosolic translocation of cytochrome c and activated the survival protein Akt	
Curcumin and quercetin	Male laka mice in the weight range of 18–20 g	Orally in drinking water curcumin and quercetin, thrice a week for 22 weeks	↓Bcl-2	[157]
			↑Bax	
			↑number of apoptotic cells	
			↑enzyme activities of caspase 3 and caspase 9	
Curcumin	Female CD-1 mice (6 weeks old)	Topical application of curcumin 5 min prior to the application of benzo[a]pyrene, once weekly for 10 weeks	↓ the number of tumors per mouse	[158]
			↓ percentage of tumor-bearing mice	
Curcumin	Males of 7-weeks-old Sprague–Dawley rats, weighing 200–250 g	Oral administration of 50 mg/kg bw 4 h before benzo[a]pyrene treatment, for 9 consecutive weeks	↓TNF-α	[159]
			↓IL-6	
			↓CRP	
			↓tunnel staining and p53 expression	
			↓apoptosis in lung epithelial cells	
			↑antioxidants level	
			↓oxidative stress	
			↑cell proliferation	
			↓inflammation	
Curcumin	Male Swiss albino mice	2% curcumin in the diet, for 14 days	↓histopathological deviations in the lung activation of MAPK signaling and NF-κB, ↓Cox-2 transcription in lung tissues	[160]

tive damage [99]. In another study by Uzunhisarcikli et al., protective effects of curcumin on BPA-induced hepatotoxicity were evaluated. Results demonstrated that BPA increased the levels of MDA and decreased the activities of antioxidant enzymes. Curcumin treatment reversed these oxidative damages. Besides, histopathological alterations in the liver were minimized by curcumin administration [100]. Curcumin prevented BPA-induced hippocampal neurotoxicity and impaired neurogenesis by enhancing the neurogenic expression/levels and the Wnt pathway genes/proteins and activation of the Wnt/β-catenin signaling pathway [101].

11 Perfluorooctane Sulfonate

Perfluorooctane sulfonate (PFOS) is a highly persistent chemical that has toxic effects [102]. Unlike the high water solubility, its bioaccumulation, biomagnification, and long half-lives in mammals made it listed as the first perfluoroalkane sulfonic acid in the Stockholm Convention Persistent Organic Pollutant (POP) [103]. For many years, this substance has been used to produce products that are highly resistant to heat, grease, oil, and water. PFOS is a toxic food contaminant, which has been used in the manufacture of many daily necessities like food packing and nonstick cookware, for example, coated paper packaging used for microwave popcorn and sandwich or snack bags. The main routes of PFOS exposure are food, water, and dust, and studies have demonstrated that PFOS was found in blood, body tissues, and breast milk of exposed people [104, 105]. Investigations revealed that PFOS caused toxicity in laboratory animals, leading to carcinogenicity like hepatocellular carcinoma, developmental toxicity, and affecting the lipid metabolism and disturbing the immune system [106].

In the study by Çelik et al., the protective effect of curcumin on PFOS-induced genotoxicity and DNA damage in bone marrow tissue and peripheral blood was evaluated [107, 108]. Results demonstrated that curcumin effectively antagonized the genotoxic effect and DNA damage caused by PFOS. PFOS caused an increase in apoptotic gene expression; curcumin inhibits apoptotic pathway proteins such as caspase 3 and 8, leading to reduce apoptotic cellular death [42]. Therefore, curcumin could be considered a potent protective natural compound that can prevent PFOS-induced genotoxicity.

12 Nitrosamines

Nitrosamines are produced by the chemical reactions between nitrites and nitrates and proteins or secondary amines such as dimethylamine [109]. They are toxic and mutagenic and produced reactive oxygen species and oxidative stress. Besides, they caused malignancy, mutagenicity, and genotoxicity by damaging the DNA [110]. These effects lead to a wide range of complications such as obesity, T2DM, NAFLD/NASH, and different types of malignancies [111, 112]. It is undeniable that the consumption of processed foods has become very popular in recent decades. The main routes of nitrosamines exposure are processed foods, preservatives, and nitrogen-containing fertilizers [113]. Diethylnitrosamine is one of the hepatic carcinogen and mutagen present in water and processed food like cheddar cheese [114]. Several studies showed the hepatoprotective effects of curcumin against diethylnitrosamine-induced HCC [115–117]. Possible mechanisms for the anti-HCC effect of curcumin include reducing the expression of proliferating cell nuclear antigen, cyclin E and p34 [118], inhibiting hypoxia-inducible growth factor-1α hepatic expression [119, 120], protecting hepatocytes against oxidative stress through NF-E2-related factor 2 mediated induction of heme oxygenase-1 and modulating AKT, TGF-β, and caspase-3 expression [121–124]. Curcumin protects the liver against oxidative damages through increasing the expression of heme-oxygenase 1 via activation of NF-E2-related factor 2 signaling [125, 126]. Oral nanocapsulated curcumin in diethylnitrosamine-induced HCC showed protection and restored redox homeostasis in liver cells by inducing cancer cell apoptosis [127]. In a study by Sreepriya et al., curcumin

had chemopreventive effects against N-nitrosodiethylamine-induced hepatocarcinogenesis by preventing the induction of hepatic hyperactive plastic nodules, bodyweight loss, increasing the levels of hepatic diagnostic markers, and hypoproteinemia [128, 129]. Curcumin administration decreased hepatocellular lipid peroxidation, increased glutathione antioxidant defense, and limited the histological alterations induced by N-nitrosodiethylamine [130]. Furthermore, curcumin reduced the liver VEGF, CyclinD1, and CDK4 mRNA expression levels and CyclinD1 and CDK4 proteins levels in liver cancer caused by N-nitrosodiethylamine [131]. Besides, curcumin could be considered a preventive and chemotherapeutic substance in lung and liver cancer induced by N-bis(2-hydroxypropyl) nitrosamine [132].

N-nitrosodimethylamine induces malignant tumors in the gastrointestinal tissues through DNA adductions and gene mutation [133]. A study by Waly et al. curcumin prevented the nitrosamine-induced oxidative stress in gastric tissues by increasing glutathione reserves and total antioxidant capacity, decreasing the level of lipid peroxides and nitric oxide release, suppressing DNA oxidative damage, and promoting the antioxidant enzymes. Furthermore, abnormal gastric architecture in histopathological findings caused by nitrosamine was recovered by the administration of curcumin [134]. Besides, curcumin could be useful in treating N-nitrosomethylbenzylamine-induced esophageal carcinogenesis when administered in the initiation and post-initiation phase [135]. In a study by Azuine et al., turmeric was effective in oral mucosal tumors induced by methyl-(acetoxymethyl)-nitrosamine as chemopreventive agents [136].

13 Benzo[a]pyrene

Benzo[a]pyrene is a well-known polycyclic aromatic hydrocarbon, considered a human carcinogen [137]. Benzo[a]pyrene is a food contaminant used in the cooking process through the pyrolysis of carbohydrates, amino acids, and fatty acids [138, 139]. For example, grilled meats and smoked fishes are the most important sources of benzo[a]pyrene [140]. Based on previous studies, scientists have shown that benzo[a]pyrene causes lung, mammary glands, skin, cervix, and forestomach tissue malignancies [141–145]. Turmeric and its main constituent, curcumin, prevent benzo[a]pyrene-induced DNA damages by inhibiting the expression of phase I enzymes in the liver and stomach [146–150]. Dietary curcumin reduces the number of squamous cell carcinomas and papillomas of the forestomach and the number of adenocarcinomas of the duodenum and colon [151]. Furthermore, curcumin inhibits DNA adducts formation in the target organs and protects them [152–154]. Curcumin-free aqueous turmeric extract is also effective on benzo[a]pyrene-induced forestomach tumors [155]. In a study by Banerjee et al., curcumin prevents benzo[a]pyrene-induced expression of p53-dependent apoptotic genes. Therefore, it prevents testicular germ cell apoptosis and restores male reproductive health [156]. Many studies confirm the protective effect of curcumin against lung malignancies induced by benzo[a]pyrene. Curcumin has a chemopreventive effect against benzo[a]pyrene-induced lung carcinogenesis through stimulating the apoptotic proteins like Bcl-2, caspase 3, and caspase 9 [157–159]. Furthermore, curcumin decreased Cox-2 transcription and benzo[a]pyrene-induced activation of NF-κB and MAPK signaling in lung tissues [160]. Modulation of p53 posttranslational modifications is another chemopreventive mechanism of curcumin against benzo[a]pyrene-induced lung carcinogenesis, especially when co-administrated by quercetin [161]. Curcumin has beneficial effects on benzo[a]pyrene-induced lung carcinogenesis by decreasing lipid peroxidation and reactive oxygen species generation. Besides, curcumin prevented benzo[a]pyrene-induced increase in the activities of drug-metabolizing enzymes (cytochrome P450 and b5). Furthermore, curcumin increases GSH levels and decreases glutathione-s-transferase, superoxide dismutase, and glutathione reductase [162]. In a study by Malhotra et al., curcumin and resveratrol co-treatment decreased the benzo[a]

pyrene-induced micronuclei formation in the lungs. Moreover, curcumin and resveratrol in the combination reduced the apoptotic protein expression such as bcl-2 in benzo[a]pyrene-treated animals [163]. In another study, curcumin and vitamin E co-administration reduced the activation of p53 and PARP-1 induced by benzo[a]pyrene. Therefore, it protects benzo[a]pyrene-induced complications in lung epithelial cells [164]. Curcumin administration reduces the side effects of benzo[a]pyrene, such as increased levels of lipid peroxides, protein carbonyl content, and decrease in the levels of tissue antioxidants. These effects were enhanced by the addition of piperine and resveratrol [165–170].

14 Mycotoxins

As a diverse category of secondary mold metabolites, mycotoxins can lead to a wide variety of toxicological impacts such as genotoxic, nephrotoxic, carcinogenic, hepatotoxic, teratogenic, and immunotoxic effects [171]. Mycotoxins are considered a major threat to public health and a factor moderating the marketable quality and nutritional value of contaminated products, resulting in substantial economic losses [172] (Table 3).

15 Aflatoxins

Aflatoxins (AFTs) are mycotoxins produced as secondary metabolites of *Aspergillus* species. AFTs are considered as potent hepatotoxins, immunotoxin teratogens, and mutagens [171, 173]. AFB1 toxicity indicated by a significant elevation in hepatic transaminases, elevation in lipid peroxide biomarkers, reduction of GSH concentration, reduction in antioxidant and down-regulation activities of gene expression of these antioxidant enzymes. Inhibitory effects of turmeric and its active ingredient, curcumin on toxicity induced by AFB1 were conducted in different in vivo and in vitro models [174–177]. The administration of turmeric and curcumin inhibited AFB1-induced hemolysis, histopathological damages, hepatic enzymes, lipid peroxidation products, pro-apoptotic proteins and pro-inflammatory gen, total protein, calcium, and immunoglobulin biomarkers values increased significantly [178, 179]. Furthermore, turmeric's partial protective effects on growth performance, feed intake, liver weight, and expression of antioxidant, biotransformation, and immune system genes were shown in different studies [175, 180–183].

Studies have shown that curcumin reduced aflatoxin-induced mutagenicity and reduced AFB(1)-N(7)-guanine adduct excretion in the urine, albumin adduct in the serum, DNA adduct in the liver, along with the reduction in the levels of AFB1–lysine adduct in the peripheral circulation. This suggests that curcumin significantly inhibits macro-molecular adduction. Dietary administration of turmeric reduced the gamma-glutamyl transpeptidase-positive foci induced by AFB1, which is the precursor of hepatocellular neoplasm [184–186].

The therapeutic effects of curcumin are probably mediated through its anti-inflammatory and antioxidant action and modulation of hepatic xenobiotic enzymes. Curcumin by its ability to scavenge free radicals restores the antioxidant status. Curcumin was also shown to induce several enzymatic antioxidants and induce de novo synthesis of GSH [187, 188].

16 Zearalenone

Zearalenone (ZEN) is a mycotoxin and has hepatotoxic effects. Smaiel et al. (2015) showed curcumin nanoparticles execute adequate protection against hepatotoxicity induced by ZEN mycotoxins. The combined treatment with curcumin nanoparticles plus ZEN resulted in improved all tested parameters in a dose-dependent manner [189]. In another study, curcumin pretreatment effectively reduced the dysregulation of cellular redox balance in porcine granulosa cells [190].

Table 3 Protective effects of turmeric/curcumin against mycotoxins in in vivo and in vitro studies

Substance	Model	Dosage and duration	Key effects	Ref.
Mycotoxins				
Aflatoxins				
Cell culture studies				
Turmeric Extracts/ curcumin	Human RBC suspension	Aflatoxin with and without turmeric extracts/curcumin	↓ Hemolysis	[182]
Animal studies				
Curcumin	Rats/oral gavage	Curcumin (15 mg/kg bw), single i.p. dose of AFB1 and combination of single i.p. dose of AFB1 with oral curcumin treated 5-week	↓ Hepatic transaminases, TBARS	[188]
			↑GSH, CAT, SOD, GPX, GST, up-regulation of antioxidant enzyme gene.	
Curcumin	Adult male Fischer rats/oral gavage	Curcumin (200 mg/kg bw), and AFB1 (25 µg/kg bw).	↑ GSH, SOD, CAT, and GPx	[187]
		Cotreatment 90 days	↓ Serum marker enzymes, lipid peroxidation	
Curcumin	Adult male Fischer rats/oral gavage	AFB1 (20 g/day)	↑ GSHT, UGT1A1	[186]
		For 6 weeks with 0.05% (w/w) of pure curcumin from 3rd week to 6th week	↓ CYP1A1, ALT, LDH, DNA adduct	
Cur-NPs-Hgs	Old male Sprague-Dawley rats/oral gavage	AFB1 plus Cur-NPs-Hgs for 3 weeks	DNA fragmentation, chromosomal aberration, Bax and caspase-3	[185]
			↑ Bcl-2	
Curcumin	Adult male albino rats/oral gavage	AF(250 µg/kg bw/day) and curcumin (200 mg/kg) for 4 weeks	↓ Histological, immunohistochemical changes, desmin	[183]
			↑ Bcl-2	
Turmeric extract	Broiler chickens/oral	0.05% ethanolic turmeric extract plus 3 ppm AF for four weeks	↑ feed intake and feed conversion ratio, body weight gain	[179]
Turmeric powder	Old male broiler chicks/oral for 21 days	AFB1 (2 mg) plus turmeric powder (200 mg/kg)	↑ Body weight gain, feed intake, glucose, albumin, total protein calcium	[180]
			↓ Relative liver and kidney weights	
Ethanolic turmeric extract	Chickens/oral	AF plus turmeric extract (3 vs. 5 mg/kg diet) 28 day	↓ ALT, AST, and uric acid, pathological changes	[179]
			↑ total protein, calcium, and HDL	
Curcumin	Male Sprague-Dawley rats/oral	Curcumin 1000 mg/kg bw/day for 6 weeks after single intraperitoneal injection of AFB1 at 1 day.	↓ ALT, AST, ALP	[178]
			, LDH, urea, creatinine, uric acid, total protein	
			↑ IgG, IgM, IgA	
Curcumin	Broiler chickens/oral	300 ppb AF plus curcumin (1.5 and 2.0 g/kg)	↑ Weight gain, glucose, protein, cholesterol, hemoglobin, PCV and erythrocytes	[177]
			↓Mortality rate, ALT. AST, ALP	

(continued)

Table 3 (continued)

Substance	Model	Dosage and duration	Key effects	Ref.
Curcumin rhizome powder	Broiler birds	AF (1 ppm) curcumin (1%) for 6 week	↓Hematological alterations, pathological lesions of liver and kidneys	[176]
Curcumin rhizome powder	Chicks	74 mg/kg of curcumin with 1.0 mg of AFB1/kg for 3 weeks	↑ Feed intake and BW gain, growth performance, SOD, GPx, GST, EH	[175]
			↓ IL-6, CYP1A1 and CYP2H1	
Zearalenone				
Cell culture studies				
Curcumin	Porcine granulosa cells	20 μM for 12 h afterwards co-treated with 60 μM ZEA for 24 h	↑SOD1 and CAT	[197]
			↓ROS	
Animal studies				
Curcumin nanoparticles	Male Sprague-Dawley rats/oral gavage	Curcumin nanoparticles (100, 200 mg/kg bw) plus ZEN (40 μg/kg bw) alone or in combination for 3 weeks	↑ Body weight gain, antioxidant capacity, GPX mRNA gene expression	[198]
			↓ MDA, DNA fragmentation	
Ochratoxin A				
Animal studies				
Curcumin rhizome powder	Broiler chicks/oral	OTA 2 ppm plus 5%, curcumin rhizome	↑ GPx, GSH reductase, CAT and TBARS	[194]
Curcumin rhizome powder	Broiler chicks/oral	Curcumin rhizome powder 2 g/kg plus	↓ uric acid, urea, creatinine, sodium and potassium	[195]
		OTA (0.5 ppm)		
Turmeric powder	Broiler chicks/oral	2.5 mg OTA/kg plus turmeric powder (150. 225 mg/kg) for 21 days	↑ Feed intake and weight gain	[196]

17 Ochratoxin A

Ochratoxins (OT) are toxic secondary metabolites produced by *Aspergillus* and *Penicillium* species. Ochratoxins have been found in cereals, horticultural crops, mixture of species, tree nuts herbs, herbal teas, and cocoa powder [191]. Ochratoxin A (OTA), the most toxic form of ochratoxins, has been nephrotoxic, immunosuppressive, teratogenic, and carcinogenic [192] [193].

Results of the Rani et al., 2009 study suggest that OTA induces oxidative stress and supplementation of *Curcuma longa* showed amelioration by stabilizing the antioxidant defenses [194]. A trial on broiler birds indicated that turmeric's use of powdered rhizome decreased the severity of toxicity. The complete recovery of birds to a state comparable to control birds could not be achieved, as there was the persistence of histological, biochemical, and ultrastructural alterations given OTA at 0.5 ppm. Thus, turmeric powder feed could achieve partial amelioration of the toxic effect of OTA [195]. This finding is contrary to other studies that have reported curcumin was not effective in protecting chicks from the toxic effects of OTA [196].

18 Conclusion

Nowadays, herbal medications and nutraceuticals are increasingly recognized in the management of most of the diseases due to their beneficial efficacies, such as antioxidant and anti-inflammatory effects. Furthermore, they are inexpensive, readily available, and easy to use. Exposure to food contaminants is inevitable these

days. Therefore, adding turmeric/curcumin to foods, in addition to the excellent taste and color, could potentially reduce the harmful effects of food contaminants.

Conflict of Interests None.

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The Effects of Curcumin Plus Piperine Supplementation in Patients with Acute Myocardial Infarction: A Randomized, Double-Blind, and Placebo-Controlled Trial

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Abstract

Background: Acute myocardial infarction (AMI) is a leading cause of death and disability worldwide. Previous investigations have demonstrated that curcumin has a cardioprotective effect and may improve myocardial injury. So this study was performed to assess whether supplementation with curcumin could diminish myocardial injury following AMI.

Methods: To conduct this randomized, double-blinded, and placebo-controlled clinical trial, seventy-two patients with acute myocardial infarction, aged 18–75 years, were enrolled and randomly divided into the active intervention and control groups. The active intervention group (n = 38) received curcumin capsules with piperine supplement (500 mg/day, 95% curcuminoids) for 8 weeks, whereas the control group (n = 34) received a placebo capsule. At the baseline and end of the study, ejection fraction was assessed, and blood samples were taken from all patients to measure the levels of cardiac troponin I(cTnI), lipid

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profile, FBG, HbA1C, liver enzymes, renal function parameters, and electrolytes.

Results: In this trial, curcumin supplementation significantly reduced the levels of HbA1C (−0.3 ± 2.2 vs. +1.1 ± 1.3, P = 0.002), LDL (−10.3 ± 20.7 vs. +0.2 ± 22.5, P = 0.039), ALT (−10.2 ± 28.5 vs. +7.3 ± 39.2, P = 0.029), and ALP (+6.4 ± 39.5 vs. +38.0 ± 69.0, P = 0.018) compared to the placebo group. Moreover, the serum concentration of HDL significantly improved in comparison with the placebo group (+4.5 ± 8.9 vs. -1.6 ± 7.7, P = 0.002). However, no substantial difference was perceived between the groups regarding the ejection fraction and serum levels of cTnI, FBG, renal function parameters, and electrolytes.

Conclusion: Our results indicated that daily intake of 500 mg of curcumin capsules with piperine supplement for 8 weeks modified lipid profile, liver enzymes, and glycemic status, but did not have any effect on ejection fraction and serum concentration of cardiac troponin I, renal function parameters, and electrolytes in acute myocardial infarction patients.

Keywords

Cardiac troponin I · cTnI, ejection fraction · Lipid profile · HbA1C, piperine · Curcumin · Myocardial infarction

1 Introduction

Based on the Global Burden of Disease Study, cardiovascular diseases (CVD) are among the most leading causes of death and are responsible for 31.8% of worldwide mortality [1, 2]. Acute myocardial infarction (AMI) and cardiomyocytes degeneration are the leading cause of morbidity and mortality among ischemic heart diseases [1, 3].

Reduced left ventricular ejection fraction (LVEF) following AMI is one of the most compelling predictors of sudden cardiac events, and its evaluation has both prognostic and therapeutic value [4–6]. Though aggressive revascularization and medical therapy in patients with MI improve LV systolic function, up to 50% of subjects do not show an elevation in LVEF several months post-MI [4, 7, 8]. Moreover, according to both short- and long-term follow-up investigations, abnormal levels of troponins have been consistently linked with the risk of poor outcome and death in patients with ST-elevation or non-ST-elevation acute coronary syndrome [9–12]. Cardiac troponin I (cTnI) is the inhibitory subunit of the troponin complex regulating the calcium-mediated interaction between actin and myosin. It is a preferred biomarker for identifying AMI when acute myocardial infarction is suspected clinically [13–16].

Herbal remedies are gaining popularity to treat or prevent various disorders such as CVD [17, 18]. Curcumin [1, 7-bis-(4-hydroxy-3-methoxy-phenyl)-1, 6-hepta diene-3, 5-dione], also called diferuloylmethane, is a natural polyphenolic compound extracted from the rhizomes of the plant *Curcuma longa*. Several studies have shown that curcumin is safe and can exert a variety of potent beneficial effects including cardioprotective, antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, lipid-modifying, anti-tumor, and anti-diabetic actions [18–30]. Despite the evidence of benefits and safety of curcumin, poor bioavailability due to low absorption, rapid metabolism, and rapid systemic elimination limits its widespread [19, 31, 32]. One of the effective techniques to elevate curcumin bioavailability is the co-administration of the piperine, a major active component of black and long peppers, a potent inhibitor of glucuronidation and can increase bioavailability by 2000% [19, 33–35]. Increasing evidence from clinical and preclinical investigations indicates that curcumin exerts its potential benefits in CVD by its antioxidant, anti-inflammatory, anti-atherosclerotic, anti-apoptotic, and lipid-modifying activities [18, 36]. Therefore, we conducted this study to investigate whether 8 weeks of curcumin capsules with piperine supplement can improve myocardial injury in patients with acute myocardial infarction.

2 Method

2.1 Trial Design

This randomized clinical trial study was conducted in one of the northeastern city of Iran, Neyshabur. It was a double-blind, placebo-controlled, and parallel-group study with a 1:1 allocation ratio for two groups. The Institutional Review Board and the Ethical Committee of Neyshabur University of Medical Sciences approved the study (Code: IR.NUMS.REC.1394.15). The study was registered in the Iranian Registry of Clinical Trials (http://www.irct.ir) (IRCT registration number: IRCT2017010922381N3). A consent form was signed by all participants before the start of the trial.

2.2 Participants

This study included 72 cases in the age group of 18–75 years with the approved diagnosis of acute coronary syndrome (ACS). In this randomized controlled, double-blind trial, the patients were recruited from the Bahman hospital, Neyshabur, located in northeastern Iran, based on clinical findings with acute MI diagnosis. Acute myocardial infarction was diagnosed by electrocardiogram (ECG), clinical symptoms, and serum markers. The exclusion criteria were liver failure (liver enzymes AST, ALT, and CK≥ 1.5-times above normal, and a bilirubin Total <2, Direct> 0.2), receiving medications like immunosuppressants, or potent cytochrome P450 3A4 inhibitors (corticosteroids, azathioprine, mycophenolic acid, tacrolimus, etc.), renal failure (urea> 18 mg/dl, Cr> 1.5 mg/dl), pentoxifylline, and cilostazol, background diseases like inflammatory or infectious diseases, leukocytosis (WBC> 11×103), consumption of berberine extract (due to inhibitory effects on the inflammatory NF-κB pathway), a history of diseases that cause malabsorption syndromes, consumption of glucosamine (due to inhibitory effects on the inflammatory nuclear factor (NF)-κB pathway), a triglyceride (TG)> 400, heart failure stage based on the New York Heart Association (NYHA) III or IV, uncontrolled blood pressure (BP) with a systolic BP>180 and a diastolic BP> 100, and finally evidence of endocrine or metabolic disorders that affect the lipid profile.

2.3 Randomization

In this study, the subjects were randomly divided into "curcumin" and "control" groups using a balanced block randomization method. So, the letters "A" were selected for "curcumin" and "B" for "control." As a result, all possible blocks were AABB, ABAB, ABBA, BBAA, BABA, and BAAB. The number was randomly selected through a random numbers table. The whole random process was blinded to ensure that the random assignment sequence occurs. For this purpose, the drugs including curcumin and placebo were previously placed in envelopes with serial numbers from 1 to 80. Except for the test coordinator, no one knew the nature of the packets. Concealment was performed for all stages of the study.

2.4 Intervention

The curcumin capsules (500 mg, 95% curcuminoids plus 5 mg Bioperine; Sami Labs Ltd., Bangalore, India) were used for the treatment in the curcumin group. The patients in the control group received placebo capsules containing lactose powder at the same dose. All the subjects consumed drugs daily for 8 weeks. At regular intervals, patients were called to follow up on medication consumption as well as its likely side effects.

2.5 Assessment of Outcomes

The ejection fraction, biochemical, and clinical measurements were the primary and secondary outcomes.

2.6 Biochemical Measurements

Venous blood samples were collected after an overnight fasting period for each patient both before and after intervention on days 0 and 60. Samples were centrifuged at 3000 rpm for 10 min to separate serum. Fasting blood glucose (FBG), lipid profile, and liver function tests were assessed instantly after separating serum by the BT-2000 Auto Analyzer machine (Biotechnica, Rome, Italy) using Pars Azmoon kits.

Blood pressure (BP) was measured by a standard mercury sphygmomanometer and cuff appropriate to the person's arm circumference after the patient was in the supine position for at least 15 min. The assessment of blood pressure was repeated two times with an interval period of at least 5 min, and the average BP values were considered.

2.7 Statistics Analysis

Data was presented as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to assess the normality of variables, and the dependent t-test and the Wilcoxon signed-rank test were used respectively to compare the two related samples (before, after) for parametric and non-parametric variables. The independent T-test and the Mann-Whitney U test were performed for normal and non-normal distribution variables to compare patients' characteristics in two treatment and placebo groups. The categorical data, such as gender and smoking, were analyzed using chi-square and Fisher's exact test. The level of statistical significance was expressed as a p-value <0.05.

2.8 Results

A total of 80 patients with acute myocardial infarction were initially enrolled in the study, but six patients in the placebo-treated group and two patients of the curcumin-treated group did not complete the study due to low adherence to the intervention, trip, or unavailability in follow-up (Fig. 1). The baseline characteristics of 72 study participants, who were randomly assigned into two groups, are shown in Table 1. There were no significant differences in baseline characteristics of participants between the groups (all $P > 0.5$), except for sodium, which was higher in the placebo group ($P = 0.047$).

2.8.1 The Effect of Curcumin on Anthropometric Parameters and Liver Function Tests

The effects of curcumin supplementation on anthropometric parameters and biochemical tests are presented in Tables 2 and 3. After 8 weeks of intervention, the mean weight and body mass index (BMI) showed no substantial changes between the groups.

Among liver enzymes (AST, ALT, and ALP), following curcumin treatment, ALT improved and the mean of the change was statistically significant compared to the placebo group. Moreover, though ALP increased in both groups, this increase was significantly higher in the placebo group than in those who had received curcumin. But no significant difference was perceived between the groups in the mean change in AST values.

2.8.2 The Effect of Curcumin on Biochemical Parameters

In this trial, while FBG was not affected by the intervention, HbA1C significantly deteriorated in the placebo group but not in the curcumin group, and finally, the mean of change in HbA1C was statistically significant between the two groups.

As shown in Table 3, though total cholesterol and triglycerides did not alter during the intervention, a significant improvement in LDL and HDL levels were observed following 8 weeks of curcumin treatment compared with the placebo.

2.8.3 The Effect of Curcumin on Serum Electrolytes and Renal Function Tests

Among serum electrolytes (sodium, potassium, magnesium), though within-group comparisons revealed a significant increase in serum concentration of potassium in curcumin group, between-

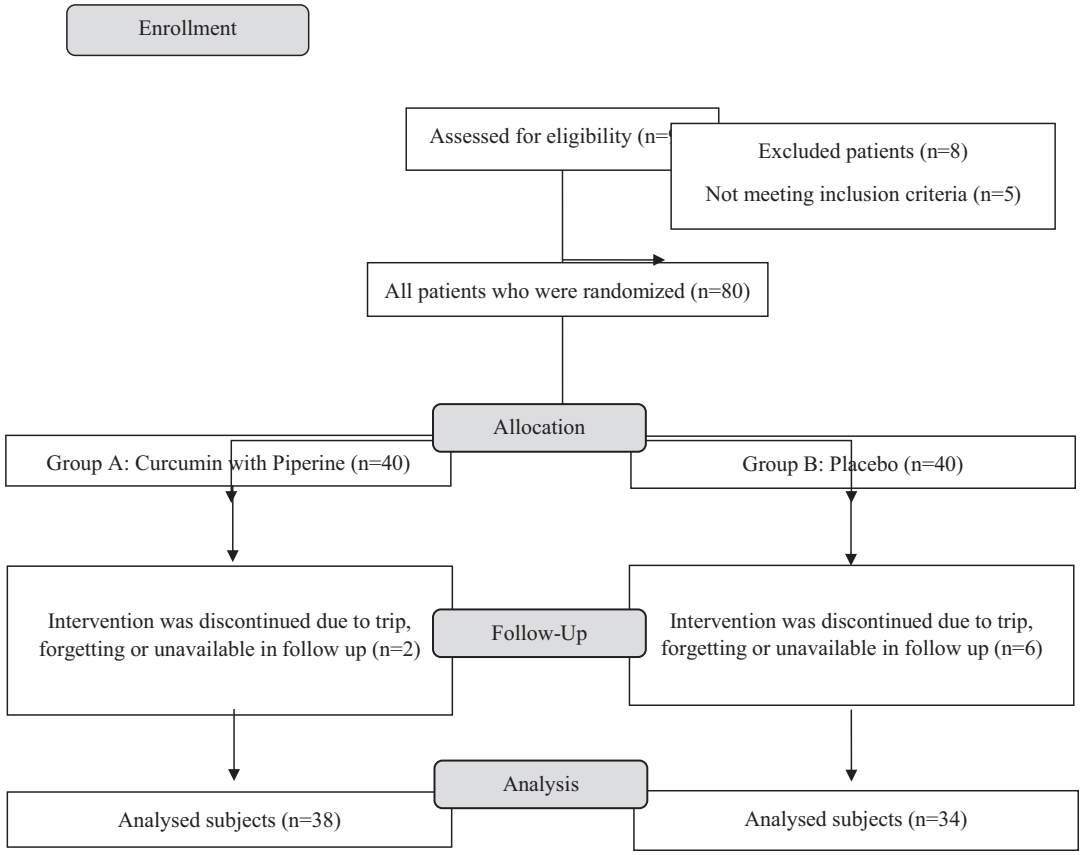

Fig. 1 Study population in the flow diagram

group comparisons exhibited no substantial differences in the serum levels of electrolytes. Also, statistical analysis showed no substantial influence of the intervention on BUN, creatinine, and urea levels.

2.8.4 The Effect of Curcumin on Cardiac Injury

Within-group analysis indicated a significant improvement in ejection fraction in both curcumin and placebo groups, but this impact was not statistically significant between the groups. Moreover, it was shown that after adjusting confounding factors including age, gender, history of diabetes, BMI, and baseline sodium, curcumin treatment had no substantial effect on ejection fraction (Table 4). Also, curcumin administration did not affect cardiac troponin I and systolic and diastolic blood pressure.

3 Discussion

The present investigation demonstrated that 8 weeks of regular ingestion of curcumin capsules with piperine did not affect patients with AMI. We evaluated ejection fraction and cTnI as markers of cardiac function and damage, respectively, and observed no substantial differences following curcumin supplementation. Earlier studies have suggested that curcumin treatment improves ejection fraction [37–42] and cTnI [37, 43–46] in cardiac injuries. In preclinical studies, curcumin treatment could diminish the size of the infarct area and protect cardiomyocytes against apoptosis-related cardiac diseases through the activation of PI3K, Akt, ERK1/2, and Bcl-2 expression and attenuation of JNK, p38 MAPK, Bax, and caspase-3 that mediated by the JAK-2 and JAK2/STAT3 signaling pathway [36, 47, 48].

Table 1 Baseline demographic, clinical, biochemical, and anthropometric data of all patients with acute MI

Characteristics	Acute MI patients		P-value[a]
	Placebo (n = 34)	Curcumin (n = 38)	
Age, (year)	59.6 ± 10.3	59.5 ± 10.4	0.984
Sex, (male, %)	52.6	72.5	0.070
Smoker (%)	23.1	27	0.640
Addiction (%)	15.4	33.3	0.069
Weight (kg)	67.9 ± 14.4	70.1 ± 10.2	0.456
BMI (kg/m^2)	26.0 ± 4.9	26.0 ± 4.4	0.991
AST (mg/dl)	62.4 ± 59.0	68.0 ± 66.7	0.731
ALT (mg/dl)	35.0 ± 24.1	40.8 ± 29.4	0.321
ALP (mg/dl)	194.5 ± 69.1	213.5 ± 46.6	0.147
FBG (mg/dl)	127.2 ± 52.3	117.8 ± 44.6	0.360
HbA1C (mg/dl)	6.2 ± 1.5	6.0 ± 1.4	0.473
TC (mg/dl)	153.0 ± 37.1	165.4 ± 52.6	0.235
TG (mg/dl)	144.7 ± 83.2	142.1 ± 86.3	0.919
HDL-C (mg/dl)	43.8 ± 13.3	42.2 ± 9.4	0.535
LDL-C (mg/dl)	77.7 ± 24.9	78.1 ± 24.6	0.946
SBP (mmHg)	137.2 ± 21.6	141.3 ± 22.7	0.427
DBP (mmHg)	83.8 ± 14.5	87.9 ± 14.4	0.222
BUN (mg/dl)	16.9 ± 7.3	16.3 ± 3.8	0.677
Creatinine (mg/dl)	1.1 ± 0.2	1.2 ± 0.3	0.081
Sodium (mmol/L)	139.9 ± 5.2	137.7 ± 4.2	0.047
Potassium (mmol/L)	4.1 ± 0.4	4.1 ± 0.3	0.613
Magnesium (mmol/L)	1.9 ± 0.5	2.1 ± 0.5	0.289
Urea (mg/dl)	5.1 ± 1.5	5.0 ± 1.3	0.912
cTnI (ng/ml)	8.0 ± 4.5	8.6 ± 4.6	0.793
EF	59.4 ± 9.2	58.2 ± 8.5	0.539

The categorical and continuous variables were presented as percentage and mean ± SD (standard deviation), respectively

[a]The categorical data was analyzed using chi-square/Fisher's exact test

The normal and non-normal continuous variables were analyzed using independent student t test and Mann-Whitney U test, respectively

AST Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *BMI* Body mass index, *HbA1C* Hemoglobin A1C, *FBG* Fasting blood glucose, *cTnI* Cardiac troponin I, *TC* Total cholesterol, *TG* Triglyceride, *HDL-C* High-density lipoprotein cholesterol, *LDL-C* Low-density lipoprotein cholesterol, *BUN* Blood urea nitrogen, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *EF* Ejection fraction

Furthermore, curcumin upregulates Na+/Ca2+ exchanger (NCX) and eNOS expression in the myocardium resulting in improved LVEF [42].

Clinical investigations assessing the effect of curcumin on EF and cTnI are scarce. In contrast to our findings, Franceschi et al. revealed that 3 months of curcumin supplementation significantly improved the EF of healthy subjects >65 years [49]. Moreover, following curcuminoids (4gr/day, pre- and postoperative) treatment in patients undergoing coronary artery bypass grafting (CABG), EF was significantly higher than the placebo group [50]. Consistent with our finding, Aslanabadi et al. reported that curcumin (single dose, 480 mg nanomicelle curcumin) pretreatment in patients undergoing elective percutaneous coronary intervention (PCI) showed no marked difference in troponin I level at 8 and 24 h after PCI [51].

A number of studies reported the effects of curcumin on obesity management [52–54]. Based on a preclinical model, curcumin exerts its anti-obesity property by decreasing the expression of PPARγ and CCAAT/enhancer-binding protein α, adipocyte differentiation, fatty acid esterification, and adipokine-induced angiogenesis in adipose

Table 2 Comparison of clinical, biochemical, and anthropometric data of patients with acute MI before and after intervention within groups

	Placebo (n = 34)			Curcumin (n = 38)		
Characteristics	Before	After	P-value	Before	After	P-value[a]
Weight (kg)	67.9 ± 14.4	68.0 ± 14.3	0.730	70.1 ± 10.2	70.2 ± 9.9	0.597
BMI (kg/m2)	26.0 ± 4.9	26.1 ± 4.9	0.602	26.0 ± 4.4	26.1 ± 4.4	0.394
AST (mg/dl)	62.4 ± 59.0	52.5 ± 47.5	0.344	68.0 ± 66.7	31.3 ± 22.3	0.003
ALT (mg/dl)	35.0 ± 24.1	42.6 ± 42.1	0.266	40.8 ± 29.4	30.6 ± 14.0	0.034
ALP (mg/dl)	194.5 ± 69.1	225.8 ± 62.2	0.003	213.5 ± 46.6	220.0 ± 54.8	0.318
FBG (mg/dl)	127.2 ± 52.3	150.1 ± 95.15	0.173	117.8 ± 44.6	110.0 ± 28.4	0.251
HbA1C (mg/dl)	6.2 ± 1.5	7.4 ± 1.5	<0.001	6.0 ± 1.4	5.7 ± 1.8	0.394
TC (mg/dl)	153.0 ± 37.1	153.4 ± 33.2	0.892	165.4 ± 52.6	157.3 ± 42.3	0.249
TG (mg/dl)	144.7 ± 83.2	140.5 ± 66.1	0.797	142.1 ± 86.3	146.8 ± 88.3	0.734
HDL-C (mg/dl)	43.8 ± 13.3	42.3 ± 10.1	0.210	42.2 ± 9.4	46.8 ± 10.1	0.003
LDL-C (mg/dl)	77.7 ± 24.9	77.3 ± 28.9	0.956	78.1 ± 24.6	68.8 ± 17.0	0.004
SBP (mmHg)	137.2 ± 21.6	136.6 ± 17.3	0.699	141.3 ± 22.7	140.3 ± 17.7	0.516
DBP (mmHg)	83.8 ± 14.5	82.8 ± 11.1	0.373	87.9 ± 14.4	86.1 ± 11.7	0.081
BUN (mg/dl)	16.9 ± 7.3	16.5 ± 5.7	0.322	16.3 ± 3.8	15.4 ± 5.9	0.487
Creatinine (mg/dl)	1.1 ± 0.2	1.0 ± 0.2	0.488	1.2 ± 0.3	1.1 ± 0.2	0.152
Sodium (mmol/L)	139.9 ± 5.2	140.6 ± 5.5	0.755	137.7 ± 4.2	139.1 ± 4.2	0.203
Potassium (mmol/L)	4.1 ± 0.4	4.3 ± 0.5	0.066	4.1 ± 0.3	4.4 ± 0.4	0.001
Magnesium (mmol/L)	1.9 ± 0.5	1.8 ± 0.5	0.689	2.1 ± 0.5	1.8 ± 0.4	0.069
Urea (mg/dl)	5.1 ± 1.5	5.0 ± 1.4	0.931	5.0 ± 1.3	7.0 ± 10.1	0.280
cTnI (ng/ml)	8.0 ± 4.5	8.9 ± 5.0	0.225	1.3 ± 4.6	9.1 ± 4.2	0.466
EF	59.4 ± 9.2	62.4 ± 7.2	0.023	58.2 ± 8.5	63.5 ± 6.0	<0.001

[a]Dependent student t and Wilcoxon test were performed for comparing normal and non-normal variables before and after intervention, respectively

Values are expressed as mean ± SD

AST Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *BMI* Body mass index, *HbA1C* Hemoglobin A1C, *FBG* Fasting blood glucose, *cTnI* Cardiac troponin I, *TC* Total cholesterol, *TG* Triglyceride, *HDL-C* High-density lipoprotein cholesterol, *LDL-C* Low-density lipoprotein cholesterol, *BUN* Blood urea nitrogen, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *EF* Ejection fraction

tissue and inducing apoptosis of adipocytes and fatty acid oxidation [55]. As shown in Tables 2 and 3, BMI was not affected by the intervention. In agreement with this finding, Akazawa et al. [56] indicated that 8 weeks of curcumin treatment in postmenopausal women did not change BMI. Based on our literature review, curcumin probably needs to be administered for a longer period of time or in higher doses to affect anthropometric parameters [52, 53, 56–60].

Since, the major organ for the metabolism of drugs and herbal supplements is the liver, hepatotoxicity [61]. In the present clinical trial, we demonstrated that 8 weeks of curcumin supplementation did not increase liver enzyme levels and explicitly and significantly reduced ALT and ALP levels, reiterating the safety of curcumin intervention in patients.

Approximately 30% of patients hospitalized with acute coronary syndromes have diabetes mellitus [62]. It has been indicated that diabetes is associated with around twofold higher long-term fatality after AMI, particularly in women [62–64]. Also, in patients with and without diabetes, a positive association between high blood glucose concentration and poor outcome subsequent to MI has been noted [65]. According to in vivo studies, curcumin exerts its antihyperglycemic effect partly by the antioxidant and anti-inflammatory mechanisms [66, 67]. Some human studies [52, 53, 57–59] have indicated the beneficial effect of curcumin on blood glucose and insulin metabolism. Consistent with these findings, we showed an improvement in HbA1C levels following 8 weeks of curcumin ingestion.

Table 3 Changes of clinical and biochemical data of patients with acute MI after intervention between groups

	Placebo (n = 34)	Curcumin (n = 38)	P-value[a]
BMI (kg/m2)	0.04 ± 0.5	0.05 ± 0.3	0.940
AST (mg/dl)	−9.9 ± 65.4	−36.6 ± 72.5	0.089
ALT (mg/dl)	7.3 ± 39.2	−10.2 ± 28.5	0.029
ALP (mg/dl)	38.0 ± 69.0	6.4 ± 39.5	0.018
FBG (mg/dl)	16.8 ± 69.5	−6.5 ± 34.6	0.071
HbA1C (mg/dl)	1.1 ± 1.3	−0.3 ± 2.2	0.002
TC (mg/dl)	1.0 ± 46.1	−0.8 ± 43.1	0.367
TG (mg/dl)	−6.1 ± 143.0	4.7 ± 86.5	0.624
HDL-C (mg/dl)	−1.6 ± 7.7	4.5 ± 8.9	0.002
LDL-C (mg/dl)	0.2 ± 22.5	−10.3 ± 20.7	0.039
SBP (mmHg)	−0.5 ± 8.6	−1.0 ± 9.1	0.843
DBP (mmHg)	−0.9 ± 6.4	−1.8 ± 6.0	0.552
BUN (mg/dl)	−1.6 ± 7.4	−0.7 ± 5.7	0.610
Creatinine (mg/dl)	−0.03 ± 0.2	−0.09 ± 0.3	0.507
Sodium (mmol/L)	0.3 ± 6.0	1.3 ± 5.9	0.457
Potassium (mmol/L)	0.1 ± 0.3	0.3 ± 0.4	0.153
Magnesium (mmol/L)	−0.07 ± 0.8	−0.2 ± 0.4	0.524
Urea (mg/dl)	0.01 ± 2.0	2.1 ± 10.5	0.267
cTnI (ng/ml)	0.4 ± 1.4	2.6 ± 6.4	0.624
EF	2.6 ± 6.4	5.0 ± 6.4	0.118

[a]Independent student t and Mann Whitney U test were performed for comparing normal and non-normal distribution variables, respectively

Values are expressed as mean ± SD

AST Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *BMI* Body mass index, *HbA1C* Hemoglobin A1C, *FBG* Fasting blood glucose, *cTnI* Cardiac troponin I, *TC* Total cholesterol, *TG* Triglyceride, *HDL-C* High-density lipoprotein cholesterol, *LDL-C* Low-density lipoprotein cholesterol, *BUN* Blood urea nitrogen, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *EF* Ejection fraction

Table 4 Linear regression to adjust for confounding factors on EF change

Variables	B	Std. error	Beta	P-value
Age	0.20	0.13	0.26	0.13
Gender	−3.41	2.65	−0.22	0.20
Group	0.44	2.13	0.31	0.83
Diabetes history	1.88	1.99	0.12	0.34
Δ BMI	1.16	2.09	0.07	0.58
Baseline sodium	−0.31	0.22	−0.19	0.16

Δ after – before

BMI Body mass index

Dyslipidemia is an important modifiable risk factor for AMI, and lipid-lowering therapy is a cornerstone in the secondary prevention of cardiovascular events after a MI [68]. As mentioned previously, several clinical trials suggested that curcumin may be a potential candidate for treating hyperlipidemia [69–71]. In agreement with these findings, present clinical trial supports that curcumin intervention improves the concentrations of LDL and HDL in patients with AMI. Curcumin upregulates the expression of the hepatic LDL receptors, cholesterol efflux regulatory protein (CERP), and ApoA1 and downregulates ApoB100 expression. The inhibition of SREBP-1c (the main nuclear receptor promoting fatty acid biosynthesis) decrease the enzymatic activity of HMG-CoA reductase, enhancement of cholesterol excretion (by increase bile acid secretion), and increase the activity of PPARα, the leading nuclear receptor in regulating fatty acids β-oxidation [26] are the other mechanisms of curcumin-mediated control of lipid biosynthesis and accumulation.

Electrolytes play a vital function in preserving the integrity of the cardiovascular system. Few studies suggest the association between serum sodium and potassium levels with long- or short-term mortality risk among ACS patients [72, 73]. The serum sodium, potassium, and magnesium levels are lower in AMI patients than in healthy individuals.

The probable mechanism for the reduction in sodium and potassium levels is the impairment of the Na/K pump and the Na/Ca exchanger. The active transport of these ions across the cell membrane requires ATPase, which in turn is dependent on Mg for its activity [74]. Though some studies indicated that curcumin can regulate the activity of Na/K pump and the Na/Ca exchanger [75–77], there weren't any substantial differences in the level of electrolytes in the present study.

Serum creatinine concentration, which is commonly used as a sensitive indicator of renal function, is one of the known predictors of adverse events of ACS [78–80]. Moreover, in two studies conducted by Saygitov [79] and Kurniawan [81], increased BUN level was a more potent risk factor for ACS outcomes than creatinine. Because urea reabsorption is a passive phenomenon associated with sodium and water reabsorption, diseases linked to enhanced water and sodium reabsorption lead to an elevation in urea reabsorption and increase in BUN levels. So, BUN levels on admission and during treatment provided extra prognostic information when added to clinical variables [82]. In our study, creatinine, urea, and BUN levels did not significantly change with curcumin administration compared to placebo. In agreement with our findings, Phrommintikul et al. [83] did not perceive any significant decrease in creatinine level in patients who underwent elective PCI following curcuminoid administration.

4 Limitations

Because of the rather small sample size and short duration of follow-up, curcumin's exact effect may not be revealed, and our results need to be confirmed in more extensive clinical trials with longer follow-up periods. The inability to provide a mechanistic view for curcumin's beneficial properties on lipid profile and glycemic status is another limitation of the current study.

5 Conclusion

In conclusion, the results obtained in this work showed that though curcumin capsule with piperine supplement did not affect cardiac function of patients with acute myocardial infarction, it demonstrated positive effects on lipids and HbA1c. Therefore, curcumin capsule with piperine supplement can be considered as an effective and safe disease preventive or therapeutic agent for the management of hyperlipidemia and diabetes. However, clinical investigations are required to confirm our results.

Conflict of Interest Muhammed Majeed is the founder of the Sabinsa-Sabinsa group. The other authors have no other conflicting interests to disclose.

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Protective Effects of Curcumin on Pulmonary Arterial Hypertension

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Abstract

Pulmonary hypertension is one of the most common diseases among older people. This disease is usually associated with complications such as vascular changes, vascular remodeling, vasoconstriction, endothelial dysfunction, right ventricular failure, and reduction in nitric oxide availability. Many chemical drugs have been used to treat pulmonary hypertension, but result in limited efficacy and several side effects, and these medications are not always available worldwide. Various studies in traditional medicine have shown that changes in lifestyle and nutritional habits can be extremely effective in both the prevention and treatment of various diseases. One treatment method related to changing nutritional habits is the use of curcumin as a nutritional supplement. Curcumin plays an important role in treating pulmonary hypertension and positively alters the aforementioned complications.

Keywords

Pulmonary hypertension · Turmeric · Curcumin

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1 Introduction

An unusual form of elevated blood pressure is pulmonary hypertension (PH). As a result of this disorder, blood pressure in the pulmonary arteries increases. Due to narrowing of the pulmonary arteries, it is difficult for blood to pass from the heart to the lungs. The heart has to contract more forcibly to compensate and ensure that there exists an adequate supply of blood to the body. This causes an increase in blood pressure in the pulmonary arteries and the heart [1, 2]. PH is a complex and hazardous disease associated with various vascular changes, vascular remodeling, vasoconstriction, and in situ thrombosis, which finally leads to right ventricular failure (RVF) and death [3]. As mentioned above, PH causes a reduction in blood flow in the small arteries and is followed by increased vascular resistance and morphological changes in blood vessels. The most abundant proteins in vascular wall tissue are collagen and elastin, which are responsible for mechanical properties, such as tensile strength and elasticity [1]. Therefore, it is possible that morphological changes in the blood vessel wall cause increased blood pressure [4–6] and, as a result, thickening of the vascular wall and destruction of collagen and elastin. This process ultimately leads to vessel wall rigidity and endothelial dysfunction [7–10]. Despite the high danger associated with PH, many studies have shown that changes in lifestyle and nutrition can be successfully employed for its management and treatment. According to one report, an important lifestyle change that can be easily implemented is the use of curcumin as a nutritional supplement [3].

Curcumin is the major phenolic compound obtained from turmeric. Turmeric is prepared from the root of *Curcuma longa*, which belongs to the ginger family Linn. Additionally, turmeric is widely used as a spice, as well as a coloring agent in foods [11–14]. Many studies have proven that curcumin can be an effective therapeutic compound for the treatment of numerous diseases, without significant toxicity or side effects [15–22]. It has been suggested that regular ingestion of curcumin may potentially prevent the development of PH, as well as the negative vascular structural changes associated with PH (Table 1).

Curcumin has various beneficial properties as it relates to the destructive effects of PH. Some of the processes positively impacted by the regular ingestion of curcumin include, but are not limited to, vascular remodeling, endothelial dysfunction, vasoconstriction, and RVF. Therefore, regular use of curcumin supplementation has been suggested to potentially slow the progression of PH. On the other hand, no literature article to date has investigated the effects of curcumin on vascular disorders caused by PH. Thus, the present review aims to survey the literature for articles reporting any protective effects of curcumin on the numerous vascular-related symptoms associated with PH.

2 Protective Effects and Molecular Mechanisms Associated with the Use of Curcumin in Pulmonary Hypertension

According to the definition proposed by the World Health Organization, the causes of PH are divided into five categories: (1) PH caused by diseases that affect the heart (specific gene mutation, HIV, and cirrhosis), (2) PH due to left-sided heart disease, (3) PH caused by lung disease, (4) PH caused by blood clots, and (5) PH due to unknown causes (idiopathic) [23]. PH is defined as an increase in the mean pulmonary arterial pressure (mPAP) to 25 mm Hg at rest, which is assessed via right heart catheterization (RHC) [24, 25]. Medications used to treat PH are not always readily available to patients worldwide, and some can be cost-prohibitive. For example, medications and therapeutics currently used to manage pulmonary hypertension include, but are not limited to, various vasodilators, guanylate cyclase stimulators to increase NO, endothelin receptor antagonists to reverse the blood vessel narrowing effects of endothelin, sildenafil to expand blood vessels, high-dose calcium channel blockers to help relax the muscles in blood vessel

Table 1 The protective effects of curcumin on pulmonary hypertension

The type of curcumin	Doses of agent	Mechanism	Investigated type of samples	Results	Ref no.
Methanolic extract of *Curcuma longa*	10, 20, and 30 mg/kg, iv	Inhibition of extracellular Ca(2+) influx	Rat mesenteric artery	Potent vasodilation	[68]
Turmeric rhizomes	4% of the diet	Phenolic compounds acting	The kidney samples	Inhibited angiotensin-converting enzyme	[59]
Curcumin	50 or 100 mg/kg/day	Nitric oxide (NO) availability and reducing oxidative stress	Rat aortic	Improves endothelial dysfunction and vascular remodeling	[28]
Curcumin	100 mg/kg/day	Prevented elastin amount in smooth muscle cells	Thoracic aorta	Preventing negative changes in blood vessel morphology	[66]
Curcumin	100 mg/L	Upregulation eNOS	Aortic tissues in mice	Improvement vascular dysfunction	[33]
Curcumin	5, 10 and 20μmol/l	Elevating PPAR-γ activity	The smooth muscle cells of the vascular wall in the rat	Suppressing oxidative stress	[52]
Tetrahydrocurcumin	50 and 100 mg/kg/day	Alleviation of oxidative stress	Aortic tissue	Prevent vascular dysfunction	[57]
Tetrahydrocurcumin	50 or 100 mg/kg/day	Enhancing NO bioavailability	Aortic medial wall	Improvement of vascular dysfunction and arterial stiffness	[67]
Curcumin	120 mg/kg/day	Increased blood velocity, altered the circulating endothelial cells, and open capillaries number	Cerebral arteries	Decreased blood pressure	[69]
Curcumin	300 mg/kg/day	Affecting SP1/AT1R DNA binding	Embryonic thoracic aortic smooth muscle cells	Downregulates AT_1R expression, reducing AT_1R-mediated vasoconstriction	[56]
Curcumin	200 mg/kg bw/day	Decrease in TNF-a levels and IL-1β	Rat smooth muscle cells	Reduction in right ventricular hypertrophy and oxidative stress	[40]
Curcumin	150 mg/kg	Mast cells regulation	Pulmonary vessel wall cells	Inhibition in the remodeling of pulmonary vessel induced by chronic hypoxia hypercapnia	[49]

eNOS Endothelial nitric oxide synthesis, *NO* Nitric oxide, *SP1* specificity protein 1, *AT1R* Angiotensin 1 receptor, *IL* Interleukin, *TNF-α* Tumor necrosis factor-α

walls, warfarin to prevent clotting, digoxin to stimulate more forceful contractions of the heart, and diuretics to remove excess fluid to reduce workload on the heart, but all of these medications/therapeutic compounds do have adverse side effects. However, in contrast to drug substances, numerous studies conducted in animals have used curcumin as a nutritional supplement for the treatment of experimentally induced PH, and the only observed side effect was diarrhea [26]. Moreover, it has been reported that curcumin exhibits beneficial effects with regard to the structural vascular changes that occur with PH (Table 1) [27, 28].

Curcumin, via a reduction in oxidative stress, upregulation of endothelial nitric oxide synthase

(eNOS) activity, suppression of angiotensin 1 receptor (AT1R) expression, and increased NO availability, causes vasodilation, a decrease in the contraction of vascular smooth muscle, an improvement in endothelial function and arterial remodeling, and, finally, a decrease in the symptoms associated with PH. The mechanisms associated with curcumin's beneficial effects are discussed below.

The main factor in the pathogenesis of PH is oxidative stress, which is characterized by an increase in reactive oxygen species (ROS) [28]. When ROS are increased due to a downregulation in eNOS, it results in a reduction in nitric oxide (NO) availability, endothelial dysfunction [29], an increase in the contraction of vascular smooth muscle, and structural remodeling of vessel walls. This spectrum of physiological processes subsequently results in a significant increase in peripheral resistance and eventually leads to an increase in blood pressure in the pulmonary arteries [30]. As mentioned above, it has been reported that curcumin improves vascular function and increases NO availability due to both its vasodilator effects and its capacity to reduce oxidative stress [28, 29, 31–34]. Specifically, curcumin induces vasodilation by reducing the levels of angiotensin-converting enzyme (ACE) and metalloproteinase (MLP), as well as increasing the availability of NO. In fact, by reducing the levels of ACE, curcumin prevents the conversion of angiotensinogen-1 to angiotensinogen-2 and, consequently, induces vasodilation [35, 36]. Importantly, and as it relates to PH, endothelial dysfunction has been shown to be correlated with an increase in oxidative stress due to increased O_2^- from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. As a result of increased O_2^-, the amount of NO available is reduced [37–39]. Furthermore, curcumin reduces right ventricular hypertrophy, although the reduction in right ventricular hypertrophy appears to be due to a decrease in the levels of tumor necrosis factor-α (TNF-α) and IL-1β. Accordingly, a decrease in the levels of TNF-α and IL-1β results in a reduction in oxidative stress [40].

As mentioned previously, another damaging effect of PH is an increase in vasoconstriction and arterial remodeling, which affects the pulmonary arterial circulation. This process contributes to decreased drainage through the small arteries, which causes enhanced arterial resistance, and ultimately results in heart failure. One of the important mechanisms involved with arterial remodeling is the proliferation of smooth muscle cells in the vascular wall. The proliferation of smooth muscle cells in the vascular wall causes pulmonary vascular remodeling, which leads to increased pulmonary artery resistance and PH [41, 42].

It should be noted that inflammatory processes are involved in arterial remodeling [43, 44]. Animal studies have demonstrated that curcumin improves endothelial dysfunction and vascular remodeling in rats by increasing NO availability, decreasing oxidative stress, and affecting the overall inflammatory process [28, 26]. Specifically, as it pertains to the inflammatory process, curcumin has been shown to inhibit the NF-κB pathway and, by affecting this pathway, regulate inflammatory cytokines such as IL-1β, IL-6, and IL-8, as well as modulate the synthesis and activation state of COX2 [45, 46]. Additionally, curcumin suppresses the activation of TNF-α, which regulates inducible nitric oxide synthase (iNOS), 5-LOX, and phospholipase A2 (PLA2). Thus, curcumin has the capacity to control, or modulate, key inflammatory pathways and numerous associated inflammatory cytokines [47]. By modulating inflammatory pathways and controlling the activation of associated cytokines, curcumin helps to regulate the arterial remodeling process. Indeed, it has been shown that inhibition of NF-κB suppresses the expression of genes involved with cell proliferation. It should also be emphasized that iNOS modulates the action of cyclic guanine monophosphate (cGMP). Importantly, the influence of iNOS on cGMP, as well as any factors that may potentially modulate 5-LOX, COX2, and PLA2, can theoretically induce vasodilation [26, 47, 48].

Mast cells are another inflammatory mediator in the body, which are involved in the con-

traction of the smooth muscles of the arterial walls. Therefore, when inflammation exists, mast cells can play an important role in contraction of vascular smooth muscle and vascular remodeling. It has been reported that curcumin, via its influence on mast cells, can prevent pulmonary vessel remodeling induced by chronic hypoxia and hypercapnia [49]. It has also been demonstrated that curcumin can inhibit the proliferation of pulmonary artery smooth muscle cells by preventing the progression of the cell cycle and, therefore, decrease pulmonary artery pressure [50]. However, inconsistent with the above studies, it has also been shown that nanoparticles with encapsulated curcumin did not improve pulmonary hypertension and the process of arterial remodeling in hypoxic rats. The results of this study demonstrated that the dose of the drug used and the method of administration were not effective in the treatment process [51]. These authors suggested that hypoxia had a major role in the localization of curcumin nanoparticles in the lungs and was possibly due to altered blood flow, increased barrier properties of the lung vasculature, and decreased endocytosis. Consequently, the target tissue level of curcumin under hypoxic conditions was much lower relative to that achieved in normoxic rats, which was suggested to occur because of differences in curcumin-nanoparticle dynamics.

Curcumin also has an effect on angiotensin-2-induced inflammation [52]. Specifically, curcumin causes a reduction in IL-6 and TNF-α production induced by angiotensin 2, which results in both decreased cell proliferation and inflammation. The anti-inflammatory and anti-proliferation effects of curcumin were shown to result from an increase in peroxisome proliferator-activated receptor gamma (PPAR-γ) activity and inhibition of NADPH oxidase-mediated intracellular ROS production [52–55].

Another relevant mechanism through which curcumin can produce vasodilation in hypertension should be addressed. This mechanism involves curcumin's capacity to inhibit AT1R in the arteries, which reduces vasoconstriction and induces vasodilation [56, 28, 33, 57, 58]. It has been reported that curcumin, by suppressing the expression of AT1R in vascular smooth muscle cells, increased vasodilation and, subsequently, prevented the progression of hypertension (induced by angiotensin-2) in rats [52, 56, 59]. Moreover, curcumin has been demonstrated to increase both the production of NO, as well as NO availability, by upregulation of eNOS in the vascular wall, which serves to reduce vascular dysfunction (i.e., improve overall vascular function) [60, 61]. Although it was mentioned above, it is important to note again that curcumin decreases cellular oxidative stress. As a result, O_2^- levels are decreased, eNOS in vascular endothelial cells is increased, and, accordingly, the availability of NO is increased [57, 62, 63]. All of these effects resulting from treatment with curcumin serve to improve endothelial dysfunction and reduce arterial remodeling [28]. Additionally, by reducing the levels of TNF-α and reducing oxidative stress, curcumin has been reported to prevent aortic remodeling and right ventricular hypertrophy in rats [64, 65]. Finally, in L-NAME-induced hypertensive rats, curcumin was shown to decrease vascular remodeling and increase elastin levels, which effectively prevented pathological changes in the walls of blood vessels [66]. Lastly, it is worth mentioning another study that evaluated cadmium-induced hypertension in mice. This particular study demonstrated that curcumin treatment prevented the negative effects of cadmium-induced hypertension as it relates to the vascular wall; specifically, curcumin treatment resulted in a decrease in vascular stiffness, vascular resistance, vascular remodeling, and cellular oxidative stress [67].

3 Conclusion

Pulmonary hypertension is a rare type of high blood pressure. This disease affects the arteries in the lungs and the right ventricle. As the disease progresses, the symptoms of PH can limit all physical activity. Pulmonary hypertension is a life-threatening condition that gets worse over time, and, presently, there is no cure.

Fortunately, various treatments can reduce the symptoms of PH and return the patient to a more normal life. Different drugs and treatment strategies have been used to treat PH. Changes to either a patient's lifestyle (e.g., regular exercise, if possible), or nutritional habits, represent two non-drug-related methods for managing PH. In fact, as it pertains to nutritional habits, several studies have shown that the regular use of curcumin helps to control PH, as well as exert beneficial effects on the vascular system to mitigate the damaging effects that PH causes in the pulmonary arteries and the arteries located in the right side of the heart. Specifically, curcumin mediates a reduction in oxidative stress, upregulates eNOS activity, and suppresses the expression of AT1R. The resultant increase in NO availability, secondary to upregulation in eNOS activity, causes vasodilation and assists in reducing vascular dysfunction. Curcumin treatment in various experimental animal models also leads to a decrease in the contraction of vascular smooth muscle and improvements in both endothelial dysfunction and arterial remodeling, which ultimately facilitates a reduction in PH. It must also be remembered that PH causes ischemia, and due to contraction of the pulmonary arteries, there is a reduction in blood flow to the lungs. Additionally, ischemia in pulmonary arteries causes inflammation and the release of inflammatory mediators, which eventually results in fibrosis of lung tissue. Due to its potent anti-inflammatory properties, curcumin would seem well-suited and potentially effective for either preventing fibrosis, or at least reducing fibrosis, in lung tissue. However, despite the positive effects observed in animal studies, the protective effects of curcumin on PH in human clinical trials has not yet been explored. Thus, it is strongly suggested that clinical studies be conducted with curcumin to test the efficacy of this dietary phytochemical as an adjunct to traditional drug therapy in the treatment of PH.

Conflicts of Interest The authors declare no conflict of interest.

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Protective Effects of Curcumin in the Reproductive System: Anti-toxic, Semen Cryopreservative, and Contraceptive Actions

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Abstract

Human daily exposure to various chemical and biological agents is growing due to modern life, and most of these chronic or acute exposures lead to important recognized toxicities. Multiple tissues and body systems could be affected following these exposures and among them is the human reproductive system, which is very vulnerable to toxins. Here we focus mainly on the male reproductive system, and available data show that various exogenous materials could have negative effects on male reproductive parameters. Interestingly, the well-known antioxidant natural product curcumin may have properties which could diminish these toxic effects. Curcumin has also shown some promise in the cryoprotection of sperm samples through its antioxidant potential. Finally, limited data exists on the putative contraceptive activity of curcumin. This narrative review aims to appraise the activity of curcumin within these topics through the available data.

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Keywords

Curcumin · Male reproductive toxicity · Antioxidant · Sperm cryopreservation · Contraceptive · Anti-toxic

1 Introduction

Infertility is a condition defined as the inability to conceive despite the couple having at least a year of unprotected and regular sexual intercourse. Infertility affects between 8% and 12% of couples worldwide, and males are responsible for approximately 20–30% of infertility cases. Besides common factors such as infection, systemic disease, lifestyle and nutritional status, and endocrine factors, there are a number of male-specific factors that affect fertility, which can be categorized into testicular or post-testicular deficiencies [1].

Generally, testes contain large amounts of polyunsaturated fatty acids and maintain a very high rate of cell division and mitochondrial oxygen consumption, which make them vulnerable to oxidative stress. In addition, humans are inevitably exposed on a daily basis to numerous natural and synthetic compounds which are potentially toxic in certain concentrations to some cells or tissues, and this of course includes the male reproductive system. It is possible that antioxidants could improve the capacity of the body to overcome oxidative stress and therefore reduce risk of toxicity to reproductive tissues [2].

Curcumin is the major curcuminoid component of turmeric (*Curcuma longa*), which is a medicinal plant and curry spice. Curcumin has been reported by some researchers to display several promising characteristics including anti-inflammatory, antioxidant, anti-carcinogenic, antiviral, and anti-infective activities along with wound-healing and detoxifying properties [3–7]. Among them, antioxidant and anti-inflammatory properties are mainly associated with the desirable preventive or putative therapeutic properties of curcumin owing to its role in free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNA and proteins in different pathological conditions [8–10]. There is a small but increasing body of evidence that supports the anti-toxic potential of curcumin against male reproductive toxicity [11], and also curcumin has been investigated for its potential to reduce oxidative damage during semen cryopreservation [12]. On the other hand, it seems that curcumin could have other important effects on the reproductive system, e.g., contraceptive actions when used at higher doses. In this review we intended to critically summarize the available evidence on the possible roles of curcumin in male reproductive health. To this end, literature searches were carried out using two main databases, SCOPUS and PUBMED. Search terms included "male reproductive," "reproductive toxicity," "sperm," "semen," "testicular toxicity," "spermatozoa," "seminiferous," "oocyte," "ovum," "oocyte," "curcumin," and "*Curcuma longa*," in order to identify and extract the available documents.

2 Male Reproductive Toxicity

Daily exposure to compounds which are potentially toxic to the male reproductive system could happen routinely in the living or working environment. Primary or secondary exposure sites of such toxic materials include the testis, epididymis, mature sperm, and the hormonal regulatory system [13]. Hazardous agents that affect these target sites determine the pathogenesis and outcome of male reproductive toxicity that includes male sexual dysfunction, semen abnormalities, and abnormal birth outcomes consisting of spontaneous abortion, stillbirth, death, congenital defect, and low or very low birth weight. The ultimate aim of male reproductive toxicology studies is to help to find practical ways to improve the overall reproductive health and potential of men and also care for the health of their potential offspring [14].

One of many mediators and contributory factors of male infertility has been identified to be oxidative stress. While normal physiological processes of sperm – for instance capacitation, hyperactivation, acrosome reactions, and signaling processes to ensure appropriate fertilization – are related to

low and controlled concentrations of reactive oxygen species (ROS), increased oxidative stress is significantly harmful to sperm function and could be related to male infertility. Therefore, the balance between antioxidant activity and ROS plays an important role in male fertility [15].

3 Curcumin and Its Major Biological Actions

Curcumin (diferuloylmethane) is a natural polyphenol and the major curcuminoid ingredient of turmeric and is extensively used worldwide, especially among Asian populations for its coloring and flavor-enhancing characteristics in curries and mustards [16]. It is also used in cosmetic products and in some preparations intended for medicinal use [9]. The World Health Organization (WHO) records that an acceptable daily intake of curcuminoids as a food additive is in the range of 0–3 mg/kg, and also curcuminoids and turmeric products have been characterized as generally safe for consumption by the US Food and Drug Administration (FDA) [16–18]. Curcumin is a multitarget natural compound with a variety of pharmacological activities ranging from anti-inflammatory and antioxidant potential [19] to anti-proliferative [20] and anti-angiogenic [21]. In addition, a number of *experimental and* clinical studies report efficacy against different disease and health problems, for instance, various cancers [22], inflammatory bowel disease [23, 24], osteoarthritis [25], metabolic syndrome [26], depression [27], respiratory [28], and neurodegenerative disease [29]. As such, curcumin is one of the most extensively studied natural products although it remains on the market as a dietary supplement rather than a medicinal product.

Two important mechanisms which have been extensively investigated in terms of the pharmacological effects of curcumin are its antioxidant and anti-inflammatory properties [9, 30] as demonstrated in a number of in vitro and in vivo studies [31]. There is evidence that curcumin increases serum SOD and catalase activities and GSH concentration and reduces serum lipid peroxides [9, 30].

4 The Possible Protective Effects of Curcumin Against Male Reproductive Toxicity

Various therapeutic effects of curcumin have been described in a number of published reports, and among these, the anti-inflammatory and antioxidant potentials are of particular interest and importance. These two main activities along with other pharmacological actions of curcumin might further reveal any anti-toxic potentials of curcumin. Herein, we will review in depth the in vivo data showing the anti-toxic potential of curcumin in the male reproductive system (Fig. 1, section 1).

Firstly, the potential for toxic effects of curcumin on the reproductive system must be evaluated. The possible toxicity of curcumin on fertility, reproduction, and multigeneration has been studied by Ganiger et al. through feeding two generations of Wistar rats with three different doses of curcumin 1500, 3000, and 10,000 ppm (categorized as low, medium, and high). The authors showed that no sign of reproductive toxicity was observed even at high dose of 10,000 ppm for 70 days oral intake of curcumin. They reported that the 10,000 ppm concentration was equivalent to the ≈ 850 to 1000 mg/kg body weight in male and females for two generations [32]. On the other hand, a study by Murphy et al. showed that some reproductive parameters including seminal vesicle weight and amount of testicular testosterone could be reduced by intravenous-administered PEGylated curcumins, though other reproductive parameters remained unchanged [33]. Studies on the adverse effects of curcumin in higher doses especially on sperm characteristics will be discussed in the next section of this review. Therefore, besides other toxicological data from curcumin, it seems reasonable to conclude that curcumin itself is probably not toxic to the reproductive system, although we note that this is based on limited published data [34].

The possible protective effects of curcumin against other toxic agents known to adversely affect male reproductive function are summarized in Table 1, and here we expand on this.

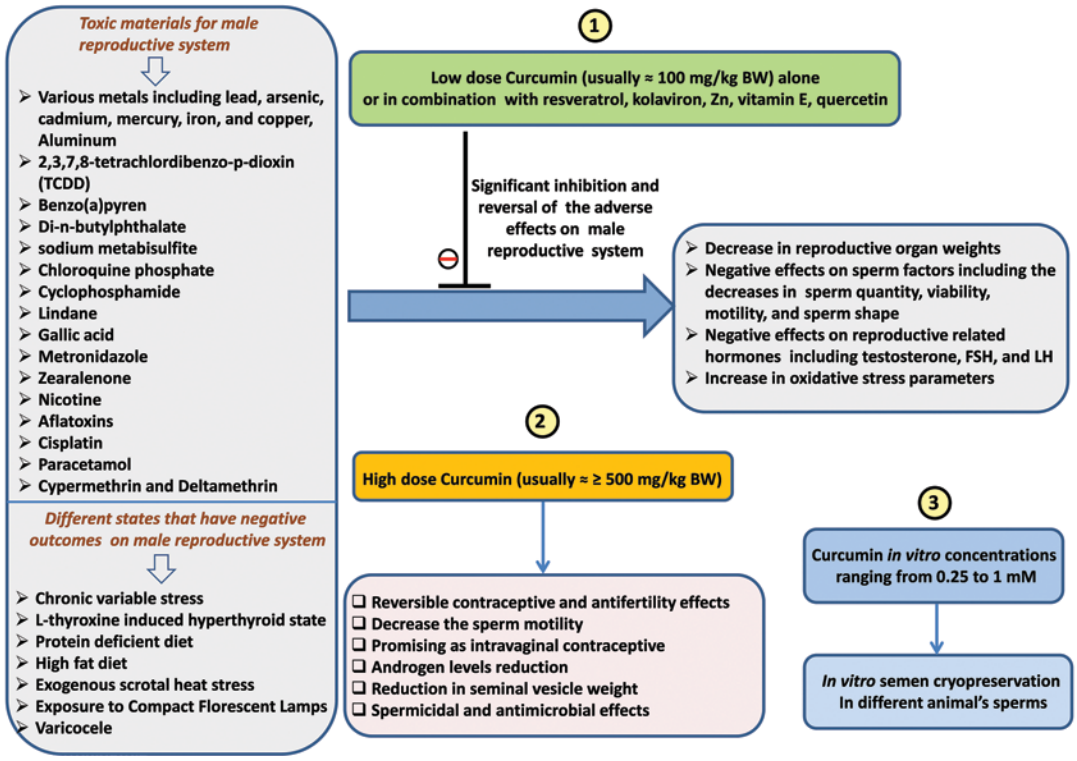

Fig. 1 An overview of the various different effects of curcumin on the male reproductive system (1). Curcumin can be used alone or in combination with other antioxidants as effective inhibitor against a wide variety of reproductive toxicants (2). Curcumin may be of value in semen cryoprotection (3). Curcumin is safe and may have effective (and reversible) contraceptive properties in higher doses

4.1 Environmental and Dietary Toxins

Aflatoxicosis is an important health concern in many developing countries, and regulatory agencies attempt to enforce careful control of aflatoxin content in foods by the suppliers. Aflatoxicosis has damaging effects on many organs, and among these, it can be very harmful to the reproductive system. Curcumin has shown some potential in ameliorating aflatoxicosis, and we have previously reviewed this topic proposing detailed mechanisms [56]. Here, the anti-toxic potential of curcumin in the male reproductive system will be discussed. In order to study the potential protective effect of curcumin, male Swiss strain albino mice were subjected to 45 days oral administration of aflatoxin (750 or 1500 μg/kg body weight/day) which significantly reduced the caput and cauda epididymis weight, while in a group of mice receiving co-administration of curcumin and aflatoxin, these changes were ameliorated in a dose-dependent fashion that might be attributable to the antioxidative properties of curcumin [35]. In addition, another group which also evaluated the protective effect of curcumin on reproduction in the same strain, with the same dose and period of aflatoxin and curcumin administration, concluded that curcumin could improve all reproductive parameters influenced by aflatoxin including sperm quantity, viability, mobilization, and morphological characteristics [36].

Zearalenone (ZEA) is another toxic compound sometimes found in contaminated food products and induces mycotoxicosis with a significant impact on the reproduction of domestic animals, especially pigs. Qin et al. demonstrated

Table 1 Animal studies on potential protective effects of curcumin against different types of male reproductive system toxicants

Toxicity induction	Evaluated toxicity parameters	Curcumin dosage	Major outcomes	Ref
Aflatoxin (750 or 1500 μg/kg/day)	Caput and cauda epididymis weight	2 mg for 45 days	Dose-dependent amelioration of weight reduction in caput and cauda epididymis	[35]
Aflatoxin (750 or 1500 μg/kg/day)	Sperm count, viability, motility, and sperm morphologic features	2 mg for 45 days	Treatment with curcumin along with aflatoxin ameliorated aflatoxin-induced sperm count, immobilization, and viability and improved the morphologic characteristics of the sperm	[36]
Metal mixtures including lead, arsenic, cadmium, mercury, iron, and copper (28 days)	Oxidative stress markers, testicular enzymes, and histopathology	100 mg/kg body weight	Counteracted oxidative stress and improved antioxidant status of the testes tissue along with the restoration of testicular enzyme activity	[37]
Aluminum (10 mg/kg BW) for 28 days	The quality of sperm (morphological normality, sperm count, motility, and viability) and testicular structure and weight as well as hormonal parameters of LH and testosterone levels	10 mg/kg body weight	Significant reversion of aluminum adverse effects on testis and sperm quality	[38]
Cadmium (1 μg/kg/day)	Oxidative stress (increased TBARS levels and decreased superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) levels), histological alterations (necrosis, edema, etc.), and spermatological damage (decreased sperm motility and sperm concentration and increased abnormal sperm rate)	100 mg/kg for 3 days	Partial reversion of toxic effects of cadmium on the reproductive system	[39]
Nicotine (0.4 mg/kg/day) for 14 or 28 days	Testicular alterations (degeneration of spermatogenic cells, Sertoli cells, and Leydig cells)	200 mg/kg for 14 and 28 days (orally)	Decreased degeneration of spermatogenic, Sertoli, and Leydig cells. Curcumin might be a potential therapeutic agent for testicular injury caused by nicotine addiction	[40]
Nicotine (0.5 mg/kg) for 28 days	Testis weight and testosterone hormone, sperm characteristics, morphologic analysis of sperm count and motility, and histological analysis	10, 30, and 60 mg/kg	Dose-dependent increase in reproductive indices in most of the groups	[41]

(continued)

Table 1 (continued)

Toxicity induction	Evaluated toxicity parameters	Curcumin dosage	Major outcomes	Ref
Metronidazole therapeutic dose (165 mg/kg/day) or high dose (500 mg/kg/day)	Testis volume and weight, total epithelium volume, and the round or long spermatid	100 mg/kg/day	Ameliorates adverse parameters in mice that received the therapeutic dose of metronidazole but not in the high-dose-treated group. Spermatocyte protection was observed in both therapeutic and high-dose-treated mice	[42]
Lindane (pesticide) 30 mg/kg for 14 and 28 days	Testes and epididymis weight, sperm head counts, sperm motility, abnormal changes in sperm morphology, biochemical changes in endogenous antioxidants and oxidative enzymes	100 mg/kg BW in pretreatment, post-treatment and combination groups	Curcumin administration was able to ameliorate lindane-induced reproductive toxicity in pretreatment, post-treatment and combination groups	[43]
Nicotine (0.5 mg/kg/day)	Decreased testosterone level, count, motility of sperms, and testis weight	10, 30, and 60 mg/kg	Nicotine administration significantly decreased testosterone level, count and motility of sperms, and testis weight compared to control group. However, increasing the dose of curcumin significantly increased reproductive indices in most of the groups	[44]
Imidacloprid 45 and 90 mg/kg for 28 days	Decrease in total epididymal sperm count, sperm motility, live sperm count, 3b-HSD and 17b-HSD enzymatic activity, and testosterone concentration Increase in gamma-glutamyl transpeptidase, lactate dehydrogenase-x, and sorbitol dehydrogenase	100 mg/kg	Most of the toxicity parameters were minimized by curcumin administration	[45]
Cyclophosphamide (CP) 100 mg/kg	Malondialdehyde (MDA) level and reductions in superoxide dismutase (SOD) activity and glutathione (GSH) content in mouse testis along with reproductive organ weight	30 mg/kg	Zn(II)-curcumin significantly ameliorated CP-induced reductions in body and reproductive organs weights. Zn(II)-curcumin dose-dependently ameliorated CP-induced reproductive system impairments, by improving sperm parameters (sperm count, viability, motility) and reducing serum testosterone and histological alterations	[46]
Sodium metabisulfite (7 and 70 mg/kg/day)	Reduction in volume reduction of seminiferous tubule, tubular epithelium, and tubule length and increase in connective tissue volume	100 mg/kg/day	Significantly ameliorated the reduction in volume of seminiferous tubule, tubular epithelium, and tubule length and the increase in connective tissue volume	[47]

Table 1 (continued)

Toxicity induction	Evaluated toxicity parameters	Curcumin dosage	Major outcomes	Ref
Chloroquine phosphate 100, 200, 300 mg/kg, and high dose of chloroquine (300 mg/kg) for 45 days	Terminal body weight and tissue weight, biochemical and histopathological analysis	80 mg/kg BW for 45 days	Mitigated chloroquine phosphate-induced oxidative damage in mice, which could be the result of its role as an antioxidant that combines free radical scavenging	[48]
Lindane pesticide 100 mg/kg BW	Testes and epididymis weight, sperm head counts, sperm motility, abnormal changes in sperm morphology, biochemical changes in endogenous antioxidants and oxidative enzymes	100 mg/kg BW for 14 and 28 days	Ameliorated lindane-induced reproductive toxicity in pretreatment, post-treatment, and combination groups	[49]
2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) 50 ng/kg BW per day	Reproductive organ weights, sperm concentration, and sperm motility	80 mg/kg/BW per day	While reproductive organ weights, sperm concentration, and sperm motility tended to decrease with TCDD exposure, these effects tended to be close to normal levels with curcumin treatment	[50]
Cypermethrin (2 mg/kg BW) and deltamethrin (2 mg/kg BW)	Reproductive organs weight, sperm count, sperm motility, level of sex hormones viz. testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), steroidogenic enzymes viz. 3β-hydroxyl steroid dehydrogenase (3β-HSD) and 17β-HSD, non-enzymatic antioxidant glutathione (GSH) and enzymatic antioxidants viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) activity, sperm abnormalities, lipid peroxidation (LPO), and the histoarchitecture of testes	100 mg/kg BW	Curcumin and quercetin protected against cypermethrin- and deltamethrin-induced reproductive system toxicity and oxidative damage in rats. The increases in activities of 3β-HSD and 17β-HSD with concomitant increases in testosterone were mainly responsible for ameliorating effects of curcumin and quercetin. Curcumin showed slightly better activity compared to quercetin	[51]
12 h daily exposure to compact florescent lamps (CFLs) for 45 days	Gonadotropin hormones and prolactin levels, histopathological and histomorphometrical analysis of the testis	20 μM	Curcumin supplementation following CFL exposure reversed changes to serum levels of follicle-stimulating hormone, prolactin, testicular weight, sperm motility, tubular differentiation index, and spermiation index	[52]

(continued)

Table 1 (continued)

Toxicity induction	Evaluated toxicity parameters	Curcumin dosage	Major outcomes	Ref
Lead acetate 50 mg/kg BW orally once a day for 35 days	Levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in testicular tissue, and sperm count, motility and viability in the epididymis, and histopathological studies of testes	100 mg, 200 mg, and 400 mg/kg BW orally once a day for 40 days	Administration of curcumin significantly improved the histopathology in testis, increased the sperm count, motility, and viability and also significantly increased the SOD, GPx, and decreased MDA in testis of lead acetate-treated rats	[53]
Cisplatin A single dose of 5 mg/kg	Histological, stereological, and immunohistochemical analysis, and transmission electron microscopy	100 mg/kg/day	Curcumin prevented caspase-3 activation and protected both testicular tissue and spermatogenesis	[54]
Cisplatin A single dose of 7 mg/kg	Body and testicular weight, plasma testosterone level, LPO level, glutathione peroxidase activity, GSH level, NO level, evaluation of testicular spermatogenesis, evaluation of testicular fibrosis and immunohistochemical studies	200 mg/kg/day	Curcumin administration prevented a reduction in testicular weight, significantly increased the testosterone level, reduced the increase of iNOS expression in the testicular tissue of cisplatin-treated rats	[55]

that curcumin pre-treatment significantly suppressed ZEA-induced oxidative stress in porcine granulosa cells [57], providing another example of its protective effect.

Chronic or acute exposure to a wide variety of metals has been proposed to be a significant factor in the pathogenesis of male or female reproductive toxicity [58, 59]. Curcumin has shown noteworthy actions to ameliorate this kind of toxicity. One study examined the effectiveness of curcumin (100 mg/kg body weight) in attenuating alterations in histoarchitecture of testes due to oxidative stress induced by a 28 days exposure of male rats to a mixture of metals including lead, arsenic, cadmium, mercury, iron, and copper. The analysis of oxidative stress markers, testicular enzymes, and histopathology revealed that metal mixture-induced oxidative stress could be counteracted by co-administration of curcumin and also that the antioxidant status of the teste tissue could be improved along with the restoration of testicular enzymes activity [37]. In addition, curcumin had a protective effect on aluminum-induced male reproductive toxicity in an experimental rat model, which is possible to attribute to its antioxidative and antiapoptotic properties. Curcumin was able in improving the quality of sperm (morphological normality, sperm count, motility, and viability) and testicular structure and weight, as well as LH and testosterone levels in the curcumin-treated aluminum-exposed group compared to the curcumin-untreated arm [38]. An ameliorating effect of curcumin was also observed in the reproductive toxicity of another metal, cadmium. A rat model of acute cadmium toxicity induced by 3 days administration of 1 mg/kg/day of this heavy metal was either treated or untreated with 100 mg/kg curcumin. Evaluation of oxidative stress, sperm quality, and tissue damages in male rats showed that cadmium infertility could be partially prevented or reversed using curcumin. Again, the mechanisms behind these effects of curcumin may be attributable to reduction in oxidative stress and histopathological changes [60]. Aktas et al. showed that the protective effect of curcumin against cadmium toxicity in rat testes is through the anti-apoptotic effects of curcumin [39].

Another heavy metal in the form of lead acetate induces testicular damage in rats manifested by decreased SOD, GPx, and increased MDA levels along with decreased sperm count, motil-

ity, viability, and altered testis histopathology (testicular damage, necrosis of seminiferous tubules, and loss of spermatid). Administration of curcumin significantly improved the histopathological changes in testes, increased the sperm count, motility, and viability, and also significantly increased concentrations of SOD and GPx and decreased MDA in testis of lead acetate-treated rats [53].

Nicotine also has detrimental effects on sperm quality in terms of motility and morphological characteristics, as well as sperm quantity. It also impairs testicular structure and function which leads to reduction in the levels of serum testosterone and estradiol [61–63]. Oxidative stress and DNA damage induced by nicotine use (e.g., in tobacco products) or exposure could result in a reduction in male fertility. Jalili et al. showed that curcumin dose dependently reversed the nicotine-induced reduction in testosterone level, sperm number and motility, and testis weight [41].

In another study, the potential ameliorating effects of curcumin on nicotine-induced testis damage in mice has been evaluated. Both blood level of testosterone and testis tissue samples were compared among groups that were treated with nicotine alone or treated with a combination of nicotine and curcumin (200 mg/kg). It was revealed that the nicotine-/curcumin-treated group showed time-dependent lesser testicular alterations in the form of spermatogenic cell, Sertoli cell, and Leydig cell degeneration. Testosterone level was also higher in the nicotine-/curcumin-treated group in comparison with the nicotine-alone arm [40].

2,3,7,8-Tetrachlordibenzo-p-dioxin (TCDD) is an environmental contaminant which belongs to the category of highly toxic, persistent organic pollutants that accumulate in animal fat and plant tissues [64]. Curcumin (80 mg/kg BW per day) was shown to efficiently reverse the male reproductive adverse effects of subchronic doses of TCDD in rats to an almost normal level in terms of reproductive organ weight, sperm concentration, and sperm motility parameters [50]. Interestingly, Sharma et al. investigated the adverse effects of cypermethrin and deltamethrin (two widely used insecticides) on the reproductive systems of male Wistar rats and showed that curcumin alone or in combination with quercetin could significantly inhibit many of the toxic effects of these insecticides mainly through the influence on regulation of sexual hormones [51].

The pesticide lindane induces adverse effects on the male reproductive system manifested by decreased sperm motility, testes and epididymis weight, sperm head counts, and increased abnormal head or tail morphology. Curcumin (100 mg/kg bw) in all forms of pretreatment, combination, and post-treatment ameliorated reproductive adverse effects induced by lindane in a study using rats [49].

Another type of environmental stressor is UV radiation, and Khalaji et al. [52] showed that 12 h daily exposure to compact florescent lamps (CFLs) for 45 days could have negative outcomes on sperm motility and sperm shape, follicle-stimulating hormone (FSH), prolactin, and testicular weight in rat models. Their study showed that daily IP administration of curcumin (20 μM) could reverse all of the reported adverse effects of CFL exposures.

Testicles are also susceptible to sodium metabisulfite, which is a commonly used disinfectant and preservative agent. It has been shown that curcumin (100 mg/kg/day) significantly ameliorates the reduction in volume of the seminiferous tubule, tubular epithelium, and tubule length while increasing connective tissue volume in Sprague-Dawley rats challenged by sodium metabisulfite at doses of 7 and 70 mg/kg/day [44].

4.2 Nutritional and Lifestyle Factors

Both female and male infertility conditions are suggested to be in part related to some of lifestyle and nutritional factors [65, 66]. Interestingly, curcumin has shown beneficial effects in these types of infertilities. In this context, a positive effect of curcumin on the male reproductive system has been revealed by Ahmed-Farid et al. In this study, the effectiveness of 141-nm-sized curcumin nanoemulsion on reproductive performance of

male juvenile rats was investigated. Subjects were fed a protein-deficient diet for 75 days, with curcumin treatment added in the last 15 days in three daily doses of 50 mg/kg curcumin or 2.5 or 5 mg/kg of curcumin nanoemulsion. It has been concluded from reproductive performance (mass motility, percentage of progressive motility of spermatozoa and normal spermatozoa) and biochemical and histopathological examinations that curcumin (50 mg/kg) and nanoemulsion curcumin (5 mg/kg) considerably improved the adverse effects of a protein-deficient diet that impacted on the male reproductive system [67].

Another malnutrition state which adversely affects male reproductive function is the high-fat diet [68]. Obesity brings about sperm number reduction and overall dysfunction in the reproductive system [69, 70]. A study set out to investigate whether or not curcumin can ameliorate high-fat-induced dysfunction in spermatogenesis. Accordingly, spermatogenesis in male Sprague-Dawley rats on 8 weeks of a high-fat diet was evaluated based on testis-to-body weight ratio, estradiol, testosterone, follicle-stimulating hormone, luteinizing hormone, and leptin blood levels as well as histopathological examinations and evaluation of germ cell apoptosis. While high-fat diet leads to disturbances in spermatogenesis as a result of abnormality in the hormone level, decrease in testis-to-body weight ratio, atrophy in seminiferous tubules, and decrease in the number of spermatogenic and interstitial cells, 8 weeks of orally administered treatment with 100 mg/kg/day curcumin alongside the high-fat diet beneficially improved the atrophied testes, reversing all the abovementioned parameters [68].

Another situation that could be harmful for male reproductive capacity and in turn leads to male infertility is exogenous scrotal heat stress-induced testicular injuries. The potential protective effect of curcumin in this damaging situation has been evaluated in the following mouse model: the scrotums of adult mice fed for 14 consecutive days with curcumin (20, 40, or 80 mg/kg.day) were subjected to a 43 °C heat stress for 20 min on 7th day of the treatment. It was shown that adverse effects of heat-induced stress such as testicular weight reduction, spermatogenic cell failure, and change in oxidative balance were substantially improved by curcumin in a dose-dependent manner [71]. Varicocele is also a common reason of infertility in men, and some reports have shown a relationship between elevated nitric oxide (NO) within dilated spermatic veins and idiopathic varicocele. In yet another study, administration of curcumin decreased the excessive release of nitric oxide in varicocelized male rats, which led to improvement of sperm parameters but no effect on epididymis and testis weights [72].

Curcumin could also have beneficial effects in suppressing male infertility induced by protracted and repeated exposure to chronic variable stress (CVS) such as cold restraint, the inclination of home cages, flashing light, isolation, damp bedding, and water deprivation. It is reported that curcumin did the protective effects through modulation of reproductive-related hormones including testosterone, FSH, and LH and apoptosis of seminiferous tubule cells [73].

4.3 Drugs

Besides other mentioned causes of male infertility, drug consumption (especially cytotoxic drugs) can be an important reason that interacts with male fertility through hormonal or non-hormonal mechanisms which have been reviewed in depth by Semet et al. [74]. Interestingly, there are some reports to show the positive potential of curcumin in reversing the toxic actions of drugs to the male reproductive system. Metronidazole, a widely used antibiotic with anti-anaerobic and antiprotozoal effects [75], negatively affects the quality of sperm and the structure of testis by reducing the number of spermatocytes and spermatids along with reducing the germinal epithelium volume. A group of researchers evaluated the ameliorating effect of curcumin in balb/c mice which had received therapeutic (165 mg/kg/day) or high-dose (500 mg/kg/day) metronidazole. Analyzing the testis volume and weight, total epithelium volume and the round or long spermatid, it was shown that curcumin (100 mg/

kg/day) ameliorates these parameters in the mice receiving the therapeutic dose of metronidazole but not in the high-dose-treated group, while spermatocyte protection was present in both therapeutic and high-dose-treated mice [42].

Chloroquine phosphate, one of the most widely used drugs against malaria, displayed adverse male reproductive effects in Swiss albino mice. Co-administration of curcumin (80 mg/kg b.w.) with chloroquine phosphate for 45 days alleviated the toxic effects of chloroquine [48].

Besides being an effective, widely used antineoplastic drug, cisplatin has reproductive adverse effects. Curcumin has been shown to decrease the expression of nuclear factor-κB (NF-κβ)/p65, caspase-3, and 8-deoxyguanosine (8-OHdG) in germinal epithelium and Leydig cells of rats to successfully counter the cisplatin-induced damage [54]. Another study on this subject also concluded that the cellular/biochemical mechanisms of cisplatin-induced testicular toxicity is through MAPK and NF-kB activation, causing a decrease in testicular weight, plasma testosterone levels, activities of glutathione peroxidase (GSH-Px) and glutathione (GSH) levels, and increase in the level of malondialdehyde (MDA) and nitric oxide (NO), and that these gonadotoxic effects of cisplatin could be reversed by curcumin administration in rats [55]. Testicular apoptosis of germ cells through Cas-3 and Bax pathways is one of the toxic side effects of cisplatin, and Gevrek et al. showed this could be prevented to some extent by administrating a combination of curcumin and vitamin E [76].

Male reproductive toxicity is one of the side effects of another chemotherapy drug, cyclophosphamide (CP), which is associated with oxidative stress. CP also has a reducing effect on the serum and testis levels of Zn, which is an essential trace element required for maintenance of germ cells, progression of spermatogenesis, and regulation of sperm motility. It has been shown that combination of Zn and curcumin is effective for protecting against reproductive damage of CP due to a synergistic effect of this combination in reducing oxidative damage [46]. Another study described the co-administration of curcumin (100 mg/kg/day) and gallic acid (100 mg/kg/day) for 30 days in male rats and the in vivo evaluation of testis oxidative stress, sperm quality, histopathology, and steroid production. Curcumin was reported to prevent impairment in sperm quality induced by gallic acid treatment and also blocked the inhibitory effect of gallic acid on plasma testosterone level, glutathione level, and activities of glutathione peroxidase, catalase, superoxide dismutase, and the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD in the rat testis. The in vitro evaluation of the expression of inflammatory responsive genes in a Sertoli cell line treated with (25–100 μM) concluded from the obtained results that curcumin with stimulatory reproductive effects could positively protect testis from the toxic effects of gallic acid via the antioxidative properties that are related to decreased lipid peroxidation and not its effects on the expression of inflammatory cytokines [77]. The effectiveness of curcumin in alleviating paracetamol-induced testis toxicity has been compared with that of N-acetyl cysteine (NAC). Despite being a safe antipyretic analgesic drug in therapeutic doses, paracetamol (PCM; acetaminophen; N-acetyl-p-aminophenol) is hepatotoxic and neurotoxic, and also it could be gonadotoxic in overdose. NAC and curcumin are both well-known potent antioxidants. The researchers concluded from tissue oxidative stress, testosterone level, sperm quantity, motility, and morphological analysis that both curcumin and NAC have reproductive protective effects, though this was shown to be stronger for NAC [78].

In conclusion, it could be suggested that in experimental studies, curcumin is able to alleviate and sometimes reverse the toxic or adverse effects of many substances or abnormal conditions and situations in male reproductive systems.

4.4 Co-administration of Curcumin with Other Protective Agents

Curcumin has also shown some therapeutic promise in male reproductive system toxicities

when co-administered with a number of other compounds. The combination of two natural products with cryoprotective and antioxidative properties, curcumin and resveratrol, has shown that they could synergistically protect the male germ cells from benzo(a)pyrene. This is an environmental toxicant resulting from incomplete combustion of organic fuels and also can be found in tobacco smoke, coal tar, and some foods (especially barbecued). This particular study was done on an isolated testicular germ cell population from adult male Wistar rats. This co-treatment protects the germ cells from P53-mediated apoptosis, modulates MAPKs, prevents oxidative stress, regulates the expression of pro- and anti-apoptotic proteins, and also improves sperm cell count and motility as well as serum testosterone levels [79].

Another substance which has been co-administered with curcumin, and evaluated for ameliorating testicular damage, is kolaviron, a biflavonoid derived from the seeds of *Garcinia kola*. Testicular damage was induced in male rats through 9 days administration of 2 g/kg di-n-butyl phthalate (dibutyl phthalate (DBP)). This toxicant is a widely used solvent dye in cellulose plastic with considerable population risk of environmental release during use or disposal through consumer products, diet, and medical devices. Di-n-butyl phthalate toxicity can lead to a significant reduction in the relative testicular weight and a marked necrosis of testicular epidermal cells and degeneration of seminiferous tubules. Kolaviron and curcumin combined had protective effects on the testes manifested by evident maintenance of structure and function of active seminiferous tubules similar to that of the control group [80]. In a study by Sahoo et al., it has been shown that curcumin and vitamin E both effectively protect the testis of L-thyroxine (T4)-induced hyperthyroid rats from oxidative stress-induced damage manifested by decreased number and increased mortality of epididymal sperm. Interestingly curcumin and vitamin E were capable to increase the glutathione peroxidase (GPx) activity in the postmitochondrial fraction (PMF) when they administered in combination, while none of them were able to express this kind of increment. Curcumin could not increase total sperm count or the impaired percentage of live sperm resulting from the hyperthyroid state (unlike vitamin E), but curcumin did appear to protect testis from T4-induced oxidative stress damage by restoring antioxidant enzymes to the level of a euthyroid rat [81].

5 The Potential Application of Curcumin in Sperm Cryopreservation

Semen cryopreservation has become very important in various reproductive-assisted technologies. Different cryoprotectant materials are currently available, but finding the best material is essential to preserve reproductive capacity. Reactive oxygen species (ROS) could be generated during the freezing protocols and therefore addition of effective antioxidants during this process is vital. Curcumin, as a natural phenol with well-known antioxidant properties, has attracted the attention of researchers as a potential supplement with efficient semen cryoprotective capability. There is experimental evidence regarding the positive impact of curcumin on the quality of cryopreserved boar, bovine, Merino ram, rat, and human sperm (Fig. 1, section 2). Cryoprotection of semen in animals is important not only from a financial point of view in artificial insemination but also in preserving endangered species. We review experimental findings on animal and human sperm cryoprotection in the following section. Supplementation of curcumin into freezing extender, which is a liquid diluent that is added to semen in order to preserve its fertilizing ability [82], improved the criteria of progressive motility and acrosome integrity but not overall viability in freeze-thawed semen samples. The optimum concentration of curcumin in boar sperm cryopreservation was determined to be 0.25 or 0.50 mmol/L [12]. There are also some studies on the cryoprotective effect of curcumin on bovine semen. One of these, which was conducted to evaluate the effect of curcumin on sperm parameters of bull spermatozoa following the freeze/thawing process, found that adding

0.5 mM curcumin to the semen extender decreases the total spermatozoid abnormality and increases the maintenance of total glutathione level. When it was combined with dithioerythritol, the protective effect of curcumin on membrane functional integrity was significantly higher compared to the control group, while these combined antioxidants did not significantly affect the lipid peroxidation and antioxidant potential levels [83]. In another study, the protective effect of curcumin (50 μmol/L) on bovine spermatozoa during the semen freezing and thawing process was shown based on preventive effects on oxidative stress related to lipid peroxidation, reactive oxygen, and superoxidase overgeneration, which finally leads to enhanced functional activity of spermatozoa [84]. In other study by Omur et al., curcumin (1 mM) combined with ellagic acid (1 mM) and methionine (1 mM) showed a remarkable positive effect on sperm parameters of Merino ram semen which was undergoing a freeze/thaw process [85]. There are also some studies of note on rat spermatozoid. For instance, in a study conducted by Soleimanzadeh and Saberivand, the effects of curcumin on DNA integrity and the quantity, motility, and viability of sperm along with the total antioxidant capacity of semen during the semen freeze-thawing process were evaluated. They concluded that supplementation of 2.5 mM curcumin significantly improves all sperm- and semen-related parameters [86]. It has been shown that reactive oxygen species-induced alterations in bull spermatozoa could be reversed by 25–50 μmol/L curcumin treatment manifested by preservation of spermatozoa viability and motility, mitochondria activity, and antioxidant characteristics that are related to reactive oxygen species scavenging characteristics of curcumin [87]. When 2.5 mM curcumin was added as a supplement to the freezing extender, the sperm motility of Angora goat following freeze/thaw was improved. In addition, superoxide dismutase activity was higher, and sperm morphology was improved in all doses of curcumin (2.5, 5 or 10 mM) compared to the control, while curcumin was not effective in the elimination of malondialdehyde (MDA) formation and the maintenance of glutathione peroxidase (GSH-PX) activity in cryopreserved goat semen [88].

6 Contraceptive and Antifertility Effects of Curcumin

In contrast to the evidence reviewed above, there are some reports on the inhibitory effect of curcumin on human sperm motility, which propose the potential of developing a new intravaginal contraceptive based on curcumin (Fig. 1, section 3). The question of whether curcumin can act as an effective contraceptive agent has been asked in some studies (Table 2). Following the first reports on the influence of curcumin on sperm motility, function, and in vitro fertilization both in human and murine models along with reversible contraceptive effect of intravaginally administered curcumin in mice [89], further investigations have been done that led to the proposition that curcumin may act as a putative novel nonsteroidal contraceptive, which – if found to be true – would be of great benefits with both spermicidal and microbiocidal properties [96]. Curcumin was evaluated in two aspects, first its inhibitory potential on forward motility of human sperm and second its antibacterial and antifungal effect on common aerobic and anaerobic bacteria as well as yeast strains which are responsible for vaginal disorders including vaginitis, vaginosis, and infection. The in vitro results revealed that curcumin dose-dependently inhibited sperm forward motility and also the growth of all examined bacteria and yeasts which cause vaginosis/vaginitis/infertility/miscarriage, with complete inhibition at concentrations higher than 250 μM [90].

Effects of curcumin on the male reproduction system, including effects on morphology, viability, mobility, and quantity of spermatozoa, testosterone level, and fertility, were seen after 56 and 84 days of daily oral administration of 600 mg/kg aqueous rhizome extract of *Curcuma longa* in male Parkes mice [91]. Also a similar dose of 517 mg/kg turmeric rhizome decoction fed orally to male mice reportedly resulted in decreased sperm motility and caused sperm abnormality

Table 2 Studies (in vitro and in vivo) on potential antifertility effects of curcumin

Curcumin dose – target animal	Main effects	Ref
31.25–500 mM, final concentration (in vitro in murine and human sperm) and 10–500 mg in 10–100 ml volume (in vivo in female mice)	Curcumin affects sperm motility, function, and fertility both in vitro as well as in vivo	[89]
1–1000 mM final concentration in human sperm	Curcumin caused a concentration-dependent inhibition of sperm forward motility with a total block at ≥250 μM concentration and can block bacteria/yeast growth	[90]
600 mg/kg body weight per day for 56 and 84 days, in male mice of the Parkes (P) strain	Reversible suppression of spermatogenesis and fertility	[91]
517.4 mg/kg body weight for 30 days, in male mice	A significant effect in decreasing the sperm motility and morphology. The decoction caused sperm abnormality or asthenoteratozoospermia	[92]
50% EtOH extract of *Curcuma longa* at the dose of 1 g/kg body weight in male rats	Arrest of spermatogenesis and depletion of androgen level	[93]
2 and 4 g/kg feed for 84 days, in New Zealand white male rabbits	Turmeric powder had mild contraceptive effect in male rabbits without deleterious effect on blood characteristics	[94]
30–300 g/ml in washed human healthy sperm	Curcumin had a selective sperm-immobilizing effect	[95]

[92]. Another group also showed that oral administration of 50% ethanol extract of *Curcuma longa* (1 g/kg) comes with 80% reduction in reproductivity of male rats manifested by spermatogenesis arrest and androgen level reduction [93]. The effect of curcumin on semen quality also has been evaluated in rabbits orally fed 2 and 4 g/kg curcumin per day for 84 days. It has been shown that the treated groups had higher number of abnormal sperm and also curcumin in male rabbits had a mild contraceptive effect while its effect on blood characteristics was not deleterious [94].

Some mechanisms were proposed to try to understand these effects. For instance, *Curcuma longa* effects on androgen synthesis was explained by an inhibitory effect on the function of Leydig cells or by inhibiting the hypothalamus pituitary axis, but the important point that should be noted is reversibility of these adverse effects of long-term and high-dose consumption of *Curcuma longa* extract.

It can be concluded that curcumin may act differently in low and high doses, so through the management of administered doses and route of administration, we could potentially benefit from both effects of curcumin in male reproductive system, in form of a potent natural contraceptive or as a sperm cryopreservator in vitro or male reproductive protector against many toxicants in vivo. In addition to the male contraceptives that have been designed based on spermicidal characteristics, inhibitors of testosterone biosynthesis could be another group of male contraceptives with focus on spermatogenesis suppression. Curcumin and its derivatives have been shown to have both effects. A group of researchers screened many natural products and their derivatives including many synthesized curcumin derivatives in an attempt to inhibit 17b-HSD3, an enzyme that catalyzes the last step of testosterone biosynthesis. These compounds were compared with curcumin, and the results showed that while interactions of curcumin with enzymes require relatively high concentrations (IC50 > 10 μM) of curcumin, some of these curcumin derivatives are more potent than the original compound and also that there is a species-dependent difference for the potency and ability of 17b-HSD3 inhibition as the IC50 of curcumin on human testis microsomes is almost 30 times higher the rat equivalent. This inter-species difference was also observed in curcumin derivate potency of enzyme inhibition, and it should be investigated whether the inhibition of 17β-HSD3 can be achieved under normal dietary consumption [97]. Another possible mechanism by which curcumin affects the spermatogenesis could be its histone acetylase inhibitory effect, as it has been shown before that histone acetylation is a core reprogramming

mechanism in spermatogenesis. It has been shown that regulation of histone acetylation plays an important role in zygot development through sequential disassociation of chromatin-associated proteins. Curcumin dose-dependently inhibits the growth of germ cell lines in mice which shows the necessity of more comprehensive investigations on reproductive toxicity of curcumin [98].

Investigations of the effects of nanomicelle curcumin on spermatogenesis in male Wistar rats treated with 7.5, 15, and 30 mg/kg nanomicelle curcumin revealed a remarkable sperm quality reduction manifested by significant decrease in the viability and motility of spermatozoa and increase in DNA-damaged sperm which consequently decreased the in vitro fertilization [99]. The same group also concluded from another study performed on male Wistar rats treated with the same doses of nanomicelle curcumin that this formulation of curcumin reduced the endocrine profile of testis and started the intrinsic apoptosis pathway, which adversely affected spermatogenesis [100]. Another possible mechanism proposed by another group of researchers is concentration-dependent decreases in the sperm motility through modulation of intracellular *p*H and plasma membrane polarization by curcumin [101].

Curcumin has also been proposed to have some potential for clinical application as a novel intravaginal spermicidal agent based on its selective immobilizing effect on human sperm. Curcumin had a time- and dose-dependent effect from sperm motility reduction to complete immobilization in 30 g/mL to 300 g/mL, respectively [95] .Different proposed formulations of vaginal contraceptive based on curcumin have been reported in the literature. One of those is a safe and efficient topical vaginal gel of copper-curcumin b-cyclodextrin, which in its optimized concentration of 1.5% w/w (copper/curcumin) had a complete sperm motility inhibitory effect along with a highly safe in vitro (Hela cells) and in vivo (rats and rabbits) profile. Curcumin is a hydrophobic compound, so b-cyclodextrin was used in this formulation as a carrier, and water solubilizer of curcumin and copper was used as a toxic compound for sperm motility and viability [102]. Another formulation is in-situ-stabilized silver nanoparticles and (copper-curcumin) β-cyclodextrin inclusion complex which was evaluated as a topical gel with three dimensions and with contraceptive, antiretroviral, and microbicide activities based on the experiments conducted on sperm motility, HIV-1 propagation, and *Candida albicans* and *Candida tropicalis* [103].

Curcumin affects multiple cellular signaling and transduction pathways so unintended nontarget effects are not unexpected and a fascinating field of study will be evaluating the potential antifertility adverse effect of curcumin or its novel formulations in the course of administration in antitumor doses. The first report on the effect of a 0.5 mg pegylated water-soluble formulation of curcumin which was intravenously administered daily in male athymic mice revealed that seminal vesicle weight and testosterone level was reduced and spermatogenic function was disrupted. These effects could be attributed to the estrogen-mimicking effect of curcumin [33].

One group of researchers used a Box-Behnken statistical design for optimization of curcumin loaded vaginal hydrogel for gelation temperature, gel strength, mucoadhesive strength, viscosity, and drug release, and then they have evaluated the optimized formulations with a human sperm immobilization test. The optimized formulation was a stable thermosensitive formulation which contained 19.96% poloxamer 407, 3.83% poloxamer 188, and 0.91% HPMC K4M that totally immobilized human sperms in 15 min [104].

7 Conclusions

It can be concluded that curcumin may have a dual function on the male reproductive system. It can be a protective agent when used in animals or humans in low doses (about 100–200 mg/kg) against many toxicants or dangerous situations for spermatogenesis. In addition, there is evidence that support the in vitro protective effect of curcumin in preserving animal and human sperm for further uses in insemination or in vitro fertilization. Finally, curcumin may find a future

application as a safe and efficient contraceptive agent in higher doses of 500 mg to 1 g, and future studies should be designed to explore these possibilities further using robust experimental and clinical methods.

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The Protective Role of Nutraceuticals in Critically Ill Patients with Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) has become a leading health problem with no effective treatment. TBI imposes a significant burden of morbidity and mortality and is a major challenge in the intensive care unit (ICU). The lack of proven effective treatments for TBI is related to the range of severity of injury, the complexity of approaching a disease that involves multiple tissue and cell types, rapid onset of pathophysiology, common comorbidity presentation, and other environmental and developmental factors. However, prompt treatment for TBI is critical, including surgery, intensive care, drugs, and alternative treatments, since cerebral edema can result in a variety of pathologies associated with primary and secondary injuries, as well as death. There is a need for interventions to be performed with the aim of preventing or treating the complications and accelerating the recovery of patients with TBI. Considering that nutritional support, when combined with other TBI treatments, is very effective, in this narrative review we focused on the role of herbal and nutrient supplements, identifying their protective effects on TBI outcomes. Combination of vitamins, amino acids, plant extracts, and herbs as a nutritional support may reduce recovery time in people with TBI, which work synergistically to repair TBI damage and improve areas of brain and body function that are most affected by TBI. Effective nutritional support is an emerging factor that may be added to help improving outcomes of TBI, but further clinical trials and empirical studies are definitely needed in this rapidly progressing field.

Keywords

TBI · Dietary supplement · Intensive care unit · Nutritional support

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1 Introduction

Traumatic brain injury (TBI) is characterized as brain damage caused by an external mechanical force, and is one of the leading causes of morbidity and mortality, as well as a significant challenge in the intensive care unit (ICU) [1, 2]. TBI is a public health issue across the globe with almost no effective treatment, referred to as the "silent or hidden epidemic" [3, 4]. It is estimated that 69 million individuals suffer from TBI annually, with the highest incidence of the disease occurring in Southeast Asia and the western Pacific [5]. The complex nature of TBI causes primary and secondary brain disorders, and these acute or long-term changes affect the health and quality of life (QoL) of patients. As a result, treatment and recovery would place a huge burden on the healthcare system, as well as society and the economy of the country [6, 7].

Brain metabolism is altered when the brain is damaged and neurons are particularly vulnerable to damage from free radicals and dysfunction of the mitochondria. Thus, a pathological phase starts that can take years to repair occasionally. Neuroinflammation endoplasmic reticulum stress and oxidative stress in the brain have been shown to be activated in TBI [8]. Endoplasmic and oxidative stress are among the primary drivers of chronic neuroinflammation, which is itself a triggering factor for tauopathy. In addition, poor functional outcomes were observed in critically ill TBI patients admitted to the ICU [9]. These patients experience severe muscle wasting, which occurs rapidly at the beginning of the ICU stay [10]. In addition, neuromyopathy, a disease that is a major cause of functional disorders, may also occur in these patients [11]. This neuromyopathy modifies nerve conduction and muscle excitability, causing neuromuscular electrophysiological disorder (NED), which leads to muscle wasting and weakness [11–13]. Patients with muscle weakness have elevated levels of plasma cytokines such as Interlukin-6 (IL-6), Interlukin-8 (IL-8), and tumor necrosis factor-α (TNF-α), which are associated with inflammatory and catabolic responses [14].

On the other hand, long-term and persistent pain is a recurring consequence of TBI [15–17]. Chronic pain prevalence in TBI is variable and high, which can reach by up to two-thirds of patients with TBI [18–20]. Post-traumatic headache (PTH) and neuropathic pain due to spasticity and fracture are also very common in TBI patients [21]. In addition to peripheral neuropathic headache and pain, post-TBI pain can originate from several different sources [22]. Muscle spasms, which are often ignored, may lead to pain caused by joint contractions, inflammation, and painful local abnormalities [23]. Other forms of post-TBI pain comprise low back pain (46%), limb pain (39%), and complex regional pain syndrome (CRPS) (12%) [24]. Therefore, chronic pain is a debilitating complication of TBI, which is a major obstacle against effective participation of patients in rehabilitation programs and thus makes it difficult to treat patients. Chronic pain delays the patient's optimal level of activity and independence and negatively affects patient's mood.

In ICU, TBI is associated with prolongation of mechanical ventilation (MV), cognitive impairment, longer stays, muscle wasting or weakness, prolonged and persistent pain, as well as increased morbidity and mortality rates [11, 25]. Therefore, effective treatment is very important to prevent functional disorders and inflammation of the neuromuscular system and also to reduce pain in these patients. To date, the market has not provided any complementary therapies that can reduce the recovery time of TBI. As a result, there is a need for a supplement therapy to shorten TBI recovery time. An intervention that can relieve patients' pain and accelerate the recovery process of TBI patients can subsequently reduce the complications of ICU, including duration of MV and length of stay (LOS) in the ICU. Considering that nutritional interventions, when combined with other TBI treatments, are effective [26], we conducted this narrative review to introduce herbal and nutritional supplements that might exert protective effects on TBI outcomes.

Effective nutritional support, including nutrient and herbal supplement due to their analgesic and anti-inflammatory properties, may be powerful and effective in reducing or eliminating the pain and inflammation. Some of these herbal and nutrient supplements are turmeric extracted from curcumin (*Curcuma longa*), *Boswellia serrata* gum resin, Bromelain (from pineapple), quercetin (as quercetin dehydrate), DLPA (DL-phenylalanine), thiamin (as thiamin hydrochloride), and vitamin B6 (as pyridoxine hydrochloride). Although, to date, the combination of these supplements have not been used in any study in patients with TBI, these components alone have been introduced as anti-inflammatory and antioxidant agents in various diseases as well as in several interventional studies.

2 An Overview of Some Nutritional Components and Their Function

2.1 Turmeric

Turmeric contains the pigment curcumin, which is a polyphenolic phytochemical. Curcumin possesses numerous salutary effects such as anti-inflammatory, antioxidant, and immunomodulatory properties that are relevant for the prevention or treatment of human diseases [27–36]. Evidence has shown that curcumin crosses the blood–brain barrier to some extent [37, 38] and is able to reduce cerebral edema [39], reduce the inflammatory response, foster energy homeostasis, and modulate synaptic plasticity after TBI [40–42] as well as injury due to cerebral ischemia/reperfusion [43, 44]. Curcumin has also been suggested as a promising candidate for the treatment of different central nervous system (CNS) neuro-inflammatory and neurodegenerative disorders.

Cerebral edema and subsequent increase in intracranial pressure (ICP) is among the serious complications of TBI that leads to increased mortality and long-term disability [45, 46]. Larid et al. [39] found that clinically achievable doses of curcumin reduce cerebral edema, decrease pericontusional expression of aquaporin-4 (AQP4), a glial water channel that promotes brain swelling, and increase neurological outcome after TBI in mice. Curcumin's protective effect was linked to a significant reduction in the acute pericontusional expression of IL-1β, a pro-inflammatory cytokine, after TBI. Curcumin also inhibited the activation of AQP4, an astrocytic water channel implicated in the development of cellular edema after head trauma. Curcumin inhibited IL-1β-induced AQP4 expression in cultured astrocytes, which was mediated, at least in part, by reduced activation of the NFκB p50 and p65 subunits (nuclear factor kappa-light-chain-enhancer of activated B cells). Curcumin reduced glial fibrillary acidic protein expression, which serves as a marker of reactive astrocytes, and attenuated phosphorylate p65 immunoreactivity in pericontusional astrocytes, supporting this theory.

Evidence suggests that impaired memory following a TBI is linked to dysfunction in synaptic plasticity-supporting molecular structures such as brain-derived neurotrophic factor (BDNF) [47–49]. In addition, one of the hallmarks of TBI is oxidative stress, which has the ability to cause events that lead to prolonged neuronal activity and plasticity [50, 51]. The anti-inflammatory and antioxidant compound curcumin will minimize the harmful effects of TBI on synaptic plasticity and cognition, according to a study by Wu et al. [43]. These findings support the theory that oxidative stress plays a key role in TBI-related cognitive dysfunction. The study used rats that were fed either a normal diet or a diet high in saturated fat for 4 weeks (n = 8/group), with or without 500 ppm curcumin, before undergoing a mild fluid percussion injury (FPI). The findings revealed that a high-fat diet worsened the deterioration of synaptic plasticity and cognitive function due to TBI [52]. Curcumin supplementation in the diet significantly decreased oxidative damage and restored altered levels of BDNF, synapsin I, and the cAMP-response element binding protein (CREB) following TBI. As a result, curcumin supplementation protects against cognitive dysfunction caused by TBI [52].

In addition, some in vitro [53, 54] and in vivo [44, 55] studies showed the neuroprotective impact of curcumin, though the underlying mechanism remained unclear. Dong et al. [56] found that curcumin's neuroprotective function in mouse TBI was mediated, at least in part, by the NF-E2-related factor (Nrf2) pathway. Edema, cell apoptosis, oxidative damage, and inflammatory reactions were also studied to see whether Nrf2 signaling played a role after curcumin treatment. Curcumin therapy in wild mice resulted in decreased ipsilateral cortex damage, neutrophil infiltration, and activation of microglia, enhancing neuronal survival against TBI-induced apoptosis.

2.2 Boswellia Serrata (BS)

Boswellia serrata (BS) is a tree of the Burseraceae (frankincense) family that grows in India, Africa, and the Middle East [57]. The gummy oleo-resin of BS has been historically used as an anti-inflammatory herbal product and is a potential memory enhancer. Several studies have shown that BS has alleviating properties in inflammatory conditions like asthma, Crohn's disease, and peritumoral brain edema in recent decades [58]. Moreover, BS resin has been shown to have strong antioxidant activity in many conditions that include colitis [59], myocardial ischemia/reperfusion injury [60], pulmonary fibrosis [61], diffuse axonal injury (DAI) [62], and ischemic brain injury [63]. In addition, another bioactive portion of *Boswellia* called AKBA has both anti-inflammatory and neuroprotective properties [64–66]. AKBA has been shown to reduce cognitive and motor complications in mice following TBI and ischemic nerve damage [65, 66].

In TBI patients, DAI is a common brain pathology. DAI has been identified in more than half of all hospitalized patients with moderate to serious head injuries, and it is a leading cause of permanent vegetative state and long-term cognitive/motor disorders in people who have suffered from a severe TBI. A study by Moein et al. [62] suggested that BS resin could significantly reduce the cognitive outcome of patients with DAI.

2.3 Bromelain

Bromelain is one of a group of enzymes that digest proteins extracted from the pineapple fruit or stem [67]. Fruit bromelain and stem bromelain are prepared differently and contain various compositional enzymes. Bromelain is commonly referred to as "stem bromelain," which consists of a combination of various endopeptidases of thiol and other components such as phosphatase, glucosidase, peroxidase, cellulase, as well as a number of inhibitors of proteases. In vitro and in vivo studies indicate that different fibrinolytic, anti-edematous, antioxidant, antithrombotic, and anti-inflammatory properties are exerted by bromelain [68, 69]. Bromelain is highly absorbable in the body without losing its proteolytic ability or having significant side effects. Bromelain has a number of therapeutic uses, including treatment of angina pectoris, surgical trauma, sinusitis, bronchitis, and thrombophlebitis, wound debridement, and improved drug absorption, especially for antibiotics [70–72].

Bromelain stimulates inflammatory mediators in mouse macrophages and human peripheral blood mononuclear cells (PBMC), including interleukin (IL)-1β, IL-6, interferon (INF)-γ, and tumor necrosis factor (TNF)-α [73, 74]. These findings showed that, in combination with the rapid response to cellular stress, bromelain potentially stimulates the healthy immune system. On the other hand, bromelain decreases IL-1β, IL-6, and TNF-α secretion, when immune cells are already activated by inflammatory stimuli [75, 76]. Bromelain is a proteolytic enzyme mixture that decreases inflammation in tissues following trauma or sports injury in general. Bromelain also aids in the reduction of edema by regulating vascular permeability [69, 77].

2.4 Quercetin (QR)

Quercetin (QR) is a natural flavonoid found in different vegetables and fruits, which has gained interest of researchers owing to its mitigating effects against inflammation, tumorigenesis, and atherosclerosis [78, 79]. Similarly, it has the

potential to influence mitochondrial biogenesis via altering different mediators (e.g., transcription factors and enzymes) involved in the inflammation cascade [79, 80]. Furthermore, quercetin can influence mitochondrial biogenesis by lowering the reactive oxygen species (ROS) generation in various cell types.

Several studies have shown that quercetin has anti-inflammatory, anti-coagulation, anti-ischemic, and anti-cancer properties [81–84]. According to Yang et al. [8], quercetin ameliorates cognitive function in TBI rats because of its neuroprotection via suppression of oxidative stress, resulting in the reduction of inflammatory response and consequent neuronal death. In addition, Li et al. [85] found that quercetin administration can potentially reduce brain injury in a TBI mouse model through augmentation of mitochondrial biogenesis and activity mediated by the PGC-1α pathway.

2.5 Vitamins

Vitamins are involved in various processes in the brain [86]. The human body utilizes thiamine for the biosynthesis of acetylcholine and gamma-aminobutyric acid (GABA) that serve as important neurotransmitters. GABA's activity has the potential to slow or stop the neuro-excitotoxicity cascade that ensue post-TBI [87].

Moreover, vitamin B_6 (pyridoxine) is a water-soluble, easily metabolized, and excreted substance with low toxicity [88]. It contains several vitamins, including pyridoxine, pyridoxal, and pyridoxamine, all of which are primarily converted in the liver to pyridoxal 5′-phosphate (PLP) [89, 90]. PLP is a vitamin B_6 active coenzyme that is required for amino acid for the metabolism, catabolism, and transamination of amino acids [89] as well as several physiological reactions [88]. PLP may increase the availability of molecules required for normal metabolic functioning, aids in glycogenolysis [91, 92], and reduces excitotoxicity [88, 93], all culminating in neuroprotection. In the experimental stroke field, there is evidence that PLP is neuroprotective after ischemic injury [89] and that the brain upregulates processes involved in PLP production to combat depletion [94].

2.6 DL-Phenylalanine (DLPA)

Phenylalanine is an amino acid, which is present in three forms: D-phenylalanine (DPA), L-phenylalanine (LPA), and the mix made in the laboratory called DL-phenylalanine (DLPA). Phenylalanine is used for therapeutic purposes in vitiligo [95], depression [96], attention deficit-hyperactivity disorder (ADHD) [97], Parkinson's disease [98], multiple sclerosis [99], rheumatoid arthritis [100], weight loss [101], and alcohol withdrawal symptoms [102]. In addition, DPA's analgesic ability has been demonstrated to be mediated by blocking enzymes that break down endorphins and enkephalins, the body's natural painkillers. Clinical trials indicate that DPA may help alleviating some types of chronic pain [103, 104].

DPA may also provide pain relief through mechanisms that are not fully understood. DPA reduced chronic pain in animal studies within 15 min of administration and the effects lasted up to 6 days [105, 106]. It also decreased responses to acute pain. At least five other studies have independently confirmed these findings [107]. DPA appears to inhibit some types of chronic pain in human clinical studies, but it has little effect on most types of acute pain. Most of the clinical studies have been focused on the pain-relieving effects of DPA at a daily dose range of 750–1000 mg, used either continuously or intermittently for a period of several weeks [108, 109].

3 Conclusions

TBI is a major health problem with socioeconomic impacts worldwide, with almost no effective treatment. It also accounts for a significant proportion of morbidity and mortality in the ICU. In patients with TBI, ICU complications occur more frequently [110]. A basis should be provided for the intervention to be performed with the aim of preventing or treating these complications and accelerating the patient's recovery. The lack of proven effective therapies for TBI is related to the range of severity of injury, the complexity of approaching a disease that involves multiple tissue and cell types, rapid onset of pathophysiology, common co-morbidity presen-

tation, and other environmental and developmental factors [111, 112]. Due to the wide range of complications, patients with TBI may show heterogeneous features of injury including DAI, ischemia, inflammation, bleeding, oxidative damage, excitotoxicity, inflammation, mitochondrial and metabolic dysfunction, and other manifestations of pathological processes [113].

Based on the reviewed evidence, a combination of nutraceuticals can potentially support neuromuscular function to reduce recovery time in people with TBI. Effective nutritional support can be a combination of vitamins (thiamin and vitamin B_6), amino acids (D,L-phenylalanine), plant extracts (bromelain from pineapple), and herbs (turmeric, *Boswellia serrata* resin and quercetin) that work synergistically to repair TBI damage and improve areas of brain and body function that are most affected by TBI. Review of previous studies show that each of these nutrients may have one or more protective function in improving the complications of TBI, including modulation of pro- and anti-inflammatory cytokines, reduction of lipid peroxidation and oxidative stress, reduction of excitotoxicity, and modulation of mitochondrial function. Protective effects of the mentioned components on TBI outcomes are presented in Table 1. Through reduction of inflammation, modulation of methylation pathways, neurotransmitters, mitochondrial imbalances, and toxic overload as well as blood–brain barrier integrity, nutraceutical supplementation can result in improved cognition, reduced cerebral edema, and pain relief. Due to the synergistic effect of natural products, phytochemicals, and nutrient compounds, combinational use of nutraceuticals may be superior to single-component supplementation. Effective nutritional support is an important factor in the intensive care setting and helps improving outcomes of TBI. Further robust evidence from clinical trials and empirical studies are definitely needed to confirm the role of nutraceuticals in the management and care of patients with TBI.

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Table 1 Protective effects of nutrient and herbal components on TBI outcomes

NMS components	Protective effects and references
Turmeric (extracted from curcumin)	Improved motor and learning performance, blood–brain barrier integrity, anti-inflammatory, antioxidant, neuroprotective role, cognition, and reduced cerebral edema in brain injured animals [42, 55, 114]
Boswellia serrata gum resin (extracted boswellic acid)	Antioxidant activity in diffuse axonal injury (DAI) [61], antioxidant activity in ischemic brain injury [62], neuroprotective role of insulin acetate (IS) bioactive component of *Boswellia* showed reduce cognitive/motor complications after TBI [61, 115]
Bromelain (from pineapple)	Reduced inflammation in tissues after TBI via decreasing the secretion of interleukin (IL)-1β, IL-6, interferon (INF)-γ, and tumor necrosis factor (TNF)-α [74, 75], and reduced cerebral edema by stabilizing vascular permeability in brain injured animals [68, 76]
Quercetin (quercetin dehydrate)	Improved cognitive function (neuroprotective action) via the inhibition of oxidative stress [8], increasing the mitochondrial biogenesis activity mediated by the PGC-1α pathway in brain injured mouse model [84]
DLPA (DL-phenylalanine)	Decreased chronic pain and low back pain by blocking the enzymes that break down endorphins and encephalin, the body's natural pain-killing chemicals [108, 109]
Thiamin (thiamin hydrochloride)	Reduced the neuro-excitotoxicity results of TBI via gamma-aminobutyric acid (GABA) [86]
Vitamin B_6 (pyridoxine hydrochloride)	Neuroprotective effects including reducing the neuro-excitotoxicity results of TBI [87, 92]

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The Effects of Curcumin on the Side Effects of Anticancer Drugs in Chemotherapy: A Randomized Controlled Trial

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Abstract

Curcumin, the active ingredient of the spice turmeric, has been shown to have anticancer activities in several preclinical and clinical studies. The prophylactic effect of curcumin against chemotherapy-induced damage and side effects was evaluated in a double-blind, placebo-controlled randomized trial. Eighty

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cancer patients on standard chemotherapy regimens were randomly assigned to receive curcumin as adjuvant therapy (500 mg per 12 hours) and matched control group to receive placebo for 9 weeks. Pre- and post-intervention, the changes in the health-related quality-of-Life (QoL) score (based on the University of Washington Quality-of-Life (UW-QoL) questionnaire, version 3), clinical symptoms, and hematological and biochemical parameters were assessed. Comparison between groups based on total QoL score showed that curcumin supplementation was not associated with improved QoL ($P = 0.102$). Hematological and biochemical analysis showed no statistical differences between the groups at the end of the trial ($P > 0.05$). However, during the trial, significant differences were observed in hemoglobin (Hb), hematocrit (HCT), lactic acid dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT), and anaplastic lymphoma kinase (ALK) between the groups ($P < 0.05$). Future studies in a larger homogenous population of cancer patients are required to confirm the adjuvant effect of curcumin on chemotherapy-induced QoL.

Keywords

Curcumin · Cancer · Quality of life · Chemotherapy · Randomized controlled trial

1 Introduction

Cancer is still a serious threat to the health of people around the world [1, 2]. Inflammations, tumor progression, exacerbation of symptoms, metastasis of tumor cells, resistance to chemotherapy and radiotherapy, and the side effects of chemotherapy/radiotherapy are known as the factors of several types of cancers that can reduce the quality of life (QoL) of cancer patients [3–5]. Chemotherapy and radiotherapy are associated with several adverse effects and damage to normal tissues causing a deterioration in patients' quality of life and even make many patients discontinue the therapy [6–8]. Thus, to help reduce suffering and improve QoL, it is necessary to explore effective adjuvant strategies to prevent and reduce the chemotherapy-induced side effects.

Many natural products derived from various plants, such as *Taxus brevifolia*, *Catharanthus roseus*, *Betula alba*, *Cephalotaxus* species, *Erythroxylum previllei*, and *Curcuma longa*, are a rich source of anticancer molecules, which can be used as adjuvants to prevent and reduce the side effects of chemotherapy [9–12]. Among them, curcumin ($C_{21}H_{20}O_6$) is an active constituent of the natural plant *Curcuma longa L.*, which belongs to the Zingiberaceae family. Curcumin has been widely used for thousands of years as a flavoring agent in the food industry and herbal medicine in Asian countries to treat vomiting, headache, diarrhea, and many other ailments [9]. Many pharmacological studies have shown that curcumin is safe and has antioxidative, antimicrobial, anti-inflammatory, and anticancer activities [13–22].

Growing evidence shows that curcumin can prevent carcinogenesis, sensitize cancer cells to chemotherapy, and protect normal cells from chemotherapy-induced damages [23]. Despite encouraging findings in cancer cell lines in vitro and preclinical studies, clinical trials investigating curcumin's protective effect against chemotherapy-induced toxicity [24] are minimal. On the other hand, QoL has become an important endpoint for treatment comparison in randomized controlled trials. Therefore, this clinical trial study was conducted to evaluate the prophylactic effect of curcumin against chemotherapy-induced damage and its side effects on clinical symptoms, hematological and biochemical parameters, and QoL indicators in cancer patients.

2 Materials and Methods

2.1 Trial Design

This study was designed as a randomized, double-blind placebo-controlled trial and performed at the Oncology Clinic of the Baqiyatallah Hospital, Tehran, Iran, from May 2016 to

December 2016. This study was approved by the research ethics committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ir.tums.ikhc.rec.13963.2008). This trial has also been registered in the Iranian registry of clinical trials (IRCT201708021165N23). Written informed consent was obtained from each participant.

2.2 Participants

This trial was conducted on cancer patients who underwent standard chemotherapy regimens. Patients with cancer on standard chemotherapy regimens with the ability to perform their daily activities were included in the study. The patients had any surgery at least 1 month before this study period. Patients were excluded from the study if they had a history of sensitivity or intolerance to curcumin supplement, any surgical intervention within 1 month before the start of the study, exacerbation of disease to an uncontrollable level, the occurrence of severe adverse events during treatment, and not willing to participate in the study.

2.3 Randomization and Blinding

The patients who met the inclusion criteria were selected through convenient sampling and assigned into two equal groups (n = 40). The intervention group received a curcumin capsule (containing 500 mg curcuminoids plus 5 mg piperine; Sami Labs, Bangalore, India), and the control group received a placebo capsule every 12 h for 9 weeks, through the blocked randomization method. Block sizes for randomization were four and six, and the allocation ratio was 1:1. The curcumin supplements and placebo capsules were placed into the closed envelopes numbered sequentially for allocation concealment. The small envelopes were placed into the large opaque envelopes and numbered sequentially. Envelopes containing a 9-week supply of the curcumin or placebo capsules were delivered to each participant according to the individuals' entry into the study. Randomization was performed by a person who was not involved in data collection and analysis. The researchers and patients were blind to the assigned intervention.

2.4 Data Collection and Instruments

A board-certified oncologist visited all patients at baseline and at the end of treatment duration. Demographic characteristics (age, gender, qualification, and comorbidity diseases), clinical data (symptoms and biochemical and hematological parameters), and health-related quality of life (HRQoL) via questionnaire were collected for each participant before and after of intervention. Biochemical and hematological indices were recorded at the beginning, first, and second months and the end of treatment.

Symptoms including nausea, vomiting, diarrhea, constipation, anorexia, weight loss, itching, insomnia, skin lesion, mouth ulcer, neuropathy, fever, body pain, neurological, eye lesion, and dry mouth were recorded for all participants. Based on their severity, these symptoms were classified into four groups: mild, moderate, severe, and very severe, and each was assigned a score accordingly. Therefore, the severity of individual clinical symptoms was judged semi-quantitatively using an analog scale responding with values ranging from "0" (absence of symptoms) to "4" (very severe). The score was assigned by the patients with the help and diagnosis of the physician on the basis of the diary and evidence.

Assessment of HRQoL was performed using the University of Washington QoL questionnaire (UW-QoL) version 3 [25]. UW-QoL is a simple and validated scale that consists of ten domain-specific questions. The domains focus on physical symptoms, physical functioning, and social function. Specifically, the items address pain, appearance, activity level, recreation, swallowing, chewing, speech, shoulder function, taste, and saliva production. Each of the domain-specific items was scored from 0 (worst QoL) to 100 (best QoL). The "composite" score is created by averaging the scores from the ten items. They

do not include the four generic questions in the composite scoring because these represent very different constructs. The University of Washington has found that the global QoL, global HR-QoL, and transitional HR-QoL items provide different and useful perspectives from the composite score. All patients filled out this questionnaire before and after the intervention.

Venous blood samples were collected from the antecubital fossa and dispensed into a 2 ml K3EDTA and 5 ml SST tubes with gel for hematology and biochemistry analysis. Hematological analysis (complete blood count (CBC) test with three-part differential) was performed within 8 h of blood draw. Three-part differential of hematological parameters included white blood cell counts (WBC) (lymph and neutrophils/eosinophil/basophils as PMN), red blood cell count (RBC) (hemoglobin (Hb), hematocrit (HCT), and the mean corpuscular volume (MCV) of red cell), and platelet (PLT). Samples for biochemical analysis were allowed to clot for at least 60 min, centrifuged, and the serum collected. Serum was analyzed within 24 h after collection. If testing was delayed, serum was stored frozen at −80 °C and subjected to a single freeze-thaw cycle at the time of analysis. The biochemical indicators measured in this study were creatine phosphokinase (CPK) and lactic acid dehydrogenase (LDH) as the muscular function tests and erythrocyte sedimentation rate (ESR) as the inflammatory function tests. In addition, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), anaplastic lymphoma kinase (ALK), total bilirubin (TB) and direct bilirubin (DB), blood urea nitrogen (BUN), and creatinine (Cr) were conducted to test liver and kidney function.

2.5 Statistical Analysis

Statistical analysis was carried out using SPSS software (ver.17) (SPSS Inc. IL, Chicago, USA). The normality of the numeric variables was checked by the Kolmogorov-Smirnov test. Data were presented using mean (SD) for the quantitative variables and normal and frequency (percent) for categorical variables. The between-group comparisons of baseline measures and demographic variables were computed by independent t-test and/or chi-square test (with exact p-value) where appropriate. For within-group comparisons, repeated measures analysis of variance (RMANOVA) was used, where before, immediately after, and post-intervention measurements were taken, followed by Sidak post hoc tests. The assumption of sphericity was addressed by Mauchly's test of sphericity, and when the assumption was not satisfied, the Greenhouse-Geisser correction of P-value was utilized. Friedman's two-way analysis of variance by ranks followed by Dun's post hoc test was conducted to assess the difference in time for ordinal variables. To assess the effect of intervention, the analysis of covariance (ANCOVA) was used after controlling for baseline measures and confounders in a two-step hierarchical model. For the ordinal primary outcome, the ordinal regressions were utilized after controlling for baseline measures and confounders in a two-step hierarchical model. In all analyses, P-values less than 0.05 were considered as significant.

3 Results

3.1 Participants of the Study

From May 2016 to December 2016, 80 out of 120 cancer patients who were referred to the oncology clinic of Baqiyatallah Hospital, Tehran, Iran, and met the inclusion criteria were included in the study. Patients were randomly divided into curcumin intervention and placebo groups (N = 40 each). Two patients in the intervention group were excluded from the study because they did not complete the treatment for 9 weeks. Figure 1 shows the flowchart of participants in the trial study. The groups were matched for the baseline demographic, including age, gender, comorbidities, qualification, and the mean year of cancer diagnosis ($P > 0.05$). Demographic characteristics of the participants in the two groups of study are presented in Table 1.

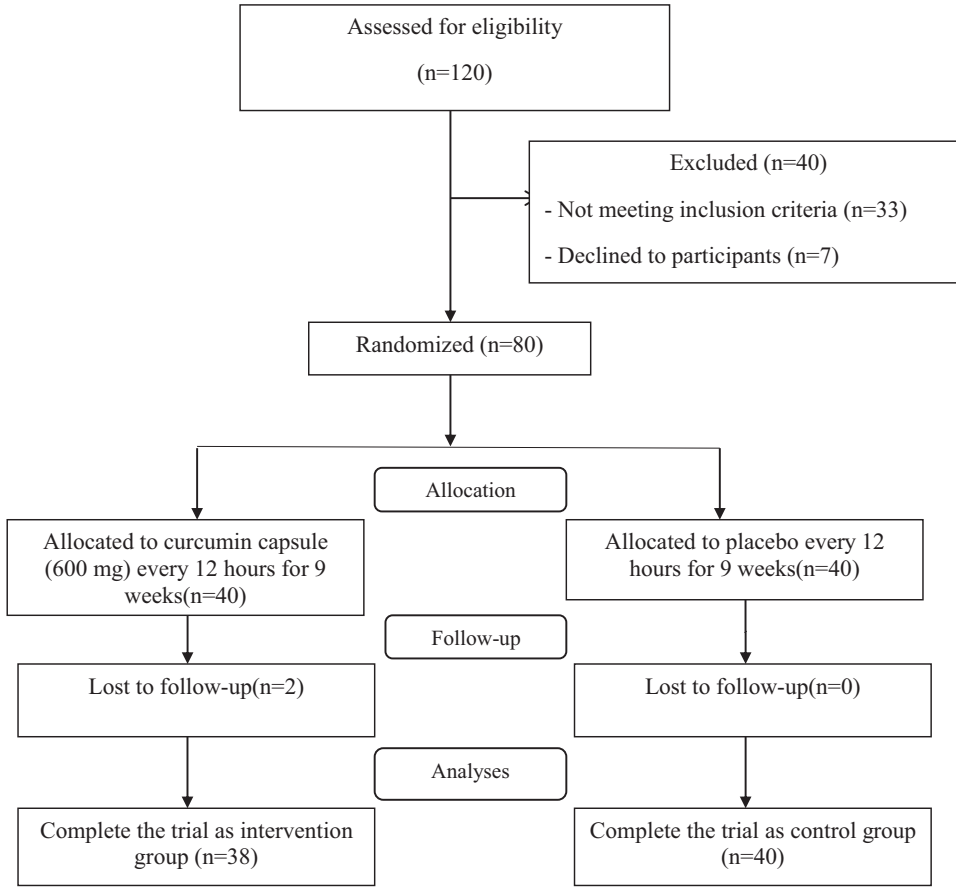

Fig. 1 Flow chart of the trial

Table 1 Demographic and clinical characteristics of the participants in two groups of study

Variables	Intervention group (n = 38)	Control group (n = 40)	*P*-value
Age (mean ± SD)	58.18 ± 10.69	56.60 ± 14.27	0.582
Gender			
Male (%)	14 (36.8)	19 (47.5)	0.368
Female (%)	24 (63.2)	21 (52.5)	
Comorbidities			
Yes (%)	5 (13.2)	8 (20)	0.547
No (%)	33 (86.8)	32 (80)	
Cancer diagnosis (mean ± SD, years)	3.16 ± 2.89	3.35 ± 1.63	0.717
Qualification			
First class (%)	6 (15.8)	9 (22.5)	0.544
Second class (%)	8 (21.1)	12 (30)	
Third class (%)	8 (21.1)	8 (20)	
Fourth class (%)	5 (13.2)	6 (15)	
Fifth class (%)	5 (13.2)	1 (2.5)	
Sixth class (%)	5 (13.2)	4 (10)	
Tenth class (%)	1 (2.6)	0 (0)	

*$P < 0.05$ was considered as significant

3.2 Effect of Curcumin on Quality of Life

Health-related quality of life for all participants was assessed pre- and post-trial according to the UW-QoL version 3, and the results are shown in Table 2. In the pre-trial, two scores of recreation (76.32 ± 23.21 vs. 63.13 ± 24.67, $P = 0.017$) and swallowing (92.89 ± 19.85 vs. 73.0 ± 32.44, $P = 0.002$) items of QoL were significantly higher in the intervention group compared with the control group. However, no significant difference was observed in other quality of life parameters between the two study groups at baseline ($P > 0.05$). The results of ANCOVA adjusted for age, gender, qualification, year of cancer diagnosis, and comorbidities, as the confounders for post-trial measures showed the significant positive effect of the intervention on swallowing score (95.00 ± 14.28 vs. 71.25 ± 37.43, $P = 0.015$), recreation score (82.89 ± 21.83 vs. 71.88 ± 24.14, P = 0.029), and chewing score (86.84 ± 27.27 vs. 78.75 ± 37.36, P = 0.015) (supplementary file, figure 1A, 1B and 1C). However, curcumin showed a significant negative effect on the quality-of-life score (35.26 ± 17.67 vs. 53.50 ± 15.28, $P < 0.001$) and quality-of-life rate score (30.53 ± 17.85 vs. 56.00 ± 14.46, $P < 0.001$) (supplementary file, figure 2A and 2B).

According to the results of two-way ANOVA with repeated measure, there was a significant time effect for pain score ($P = 0.001$), activity score ($P = 0.001$), recreation score ($P = 0.038$), quality-of-life score ($P < 0.001$), quality-of-life rate score ($P < 0.001$), socioemotional functioning composite ($P = 0.002$), and total quality of life ($P = 0.036$), which showed the effect of time trend on these results. In addition, the results revealed that the quality-of-life score ($P < 0.001$) and quality-of-life rate score ($P < 0.001$) were affected by the interaction of time and intervention. Comparison between groups based on total QoL score revealed that curcumin supplementation was not associated with improved QoL and the difference in this score between the two groups was not significant (84.16 ± 17.04 vs. 77.05 ± 14.66, $P = 0.083$). However, over time, this score has increased significantly in both groups ($P = 0.036$).

3.3 Effect of Curcumin on Hematological and Biochemical Parameters

The comparisons of hematology and biochemical parameters, before, after, and during the trial (first and second month) in the intervention and control groups, are shown in Tables 3 and 4, respectively. Based on the results of independent t-tests at the baseline, there were no significant differences between the two groups in terms of hematology and biochemical parameters, which indicates the homogeneity of participants in the study ($P > 0.05$). The results of ANCOVA for post-intervention measures adjusted for age, gender, qualification, year of cancer diagnosis, and comorbidities as the confounders showed statistical difference in Hb on the first month (11.74 ± 3.36 vs. 9.82 ± 4.30,$P = 0.042$), HCT on the second month (34.53 ± 11.25 vs. 27.53 ± 14.65, $P = 0.048$), LDH on the second month (406.2 ± 186.01 vs. 264.79 ± 216.7,$P = 0.010$), SGOT on the second month (27.05 ± 15.55vs. 18.30 ± 11.42,$P = 0.011$), and ALK on the second month (237.2 ± 269.3 vs. 144.5 ± 127.4, $P = 0.049$) between two groups of the study. According to the results of two-way ANOVA with repeated measure, there was a significant time effect on WBC ($P < 0.001$), CPK ($P < 0.001$), LDH ($P < 0.001$), ALK ($P < 0.005$), and ESR ($P = 0.045$). In addition, comparisons between groups with respect to the interaction of time and intervention showed significant differences for HCT ($P = 0.013$), LDH ($P = 0.002$), and ALK ($P = 0.020$) (supplementary file, figure 3A, 3B and 3C).

3.4 Effect of Curcumin on Symptoms

Baseline symptoms including nausea ($P = 0.179$), vomiting ($P = 0.540$), diarrhea ($P = 0.569$), constipation ($P = 0.227$), anorexia ($P = 0.583$), weight loss ($P = 0.494$), itching ($P = 0.846$), insomnia ($P = 0.565$), skin lesion ($P = 0.837$), mouth ulcer ($P = 0.228$), neuropathy ($P = 0.927$), fever ($P = 0.939$), body pain ($P = 0.682$), neurological ($P = 0.988$), eye lesion ($P = 0.487$), and

Table 2 Comparison of QoL items on pre- and post-trial between the intervention and control groups

Items	Groups	Pre-trial (Mean ± SD)	Post-trial (Mean ± SD)	*P*-value		
				Intervention effect**	Time effect***	Interaction****
Pain score	Intervention	67.76 ± 24.60	80.26 ± 21.08	0.849	0.001#	0.211
	Control	73.75 ± 23.99	79.38 ± 18.68			
	*P-value	0.280	0.480			
Appearance score	Intervention	77.63 ± 30.53	88.42 ± 22.00	0.721	0.055	0.554
	Control	80.75 ± 31.65	86.50 ± 21.07			
	*P-value	0.659	0.689			
Activity score	Intervention	63.82 ± 25.13	78.95 ± 31.04	0.116	0.001#	0.611
	Control	58.75 ± 22.66	70.00 ± 24.81			
	*P-value	0.382	0.220			
Recreation score	Intervention	76.32 ± 23.21	82.89 ± 21.83	0.029#	0.038#	0.765
	Control	63.13 ± 24.67	71.88 ± 24.14			
	*P-value	0.017#	0.068			
Swallowing score	Intervention	92.89 ± 19.85	95.00 ± 14.28	0.015#	0.960	0.589
	Control	73.00 ± 32.44	71.25 ± 37.43			
	*P-value	0.002*	0.015#			
Chewing score	Intervention	86.84 ± 25.16	86.84 ± 27.27	0.015#	0.884	0.884
	Control	80.00 ± 27.27	78.75 ± 37.36			
	*P-value	0.254	0.401			
Speech score	Intervention	90.13 ± 24.06	95.00 ± 16.52	0.205	0.712	0.406
	Control	88.00 ± 25.99	86.13 ± 25.96			
	*P-value	0.708	0.068			
Shoulder score	Intervention	73.68 ± 39.83	80.26 ± 31.92	0.434	0.905	0.282
	Control	78.75 ± 33.76	73.68 ± 36.27			
	*P-value	0.546	0.373			
Taste score	Intervention	80.26 ± 33.41	82.11 ± 28.77	0.141	0.440	0.237
	Control	79.75 ± 29.13	71.00 ± 35.36			
	*P-value	0.942	0.129			
Saliva score	Intervention	80.53 ± 32.29	78.95 ± 32.03	0.710	0.775	0.919
	Control	71.50 ± 33.48	70.75 ± 31.49			
	*P-value	0.230	0.476			

(continued)

Table 2 (continued)

Items	Groups	Pre-trial (Mean ± SD)	Post-trial (Mean ± SD)	*P*-value		
				Intervention effect**	Time effect***	Interaction****
Quality-of-life score	Intervention	56.32 ± 18.25	35.26 ± 17.67	<0.001#	<0.001#	<0.001#
	Control	51.50 ± 14.94	53.50 ± 15.28			
	*P-value	0.209	<0.001#			
Quality-of-life rate score	Intervention	52.11 ± 14.36	30.53 ± 17.85	<0.001#	<0.001#	<0.001#
	Control	50.00 ± 15.69	56.00 ± 14.46			
	*P-value	0.539	<0.001#			
Physical functioning composite	Intervention	84.71 ± 17.23	87.72 ± 16.97	0.131	0.760	0.388
	Control	78.83 ± 16.87	77.40 ± 22.87			
	*P-value	0.132	0.065			
Socioemotional functioning composite	Intervention	70.39 ± 20.33	80.59 ± 20.59	0.144	0.002#	0.282
	Control	68.59 ± 15.28	74.34 ± 14.52			
	*P-value	0.658	0.134			
Total quality of life score	Intervention	77.55 ± 15.04	84.16 ± 17.04	0.102	0.036#	0.319
	Control	73.71 ± 14.15	77.05 ± 14.66			
	*P-value	0.249	0.083			

# $P < 0.05$ was considered as significant. * Independent t-test for baseline measures and ANCOVA for post-intervention measures adjusted for baseline measurements. ** ANCOVA for after (post)-intervention measures adjusted for age, gender, qualification, year of cancer diagnosis, and comorbidities. *** Time main effect based on RMANOVA. **** Time by intervention interaction effect based on RMANOVA

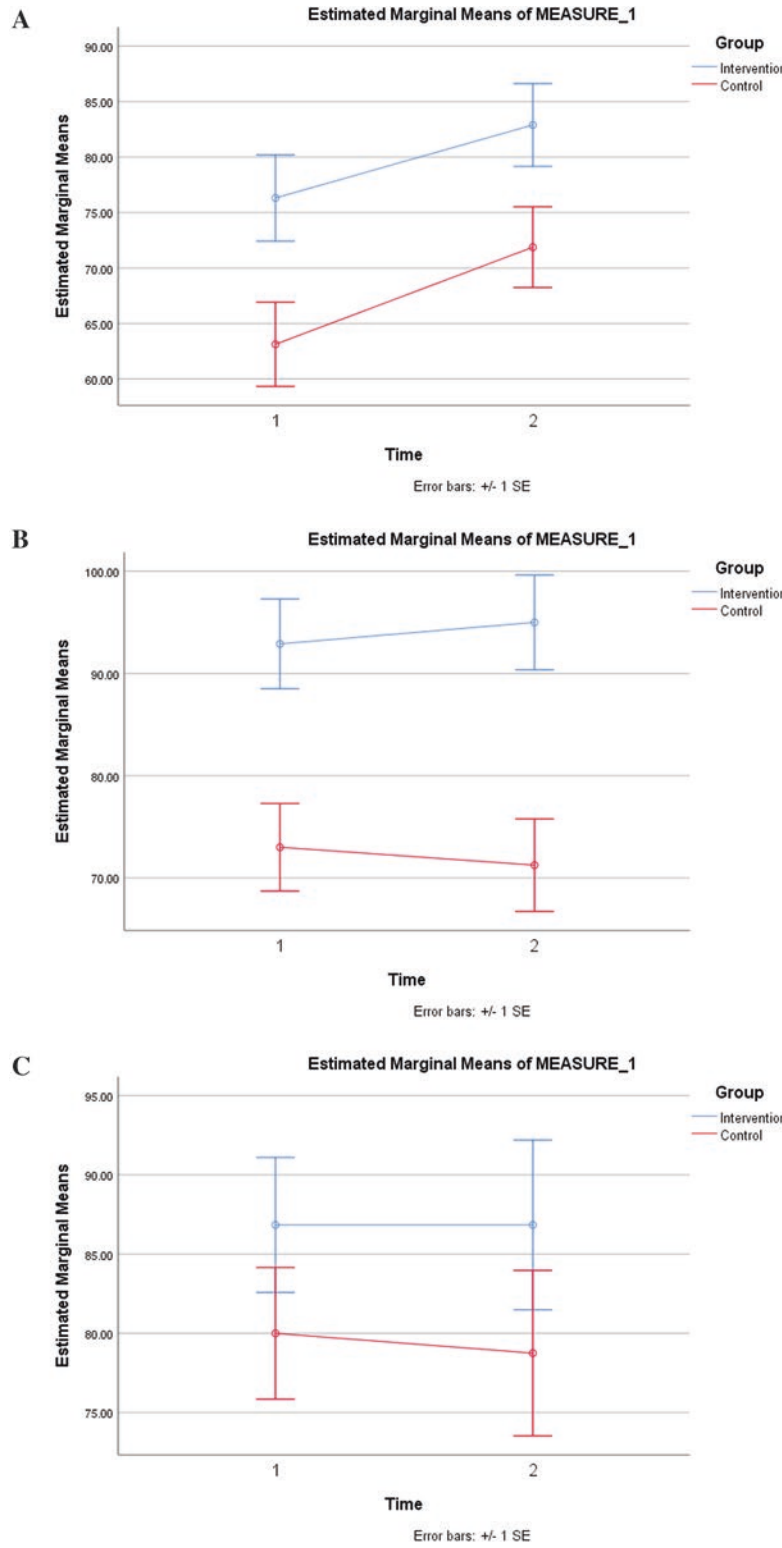

Fig. 2 Interaction of time*group for measurements of (**A**) recreation score, (**B**) swallowing score and (**C**) Chewing score

Table 3 Comparison of hematological parameters, before, after, and during the trial in the intervention and control groups

Parameters	Groups	Baseline	First month	Second month	Third month	P-value***	P-value****
WBC count							
W.B.C	Intervention	6119.9 ± 1873.1	5536.3 ± 2789.5	5363.1 ± 2616.2	4583.6 ± 3291.9	<0.001#	0.598
($\times 10^3$) U/L	Control	5852.4 ± 2512.6	4807.5 ± 2984.7	4098.5 ± 2839.1	4074.7 ± 2768.3		
	***P-value**	0.597	0.318	0.053	0.520		
	****P-value**	0.578	0.278	0.188	0.699		
Lymph	Intervention	32.87 ± 22.44	34.48 ± 16.80	33.01 ± 15.59	43.01 ± 61.20	0.257	0.861
(%)	Control	31.06 ± 15.48	29.01 ± 19.90	28.12 ± 19.18	33.78 ± 27.06		
	***P-value**	0.679	0.217	0.253	0.405		
	****P-value**	0.779	0.454	0.349	0.520		
PMN	Intervention	44.24 ± 25.79	48.06 ± 19.25	49.49 ± 18.80	42.29 ± 22.63	0.392	0.279
(%)	Control	52.65 ± 20.64	47.72 ± 25.78	45.34 ± 25.41	42.99 ± 24.81		
	***P-value**	0.115	0.844	0.355	0.770		
	****P-value**	0.215	0.477	0.392	0.865		
RBC count							
Hb	Intervention	15.11 ± 17.74	11.74 ± 3.36	11.39 ± 3.76	10.09 ± 4.83	0.141	0.396
(g/dL)	Control	11.90 ± 2.23	9.82 ± 4.30	9.42 ± 5.22	11.72 ± 18.21		
	***P-value**	0.259	0.039#	0.074	0.565		
	****P-value**	0.341	0.042#	0.168	0.467		
HCT	Intervention	29.81 ± 15.15	35.37 ± 10.10	34.53 ± 11.25	29.98 ± 14.61	0.250	0.013#
(%)	Control	34.42 ± 7.68	30.72 ± 13.63	27.53 ± 14.65	28.93 ± 16.96		
	***P-value**	0.092	0.090	0.023#	0.763		
	****P-value**	0.699	0.127	0.048#	0.705		
MCV	Intervention	68.86 ± 34.88	96.83 ± 110.76	79.23 ± 25.69	72.85 ± 33.09	0.234	0.171
(fL)	Control	81.09 ± 17.13	75.16 ± 30.07	68.87 ± 36.00	70.46 ± 36.71		
	***P-value**	0.051	0.476	0.123	0.788		
	****P-value**	0.081	0.693	0.187	0.783		
Platelets							
PLT	Intervention	287.97 ± 153.32	311.50 ± 539.35	222.13 ± 122.45	192.66 ± 103.43	0.109	0.349
($\times 10^3$) U/L	Control	249.04 ± 144.54	192.48 ± 114.83	194.70 ± 131.14	182.28 ± 126.18		
	***P-value**	0.252	0.208	0.552	0.761		
	****P-value**	0.252	0.312	0.818	0.820		

WBC White blood cell, *RBC* Red blood cell, *HB* Hemoglobin, *HCT* Hematocrit, *MCV* Mean corpuscular volume of red cell. # $P < 0.05$ was considered as significant. * Independent t-test for baseline measures and ANCOVA for post-intervention measures adjusted for baseline measurements. ** ANCOVA for after (post) intervention measures adjusted for age gender qualification year of cancer diagnosis and comorbidities. *** Time main effect based on RMANOVA. **** Time by intervention interaction effect based on RMANOVA

Table 4 Comparison of biochemical parameters, before, after, and during the trial in the intervention and control groups

Parameters	Groups	Baseline	First month	Second month	Third month	P-value***	P-value****
Cardiac function tests							
CPK	Intervention	22.50 ± 38.55	61.05 ± 63.62	74.00 ± 66.20	63.87 ± 58.72	<0.001#	0.087
(mg/dL)	Control	40.05 ± 38.70	51.38 ± 39.89	52.08 ± 38.88	58.58 ± 68.23		
	***P-value**	0.058	0.463	0.159	0.620		
	****P-value**	0.058	0.573	0.332	0.538		
LDH	Intervention	155.5 ± 212.94	338.5 ± 183.71	406.2 ± 186.01	407.68 ± 298.2	<0.001#	0.002#
(U/L)	Control	264.53 ± 253.2	317.93 ± 196.2	264.79 ± 216.7	311.3 ± 20.3.5		
	***P-value**	0.054	0.593	0.006#	0.151		
	****P-value**	0.054	0.551	0.010#	0.162		
Inflammatory function tests							
ESR	Intervention	13.50 ± 19.96	27.05 ± 27.0.6	28.39 ± 26.69	26.18 ± 24.68	0.047#	0.103
(mm/hour)	Control	27.73 ± 33.48	30.29 ± 30.36	29.65 ± 32.09	24.80 ± 25.25		
	***P-value**	0.026#	0.665	0.640	0.356		
	****P-value**	0.026#	0.749	0.692	0.341		
Liver function tests (LFT)							
SGOT	Intervention	26.03 ± 14.74	25.35 ± 14.95	27.05 ± 15.55	24.71 ± 18.37	0.138	0.136
(U/L)	Control	35.95 ± 58.99	21.60 ± 12.55	18.30 ± 11.42	21.39 ± 18.40		
	***P-value**	0.317	0.160	0.005#	0.412		
	****P-value**	0.317	0.151	0.011#	0.436		
SGPT	Intervention	25.45 ± 33.81	28.95 ± 40.46	24.08 ± 22.92	24.97 ± 30.00	0.454	0.617
(U/L)	Control	38.30 ± 84.51	33.37 ± 59.85	27.37 ± 50.91	27.08 ± 42.26		
	***P-value**	0.385	0.962	0.957	0.975		
	****P-value**	0.385	0.863	0.950	0.977		
ALK	Intervention	97.16 ± 118.05	222.8 ± 172.9	237.2 ± 269.3	183.42 ± 185.6	0.005#	0.020#
(U/L)	Control	151.13 ± 143.7	186.8 ± 127.3	144.5 ± 127.4	161.39 ± 140.9		
	***P-value**	0.075	0.114	0.022#	0.444		
	****P-value**	0.075	0.203	0.049#	0.501		
TB	Intervention	0.28 ± 0.58	0.61 ± 0.43	0.85 ± 0.99	0.56 ± 0.43	0.553	0.096
(mg/dL)	Control	0.88 ± 2.35	0.67 ± 0.42	0.60 ± 0.45	0.54 ± 0.47		
	***P-value**	0.129	0.485	0.175	0.913		
	****P-value**	0.129	0.580	0.213	0.923		

(continued)

Table 4 (continued)

Parameters	Groups	Baseline	First month	Second month	Third month	P-value***	P-value****
DB	Intervention	1.31 ± 7.61	0.18 ± 0.19	0.25 ± 0.26	0.18 ± 0.15	0.348	0.375
(mg/dL)	Control	0.25 ± 0.28	0.22 ± 0.22	0.24 ± 0.24	0.19 ± 0.18		
	***P-value**	0.378	0.473	0.729	0.785		
	****P-value**	0.378	0.413	0.867	0.667		
Kidney function tests (KFT)							
BUN	Intervention	19.05 ± 8.76	17.66 ± 11.61	19.16 ± 11.79	18.18 ± 12.12	0.234	0.275
(mg/dL)	Control	19.93 ± 16.97	17.84 ± 12.12	15.24 ± 11.12	14.71 ± 12.12		
	***P-value**	0.775	0.993	0.075	0.108		
	****P-value**	0.876	0.963	0.086	0.129		
Cr	Intervention	0.99 ± 0.30	0.92 ± 0.37	1.23 ± 2.03	1.22 ± 2.17	0.622	0.196
(mg/dL)	Control	1.05 ± 0.32	0.90 ± 0.49	0.79 ± 0.49	0.80 ± 0.46		
	***P-value**	0.416	0.626	0.157	0.214		
	****P-value**	0.416	0.503	0.294	0.416		

CPK Creatine phosphokinase, *LDH* Lactate dehydrogenase, *ESR* Erythrocyte sedimentation rate, *SGOT* Serum glutamic-oxaloacetic Transaminase, *SGPT* Serum glutamic-pyruvic transaminase, *ALK* Anaplastic lymphoma kinase, *TB* Total bilirubin, *DB* Direct bilirubin, *BUN* Blood urea nitrogen, *Cr* Creatinine, # $P < 0.05$ was considered as significant. * Independent t-test for baseline measures and ANCOVA for post-intervention measures adjusted for baseline measurements. ** ANCOVA for after (post)-intervention measures adjusted for age gender qualification year of cancer diagnosis and comorbidities. *** Time main effect based on RMANOVA. **** Time by intervention interaction effect based on RMANOVA

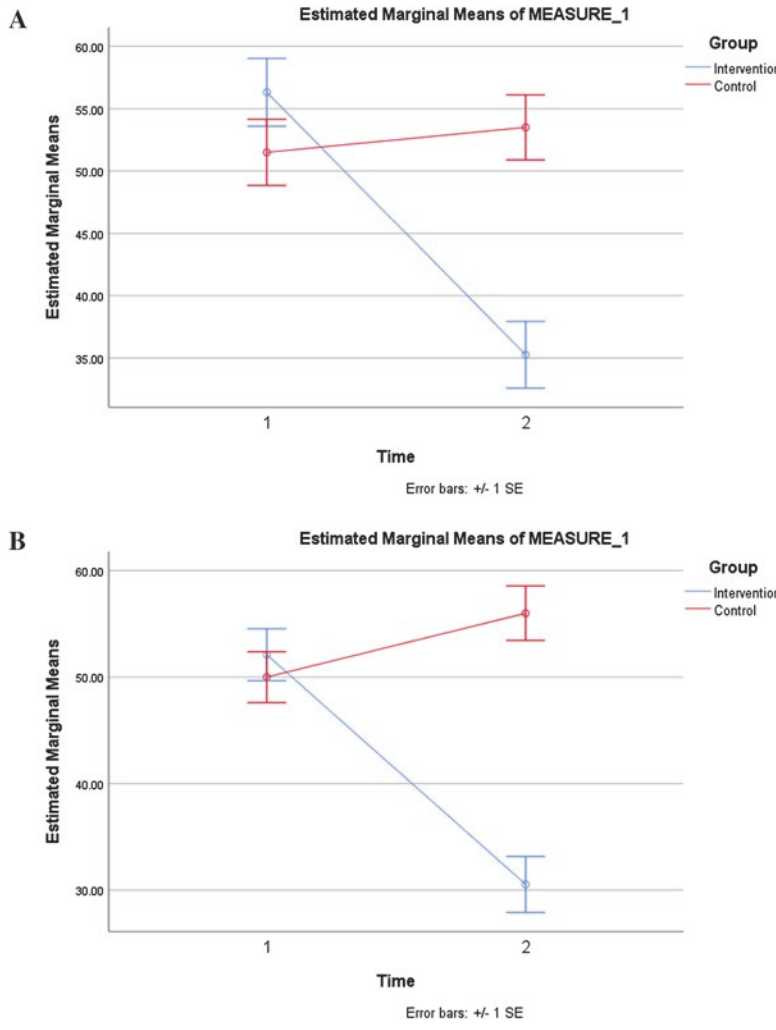

Fig. 3 Interaction of time*group for measurements of (**A**) quality of life score, and (**B**) quality of life rate score

dry mouth (P = 0.737) were well matched between the two study groups, and we did not find any significant differences between two groups of study ($P > 0.05$). After intervention, a physician evaluated all symptoms on the first, second, and third month for each patient in both groups. Within-group comparison of evaluated symptoms in the intervention and control groups is shown in supplementary file, Table 1. The results of Friedman's analysis of variance by ranks showed a significant decrease in the intervention group for nausea (P = 0.001), vomiting (P = 0.022), anorexia ($P < 0.001$), weight loss ($P < 0.001$), itching (P = 0.032), insomnia (P = 0.001), mouth ulcers (P = 0.006), neuropathy (P = 0.001), body pain ($P < 0.001$), neurological symptoms ($P < 0.001$), and dry mouth (P = 0.005). In the control group, the significant decreases were observed for diarrhea (P = 0.023), anorexia ($P < 0.001$), weight loss ($P < 0.001$), fever (P = 0.029), and neurological symptoms (P = 0.014). In addition, comparisons of changes in the symptoms between curcumin (intervention) and placebo (control) groups are presented in supplementary file, Table 2. Based on the results of ordinal regression after controlling the baseline measurements, a significant difference was observed for nausea in the second (P = 0.013)

and third month ($P = 0.011$); anorexia in the first ($P = 0.002$), second ($P = 0.009$), and third months ($P = 0.013$); insomnia in the second ($P = 0.046$) and third month ($P = 0.005$); mouth ulcers in the first ($P = 0.008$) and second months ($P = 0.005$); neuropathy in the first ($P = 0.018$), second ($P = 0.001$), and third ($P = 0.014$) months; body pain in the second ($P = 0.031$) and third ($P = 0.008$) months; neurological symptoms in the first ($P = 0.021$) and third ($P = 0.001$) months; and dry mouth in the third month ($P = 0.024$) in the curcumin-treated group after adjusting for age, gender, qualification, diagnosis, comorbidities, and CBC tests as confounders.

4 Discussion

The side effect of chemotherapy in cancer patients at intestinal (diarrhea/constipation), brain (nausea/vomiting, appetite loss, memory loss), and blood (decreased platelet and neutrophil count) and also toxicity at specific organs (heart, liver, kidney, and ears) are common and related in a predictable and specific way to the mechanism of action of the chemotherapeutic agent [26, 27]. This clinical trial study evaluated the adjuvant effects of curcumin against chemotherapy-induced injury and side effects on clinical symptoms, hematological and biochemical parameters, and QoL indicators in cancer patients. The analysis showed significant decreases in nausea, vomiting, itching, insomnia, mouth ulcer, neuropathy, body pain, and dry mouth symptoms in the curcumin intervention group. In terms of QoL, the curcumin supplementation had a positive effect on the recreation, swallowing, and chewing scores. However, curcumin supplementation was not associated with a significant improvement in overall QoL score. The hematological and biochemical analysis showed no statistical differences between the groups at the end of the trial. However, during the trial, significant differences were observed in Hb, HCT, LDH, SGOT, and ALK between the groups.

Chemotherapy has been shown to result in anemia in cancer patients [28, 29]. Anemia negatively impacts survival and accentuates fatigue in cancer patients [30]. Lower hemoglobin levels and prolonged myelosuppression are reported in cancer patients, especially in patients with head and neck cancer [31, 32]. Our results revealed that the Hb level decreased during the study, which was less in patients who took curcumin so that in the first month of the study, the difference in Hb level in the intervention group compared to the control group was significant. Some prior studies have found an association between higher Hb levels and better physical, emotional, and functional well-being in cancer patients [32, 33]. Yellen et al. [33] found that the cancer patients with the lowest (< 9.9 g/dL^{-1}) and highest (>11.5 g/dL^{-1}) Hb levels reported the worst and best QoL, respectively, and those with intermediate Hb levels generally reported similar QoL. According to our results, the levels of HCT, MCV, and PLT increased when taking curcumin in the intervention group, while it decreased in the control group. However, at the end of the study and after stopping curcumin, their levels dropped again. In the present study, we observed hematological changes in terms of the number of WBC in both groups. No statistically significant difference was observed between the groups, and the values were within the normal range in both groups. However, the reduction rate was lower in the intervention group than in the control group. These results indicate that curcumin can affect the hematological parameter, but its effect is short time and may be related to the selection of appropriate dosages and length of curcumin treatment.

Growing evidence shows that chemotherapy-induced cardiotoxicity includes oxidative stress, mitochondrial damage, calcium flux changes, and activation of proapoptotic signaling cascades, etc., which can increase serum CPK and LDH markers of cardiac toxicity [23]. In the current study, we also observed an increasing trend of these parameters in both groups, and curcumin consumption did not affect them. However, a study by Benzer et al. [34] in rats demonstrated that curcumin has multiple cardioprotective effects due to its antioxidant, anti-inflammatory,

and anti-apoptotic properties. In this study, oral administration of curcumin (100 or 200 mg/kg body weight) for 7 days with doxorubicin (DOX) drug significantly reduced serum CPK and LDH. In addition, in a study by Swamy et al. [35], curcumin (200 mg/kg) was used as pretreatment for 2 weeks and in combination with DOX for another 2 weeks. The results showed that curcumin administration remarkably reduced the elevated level of cardiac toxicity markers and protected the myocardium from Dox damage. In both studies in rats, curcumin was prescribed with DOX, a common drug in the management of malignancy. Although other similar studies have examined the cardioprotective effect of curcumin, most of them are preclinical studies in rats [36], and no studies have been performed in humans.

Hepatotoxicity and nephrotoxicity are severe side effects associated with chemotherapy that often causes liver and kidney injury by damaging the structure and function of the kidney and liver. Cisplatin, a cytotoxic drug used in cancer chemotherapy, could significantly increase serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels [37]. Histopathological observations have revealed that cisplatin could induce hepatocyte damages such as liver congestion and ground glass changes. Waseem et al. [38] found that curcumin pretreatment prior to cisplatin could prevent cisplatin-induced hepatotoxicity in a rat model. Measurements of blood SGOT, SGPT, ALK, TB, and DB were conducted to test liver function in this study. No statistical differences were observed between the levels of these parameters in the experimental and control groups at the end of the study. Contrary to the rat model results, our result indicates that curcumin has no effect on hepatotoxicity in cancer patients. In terms of nephrotoxicity, evidence suggests that chemotherapeutic agents such as mitomycin (MMC) and cisplatin can increase Cr and BUN levels, causing severe kidney damage [39, 40]. In the present study, BUN and Cr were measured as blood metabolites to evaluate renal function, and the results showed no statistical difference between the studied groups, and both parameter values remained within the normal range.

A strength of this randomized, double-blinded, and placebo-controlled study was that the curcumin and placebo groups' participants were comparable in many of their demographic characteristics and baseline values for QoL and hematological and biochemical parameters. On the other hand, some limitations deserve acknowledgment. This study had a relatively small sample size, and the patients were not categorized and compared by the type of cancer. Moreover, this study only tested a single dose of curcumin, and the duration of follow-up was not long enough to assess the impact of the intervention on hard outcomes such as mortality. Future clinical trial research is required to confirm the potential protective role of curcumin and selecting the most appropriate dosages and length of treatment as well as the possibility to include longer-term treatments.

5 Conclusion

This clinical trial study evaluated the adjuvant effects of curcumin against chemotherapy-induced injury and side effects on clinical symptoms, hematological and biochemical parameters, and QoL indicators in cancer patients. The findings of the present study showed a limited effectiveness of curcumin despite some improvements in clinical symptoms and items of QoL such as recreation, swallowing, and chewing. However, the difference in overall score of QoL between the two groups was not significant. Further studies in larger and more homogenous populations are required to ascertain the clinical efficacy of curcumin supplementation in preventing chemotherapy-induced complications. Additional investigations are also worth exploring if dose escalation could lead to a different result (Fig. 4).

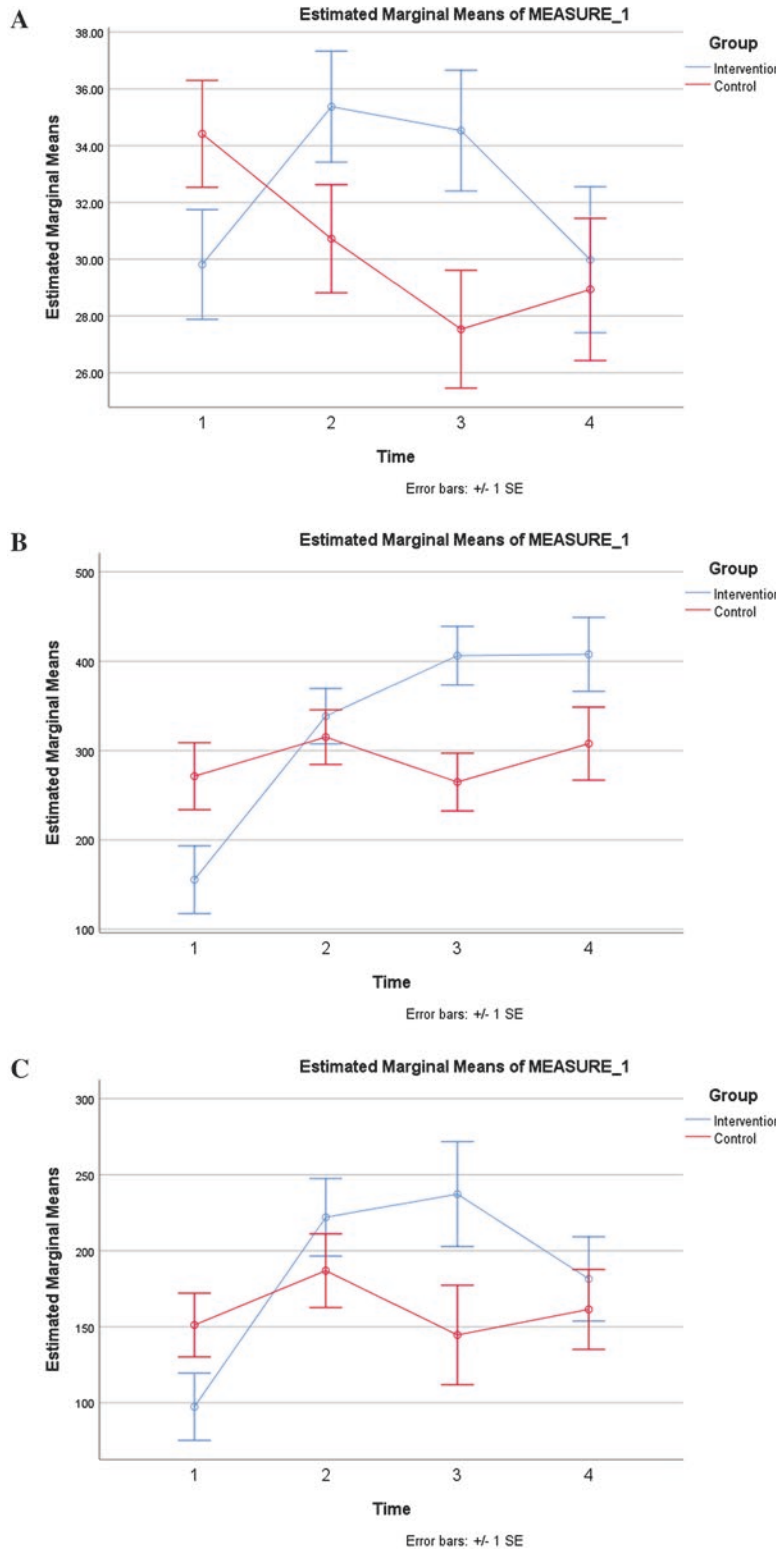

Fig. 4 Interaction of time*group for measurements of (**A**) HCT, (**B**) LDH and (**C**) ALK

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Conflict of Interest Muhammed Majeed is the founder of the Sami-Sabinsa group. The other authors have no other conflicting interests to disclose.

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Crocin Improves Diabetes-Induced Oxidative Stress via Downregulating the Nox-4 in Myocardium of Diabetic Rats

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Abstract

Background: Oxidative stress has a crucial role in the pathophysiology of cardiac dysfunction in the diabetic milieu. Crocin is a natural compound that acts as an antioxidant which could potentially ameliorate oxidative damages in various tissues. The potential role of crocin in the myocardial tissue is not clear yet. This study was aimed to evaluate the possible antioxidative properties of crocin in the myocardium of diabetic rats.

Materials and Methods: Male Wistar rats were randomly divided into four groups as normal, normal-treated, diabetic, and diabetic-treated. Diabetes was induced by a single intravenous injection of STZ (40 mg/kg). Two treated groups of animals (diabetic and non-diabetic) were treated with crocin daily for 8 weeks (40 mg/kg/IP). At the end of day 56, animals were sacrificed under deep anesthesia, and blood and tissue samples were collected. After tissue preparation, the level of nitrate, malondialdehyde, and glutathione and

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the activity of superoxide dismutase and catalase enzymes were measured via standard protocols. In addition, the level of Nox-4 mRNA expression was examined by RT-PCR method. The data were analyzed via one-way ANOVA, and $P < 0.05$ was considered as a significant difference.

Results: Diabetes induces oxidative damages by upregulating the Nox-4 enzyme and increasing nitrate and malondialdehyde levels in the myocardium. Diabetes reduced the superoxide dismutase, catalase, and glutathione activities in the myocardial tissues. Treatment with crocin reversed these changes, reduced Nox-4 mRNA expression, and reduced the nitrate and malondialdehyde content in the myocardium of diabetic rats.

Conclusion: Diabetes induces oxidative stress in myocardium via the upregulating Nox-4 enzyme, and the treatment with crocin reversed these changes. Thus, crocin could be considered as a novel agent for potentially protecting myocardial tissues against diabetes-induced oxidative damages.

Keywords

Crocin · Oxidative stress · Myocardium · Nox-4 · Malondialdehyde

1 Introduction

The prevalence of diabetes mellitus (DM) is growing rapidly globally [1]. This chronic illness will result in several complications such as retinopathy, nephropathy, and cardiovascular disease [2, 3]. It has been shown that poorly controlled diabetes contributes to the development of cardiac dysfunction through stimulating various pathologic pathways such as inflammation and oxidative stress [4, 5]. Oxidative stress refers to an imbalance between the production of free radical species and cellular antioxidative defense system in favor of the free radicals [6, 7]. In this state, the production of free radicals is increased, and their adverse pathologic effects are evident [8]. Therefore, readjusting the oxidative milieu toward normal physiologic state can effectively ameliorate various complications of diabetes including cardiac dysfunctions [8].

Oxidative stress plays an important role in the pathophysiology of cardiovascular complications such as the production of atheromatous plaques leading to progression of atherosclerosis and hemodynamic dysfunction, weakening the myocardium, platelet activation and thrombosis, inflammatory responses, and endothelial dysfunction [9–11]. It is shown that antioxidative therapy provides beneficial impacts on various cardiovascular conditions such as hypertension, ischemic heart disease, atherosclerosis, cardiomyopathies, and congestive heart failure [10, 12, 13]. With DM, a higher amount of free radicals are generated through several molecular mechanisms such as polyol and hexosamine pathways, lipid peroxidation, protein kinase C (PKC), mitochondrial dysfunction, glucose autoxidation, and free radical generator enzymes [9]. NADPH oxidase[1]-4 (Nox-4) is a potent membrane-bound enzymatic protein that is closely involved in the generation of free radicals during DM in several tissues including myocardium [4, 14, 15]. Of the seven mammalian isoforms of NADPH oxidase (Nox1–5 and Duox1–2), Nox-2 and Nox-4 are expressed in the myocardium [14]. Among them, Nox-4 is closely associated with early stages of DM-induced cardiomyopathies and is the main source of free radical species in the myocardium of patients with diabetes [4, 15]. Hence, lowering Nox-4 expression and activity could potentially improve cardiac dysfunction in the diabetic milieu [16–18].

Crocin is a natural water-soluble beta-carotenoid, which is mainly found in saffron (*Crocus sativus* L.) and *Gardenia* plants. Crocin has significant pharmacologic effects and antioxidative potentials [19–21]. This chemical compound has a potent scavenging capacity that neutralizes the activities of free radicals and pre-

[1] Nicotinamide adenine dinucleotide phosphate oxidase

vents oxidative damages [19, 22]. Moreover, it can potentiate the cellular antioxidative factors such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [22]. Emerging evidence demonstrates the antioxidative potentials of crocin in the liver, kidneys, and the pancreatic cells [19, 22, 23]. But there is little data about its possible antioxidant effects on the myocardium especially in the diabetic milieu [24, 25]. Therefore, in the current study, we have evaluated the possible antioxidative effects of crocin in the myocardium of diabetic rats.

2 Methods

2.1 Animals

Male Wistar rats (220–240 g) were purchased from Pasteur Institute (Tehran, Iran) and were kept in standard cages (two rats per cage) at standard temperature (22 ± 2 °C) and humidity (%55 ± 5) by 12 h. Light/dark cycle and free access to water and standard rodent food were provided. These animals were divided randomly into four groups as normal (N); normal treated with crocin (N + C); diabetic (D); and diabetic treated with crocin (D + C) (n = 6). On the first day, blood samples were obtained from the rat's tail.

2.2 Diabetes Induction

Diabetes was induced by an intravenous injection of streptozotocin (STZ) (Sigma Aldrich) (45 mg/kg) into the tail vein. After 72 h, blood samples were obtained from the rat's tail to assess the blood glucose using a standard glucometer (Bionime, Swiss).

2.3 Treatments

Crocin (Sigma Aldrich) was dissolved in distilled water daily, and then it is injected (40 mg/kg/day/IP) to two treated groups of experimental rats (N + C and D + C) for 56 consecutive days.

2.4 Blood and Tissue Sampling

At the end of day 56, all rats were anesthetized by ketamine, and blood samples were collected directly from the heart, and then serum was separated immediately by centrifuge (3500 rpm for 12 min). After that, animal was sacrificed and promptly heart tissues were removed for assessing the malondialdehyde (MDA), nitrate (nitrate), and glutathione (GLT) content and catalase (CAT) and superoxide dismutase (SOD) enzyme activities.

2.5 Blood Glucose Analyzing

The levels of blood glucose were calculated using the available commercial kits (Pars Azmoon, Iran) by a protocol at the beginning (day 1), day 4 (to confirm diabetes induction), and at end of the study (56th day).

2.6 Tissue Preparation

Little fractions of heart samples (500 mg) were weighed, and then homogenization medium (phosphate buffer (0.1 mol, pH = 7.4)) was added. After that, tissues were homogenized on ice by an electric tool. The remaining homogenized samples were centrifuged (20 min at 4 °C and 4000 rpm), and supernatants were removed and stored in −80 °C as the cytosolic extract of heart tissues for biochemical assessments.

2.7 Biochemical Assessments

2.7.1 SOD Enzyme Activity

The activity of SOD enzyme was determined via the method established by Winterbourn which was developed based on the ability of SOD enzyme to inhibit the reduction of nitro blue tetrazolium by superoxide [26]. About 0.067 mol of potassium phosphate buffer (pH 7.8) was added to 0.1 mole EDTA containing 0.3 mM sodium cyanide, 1.5 mM nitro-blue tetrazolium, and 0.1 ml of stored testicular sample. Then, 0.12 mM

of riboflavin was added to activate the reaction and incubated for about 10 min. Finally, the sample optic absorbance was recorded at 560 nm for 5 min on the spectrophotometer. The amount of enzyme required to produce 50% inhibition was taken as 1 unit (U). The final results were expressed as U/mL.

2.7.2 CAT Enzyme Activity

The activity of CAT enzyme in the myocardium was assessed by the Aebi method [27]. A mixture containing 0.85 ml of potassium phosphate buffer 50 mM (pH 7.0) and 0.1 ml homogenate at room temperature was incubated for about 10 mins. The reaction was activated by adding 0.05 ml of H2O2 (30 mM prepared in potassium phosphate buffer 50 mM, pH 7.0). Change in absorbance was recorded for 3 mins at 240 nm. The CAT enzyme activity was expressed as 1 μmole H2O2 decomposed U/mL.

2.7.3 GLT Content Examination

GLT content of heart tissues was examined by Tietz method through the following steps [28]. The protein content of heart sample precipitated by adding 5% sulfosalicylic acid and then centrifuged (2500 g/10 min) and removed. Then, 100 μl of protein-free supernatant, 800 μl of 0.3 mM Na_2HPO_4, and 100 μl of 0.04% 5–5′-dithiobis [2-nitrobenzoic acid] were mixed in 0.1% sodium citrate. The 5–5′-dithiobis [2-nitrobenzoic acid] optic absorbance was recorded at 412 nm for 5 min. A standard curve for GLT was performed, and sensitivity of measurement was detected to be between 1 and 100 μM. The level of GLT was expressed as nmol/mL.

2.7.4 Nitrate Content Assaying

The nitrate level of heart tissues (an index for nitrous free radicals) was assessed by the colorimetric reaction of the Griess method [29]. 0.1 ml of the cytosolic extract of heart tissues was deproteinized by adding 0.2 ml of zinc sulfate solution and then centrifuged for 20 minutes at 4000 rpm and 4 °C to supernatant separation. 0.05 ml of sulfanilamide (0.01%) and 0.05 ml N-[1-naphthyl] ethylenediamine di-hydrochloride (NED, 0.01%) were incubated at 37 °C of temperature for 30 minutes in a dark room. The optic absorbance of the mixture was determined at a wavelength of 540 nm. Nitrite concentration was assessed by a standard curve generated from the absorbance of each sodium nitrate solution. The level of nitrate content was expressed as μg/mg protein.

2.7.5 Lipid Peroxidation Assaying (MDA Content)

The level of MDA content (the end product of lipid peroxidation) was evaluated using the Satoh method [30]. 0.5 ml of tissue homogenate was added to 1.5 ml of 10% trichloroacetic acid, mixed, and incubated at room temperature for 10 mins. Afterward, 1.5 ml of supernatant and 2 ml of thiobarbituric acid (0.67%) were added and placed in a boiling water bath in sealed tubes for about 30 mins. Then, the samples remained for cooling at room temperature for 20 mins. 1.25 ml N-butanol was then added, vortexed, and centrifuged for 5 mins at 2000 g. The final supernatant was removed and its optic absorbance detected at 532 nm. MDA content was calculated using 1,1,3,3-tetraethoxypropane and expressed as nmol/ml.

2.8 mRNA Expression Assaying

For assessment of the Nox-4 gene expression, we applied the RT-PCR technique in three sequential steps: (1) RNA extraction, (2) cDNA synthesis, and (3) amplification as follows. 100 ml of tissue was mixed by 1 ml of topazol solution, and after 15 min, 200 μl of chloroform was added and then incubated in room temperature for about 10 mins. It was then centrifuged (15 min, 12,000 g, 4 °C); after that, its supernatant was removed. 500 μl of isopropanol was added and centrifuged again (15 min, 12,000 g, 4 °C). Then, the liquid was separated, and 1 ml of ethanol is added and centrifuged (8 min, 7500 g, 4 °C). By adding 70 μl of DEBS solution into the microtube and incubating in 55 °C for 5 min, the entire RNA is extracted (Table 1).

Table 1 Forward and reverse primers of Nox-4 for RT-PCR technique

Gene	Forward primer	Reverse primer
NOX-4	AGATGTTGGGCCTAGGATTGTG	AGCAGCAGCAGCATGTAGAAGA

cDNA synthesis was performed via these consecutive steps: 3 µl of RNA and 17 µl of distilled water were added to the cDNA synthesis commercial kit. Then, cDNA was synthesized through 12 rounds of 3 steps by thermo-cycler (step 1, 20 °C for 30s; step 2, 45 °C for 4 min; step 3, 55 °C for 30s) plus to one round of heat activation step (55 °C for 5 min). For cDNA amplification, 3 µl of cDNA, 2 µl of primers (both forward and reverse), and 17 µl of distilled water were added to commercial PCR kit and then inserted in thermo-cycler for 6 heating steps which steps 2–5 were repeated for 35 cycles (step 1, 95 °C for 2 min; step 2, 95 °C for 30s; step 3, 53 °C for 30s; step 4, 72 °C for 1 min; step 5, 72 °C for 10 min; step 6, 30 °C for 30s). For running amplified genes, we applied the gel arose and a housekeeping gene (beta-actin). After the running, gels were kept in ethidium bromide for 20 min, and then, photos were captured by gel doc.

2.9 Statistical Analyses

Data analyzed by one-way analysis of variance (ANOVA) and Tukey tests, as post hoc, in the SPSS software. In all steps, $P < 0.05$ was considered as a significant difference. The final results are expressed as the Mean ± SD.

2.10 Ethical Considerations

All ethical protocols about the animal studies which were approved by the local ethics committee and the NIH Guidelines for care and use of experimental animals were followed.

3 Results

Table 2 presents the values of serum glucose as mg/dL in experimental groups at day 1, 4, and 56.

Table 2 Mean values of blood glucose as mg/dL (± SD) in all experimented groups in day 1, 4, and 56 of the study

	Serum Glucose		
Groups	Day 1	Day 4	Day 56
Normal	95 ± 6	91 ± 8	94 ± 8
Normal + crocin	97 ± 7	94 ± 7	82 ± 7
Diabetes	102 ± 8	402 ± 18	385 ± 23
Diabetes + crocin	101 ± 5	395 ± 21	324 ± 22

Figure 1 presents the changes in nitrate content (as a marker of free radical content) as nMol/mL in all experimental rats. The mean value of nitrate content in the normal and normal-treated groups is 2.1 ± 0.282 and 1.65 ± 0.17, respectively. The presence of diabetes significantly increased the mean value of nitrate content to 6.23 ± 0.63 ($P = 0.001$). But, crocin significantly decreased that to 2.25 ± 0.22 ($P = 0.001$) (Fig. 1).

Figure 2 shows the amounts of CAT enzyme activities as a unit/mL in all groups. In normal and normal-treated animals, the CAT enzyme activity is 0.52 ± 0.01 and 0.041 ± 0.01, respectively. The presence of diabetes increased to 0.23 ± 0.01 ($P = 0.001$). Also, crocin significantly declined to 0.075 ± 0.005 ($P = 0.01$) (Fig. 2).

Figure 3 shows the representative values of SOD enzyme activities as a unit/mL in all experimental rats. In normal and normal-treated groups, the mean values of SOD enzyme activity were 112 ± 15.3 and 85 ± 12.6, respectively. Diabetes significantly increased that to 202 ± 14 ($P = 0.01$). Also, crocin significantly declined that to 145 ± 11 in the diabetic animals ($P = 0.03$) (Fig. 3).

Representative changes of GLT levels as nMol/mL in all experimental rats are depicted in Fig. 4. The mean value of GLT content in the normal and normal-treated groups is 0.22 ± 0.01 and 0.19 ± 0.02, respectively. The presence of diabetes markedly increased that up to 0.56 ± 0.016 ($P = 0.001$). Crocin reduced that to 0.25 ± 0.015 in the diabetic rats ($P = 0.01$) (Fig. 4).

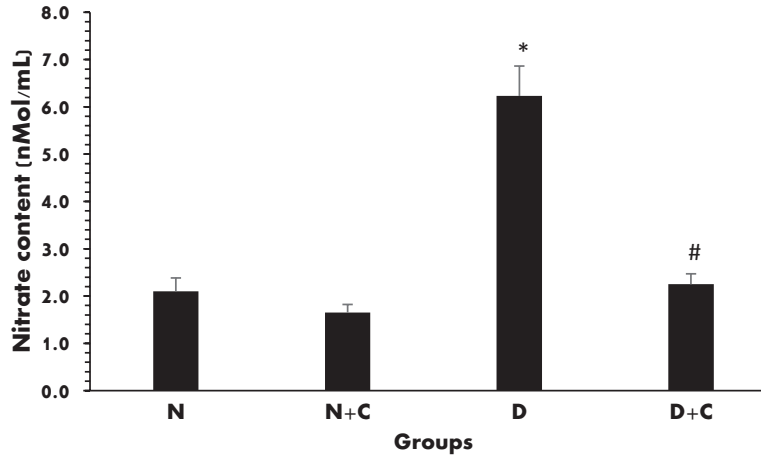

Fig. 1 Representative Nitrate content (nMol/mL) in normal (N), normal + crocin (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SEM. * (P = 0.001) significant differences with the control group (N). # (P = 0.001) significant differences with the diabetic group (D)

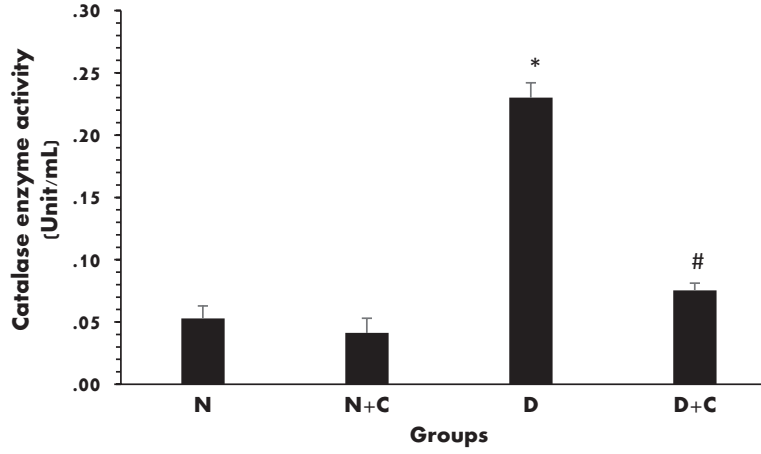

Fig. 2 Representative CAT enzyme activities as unit/mL in normal (N), normal + crocin (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SEM. * (P = 0.001) significant differences with the control group (N). # (P = 0.01) significant differences with the diabetic group (D)

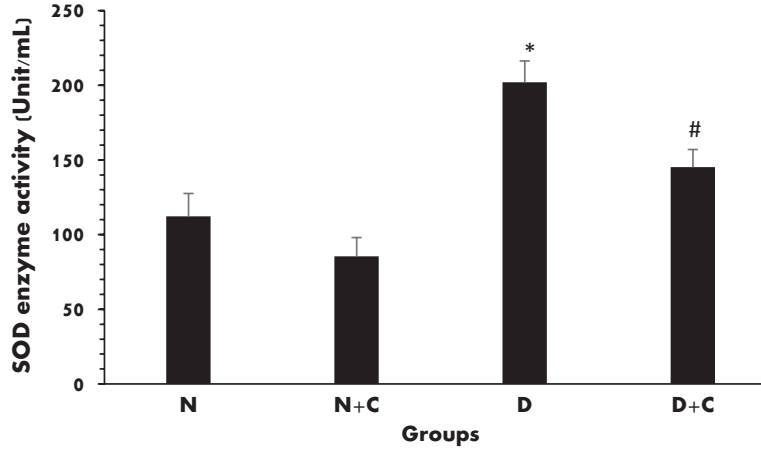

Fig. 3 Representative SOD enzyme activities as unit/ml in normal (N), normal + crocin (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SEM. * (P = 0.01) significant differences with the control group (N). # (P = 0.03) significant differences with the diabetic group (D)

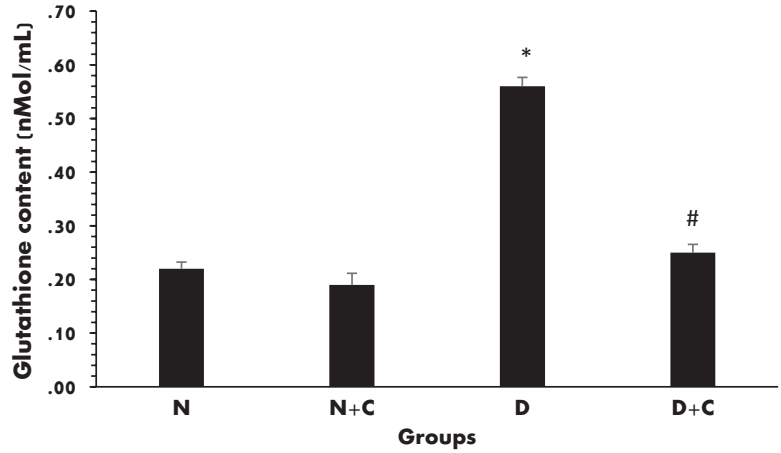

Fig. 4 Representative = GLT content (nMol/mL) in normal (N), normal + crocin (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SEM. * (P = 0.001) significant differences with the control group (N). # (P = 0.01) significant differences with the diabetic group (D)

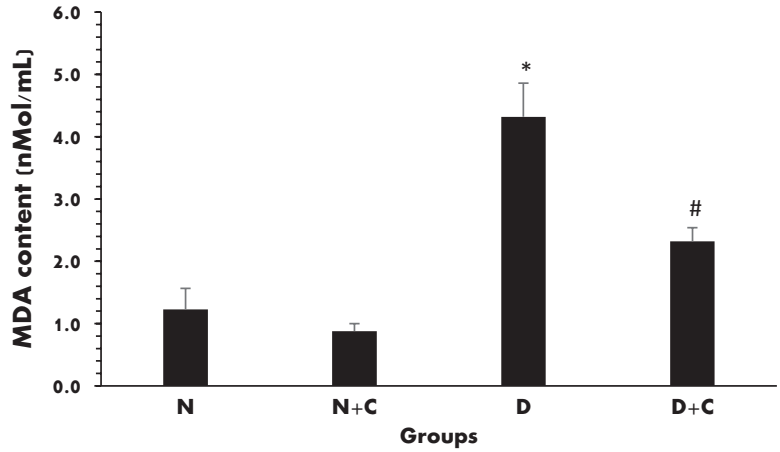

Fig. 5 Representative MDA content (nMol/mL) in normal (N), normal + crocin (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SEM. * (P = 0.001) significant differences with the control group (N). # (P = 0.01) significant differences with the diabetic group (D)

Figure 5 demonstrates the changes in MDA content in the myocardium of experimental groups as nMol/mL. The mean value of MDA content in normal and normal-treated rats were 1.23 ± 0.33 and 0.88 ± 0.12, respectively. The presence of diabetes markedly increased to 4.32 ± 0.54 (P = 0.001). Treatment by crocin in diabetic rats decreased that to 4.49 ± 0.68 (P = 0.01) (Fig. 5).

Figure 6 displays the levels of NOX-4 expression at mRNA level than to β-actin in all experimental groups. Crocin has no significant effect on this level. Diabetes increased that (P = 0.01), but crocin declined that in diabetic rats insignificantly (P = 0.05).

4 Discussion

Oxidative stress has a crucial role in the pathophysiology of various complications of diabetes including cardiac dysfunction [9–11]. In the diabetes milieu, the presence of excessive free radicals results in the breakdown of biological molecules and results in physiological dysfunction of cellular elements in various tissues [7, 31]. Moreover, it contributes to the onset and progress of other pathologic pathways involved in tissue dysfunction such as inflammation and apoptosis [32, 33]. It has been shown that crocin lowers the amount of free radical formation and

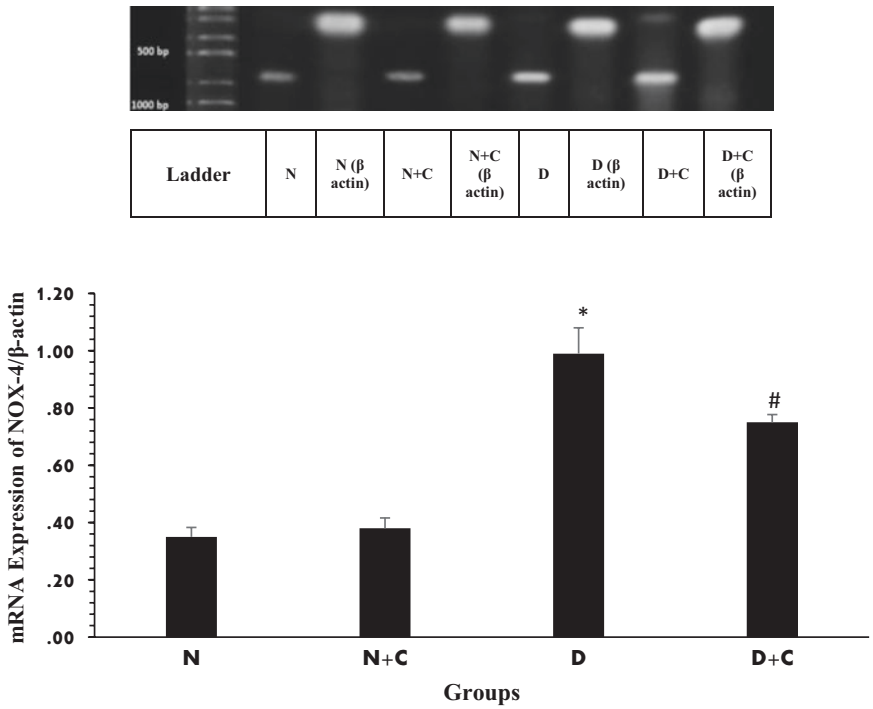

Fig. 6 Representative mRNA expression of NOX-4/β--actin in normal (N), normal-treated (N + C), diabetic (D), and diabetic + crocin (D + C) groups. All values are presented as mean ± SD. * (P = 0.01) significant differences with the control group (N). # (P = 0.05) significant differences with the diabetic group (D)

potentiates the cellular antioxidant defense system thereby protecting tissues against oxidative damages and tissue dysfunctions [22, 33]. In the present study, we demonstrated that diabetes induces oxidative damages in the myocardium of experimental rats. This was confirmed with a higher number of nitrate-free radicals and increased levels of MDA (as a toxic byproduct of lipid peroxidation and a key marker of oxidative damages) accompanied with higher levels of Nox-4 mRNA expression. We have also shown that crocin significantly improved oxidative damages in cardiac tissues of diabetic rats. This finding was confirmed with lower levels of MDA and lower levels of Nox-4 expression in the myocardium of diabetic rats.

Diabetes is a potent upstream event for developing oxidative stress [9]. In turn, oxidative stress is closely involved in the pathophysiology of cardiac and cardiovascular disorders [34, 35]. In this study, uncontrolled hyperglycemia increased the MDA content in the myocardium of diabetic animals. MDA is a well-known marker of oxidative damages produced through lipid peroxidation process in the oxidative milieu [36]. This was accompanied by more free radical production (nitrate content) and higher levels of Nox-4 expression. Previous studies have established that Nox-4 plays an important role in the generation of free radical in the myocardium in the diabetic milieu [37, 38]. This potent membrane-bound enzyme has a higher activity during DM and produces a greater amount of free radicals which in turn contributes to the development of oxidative damages [16, 38, 39]. Normal cardiac redox signaling, which gets deranged during DM, has key roles in the physiologic functions of cardiomyocytes [14]. Maher et al. in 2019 [40] and Yaribeygi et al. in 2018 [23] demonstrated that Nox-4 expression and activity increased in the diabetic milieu [23, 40]. They found that diabetes upregulates the Nox-4 accompanied by excess free radical generation and oxidative damages [23, 40]. Also, Kuroda et al. in

2010 demonstrated that Nox-4 is a major source of free radical formation in the cardiac tissues [41]. DM can upregulate Nox-4 through several pathways including protein glycation, glucotoxicity, dyslipidemia, impaired calcium homeostasis, and renin-angiotensin system activation [14]. In this study, upregulated levels of Nox-4 were accompanied by an increased free radical production and MDA content. Interestingly, the activities of antioxidative elements such as SOD, CAT, and GLT were increased. Yaribeygi et al. (2018) reported that DM-induced Nox-4 upregulation is matched with lower activities of antioxidative elements [23]. In addition, Patel et al. (2013) found that hyperglycemia induces Nox-4 expression and reduced activities for SOD, CAT, and GLT [42]. This suggests that increased levels of aforementioned antioxidant elements' activities were complementary responses against hyperglycemic milieu protecting tissue against oxidative damages.

Our findings indicate that crocin improves DM-induced oxidative damages in the cardiac tissues in diabetes. Crocin is an herbal-based compound which has potent antioxidative effects protecting tissues against oxidative damages [19, 22, 23]. In previous studies, we found that crocin exerts potent antioxidative effects in the liver, kidney, and pancreatic tissues [19, 22, 23]. However, the potential role of crocin in cardiac tissues was not clear before. In the current study, we demonstrated for the first time that crocin reduced MDA content and improved oxidative damages in cardiomyocytes by lowering the Nox-4 expression in the diabetic milieu. Also, it reduced the levels of SOD, CAT, and GLT activities in diabetic treated rats, compared with diabetic non-treated animals. We suggest that reduced activity levels of these elements are due to lower oxidative stress in the crocin-treated animals. It was suggested that crocin exerts its antioxidative properties via direct scavenging of the free radical species, potentiation of antioxidative elements, and lowering the free radical generation [23, 43, 44]. According to the results of this study, crocin improves oxidative stress by lowering the free radical production in diabetic myocardial tissue.

In conclusion, this study suggests that DM induces oxidative damages in cardiomyocytes by increasing the levels of free radical generation at least partly via Nox-4 upregulation. Treatment with the antioxidant pharmaceutical agent of crocin restored these changes and improved oxidative stress by lowering the nitrate and MDA content and Nox-4 expression in the diabetic milieu. These findings suggest for the first time that crocin could potentially be a new herbal-based pharmaceutical agent protecting cardiomyocytes against oxidative damages in the diabetic milieu.

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Conflict of Interests The authors declare that they have no conflict of interest in this study.

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Role of Herbal Medicines in the Management of Brain Injury

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Abstract

Brain is susceptible to oxidative stress due to its increased oxygen consumption and low antioxidant levels. Oxidative stress plays a crucial role in the pathogenesis of various neurological diseases. This review on the role of herbal medicines in the management of brain injury was performed by searching Web of Science, PubMed, Google Scholar, Scopus, and Iran Medex between 1976 to January 2020. The search words contained brain injury, and the total number of publications for the review study was 32. Studies with various medicinal plants such as *Acanthopanax senticosus, Bacopa monnieri,* carnosol, *Cassia mimosoides, Centella asiatica, Crocus sativus, Cuminum cyminum,* curcumin, *Feronia limonia, Gardenia jasminoides, Ginkgo biloba, Kaempferia parviflora, Mentha longifolia, Nigella sativa,* olive, orientin, pomegranate, quercetin, rice bran, *Rosa damascena, Thymus vulgaris, Viola odorata, Withania coagulans, Zingiber officinale*, and *Ziziphus spina-christi* show a significant improvement in brain injury. The different mechanisms for

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improvement in brain injury by these medicinal plants include HIF-1 (hypoxia-inducible factor 1) signaling, free-radical scavenging, reduction of nitric oxide (NO) toxicity and acetylcholine esterase (AChE) activity, decrease of pAkt and its downstream targets, downregulation of the aquaporin-4 (AQP-4) and TLR4/NF-κB/TNF-α signal, reduction in malondialdehyde and NO levels, increasing neuronal density in the hippocampus, and inhibition of oxidative stress. In this review, the neuroprotective actions and molecular mechanisms of herbal medicines are evaluated by reviewing available studies.

Keywords

Brain injury · Medicinal plants · Molecular mechanism

1 Introduction

Oxidative stress is essentially an imbalance between reactive oxygen production and the ability to detoxify their detrimental effects through neutralization by the antioxidant system. The brain is particularly sensitive to oxidative stress due to its high oxygen consumption of oxygen, low levels of antioxidants, and the presence of high content of oxidizable fatty acids. After brain injury, lipid peroxides, reactive oxygen, and nitrogen species are generated in the brain. At the same time, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and "glutathione S-transferase" (GST) levels are reduced. The above imbalance contributes directly to the development of brain injury [1]. Enhancement of this antioxidant system could potentially result in the inhibition of reactive oxygen species [2–4].

Natural products have been increasingly used for brain injury recently. Natural products reported in this study include *Acanthopanax senticosus, Bacopa monnieri,* carnosol*, Cassia mimosoides, Centella asiatica, Crocus sativus, Cuminum cyminum,* curcumin*, Feronia limonia, Gardenia jasminoides, Ginkgo biloba, Kaempferia parviflora, Mentha longifolia, Nigella sativa,* olive*,* orientin*,* pomegranate*,* quercetin*,* rice bran*, Rosa Damascena, Thymus vulgaris, Viola odorata, Withania coagulans, Zingiber officinale*, and *Ziziphus spina-christi.* The present review was aimed to assess the molecular mechanisms for the potential neuroprotective role of herbal plants in brain injury (Table 1).

2 Method

Online studies were checked using Google Scholar, Medline, Pub Med, Web of Knowledge, and Scopus till October 2020 to identify manuscripts about the effect of herbal medicines on brain injury and their possible mechanisms. For this purpose, keywords were brain injury, medicinal plants, and molecular mechanism.

3 Results

3.1 *Acanthopanax senticosus*

A. senticosus is a Chinese herbal medication that is widely found in North Asia [5]. This plant is found to be useful in the management of cerebral embolism, cerebral ischemia attacks, cerebral thrombosis, cerebral arteriosclerosis, coronary heart disease, angina pectoris, and menopausal syndrome [5]. Also, it is found to be effective in managing inflammation and stress-induced pathophysiologic changes [6]. The chemical constituents of *A. senticosus* are saponins, flavonoids, eleutheroside, and amino acids [7]. The effect of *A. senticosus* aqueous extract (235.7 mg/kg, orally) was evaluated on brain injury in old male Kun-Ming mice for 14 days. The results indicated that *A. senticosus* extracts had positive effects on the structure of nerve cells, phagocytosis, fission, and adhesion. Besides, *A. senticosus* significantly changed eight different kinds of proteins such as γ-actin, enolase 2, "heat shock protein 90β" (HSP90β), dihydropyrimidinase-related protein 2 (CRMP2), tubulin protein fam-

Table 1 The effects of medicinal plants on brain injury

Plant	Ext./Cons.	Dose	Exp. model	Effect	Ref.
A. senticosus	Aqueous extract	235.7 mg/kg, orally	Simulated spatial radiation	Positive effects on nerve cells' structure, adhesion, phagocytosis, and fission	[7]
B. monnieri	Bacopaside I	3, 10, and 30 mg/kg, orally	MCAO	↓Neurological deficits and cerebral infarct volume and edema	[10]
				↑brain ATP content, NO level, total adenine nucleotides, Ca2 + Mg2 + ATPase and Na + K + ATPase activities	
				Improved antioxidant enzyme activities	
				Inhibited the increase in MDA content of the brain	
Carnosol		1, 5, 10 mg/kg, i.p.	Restraint stress	↓The immobility time, GSH, SOD, GPX, GRD, CAT	[14]
C. mimosoides	Methanol ethyl acetate, butanol, ethyl acetate, hexane, and water extracts	200 mg/kg, i.p.	MCAO	↓Infarct size, HepG2 cell survival	[18]
		5, 10, 20 mg/kg, i.p.		Improved the cell viability	
				Prevented ischemic stroke	
C. asiatica	Ethanolic extract	100, 200, and 300 mg/kg, orally	MCAO	Prevented neuronal injury	[26]
				Improved neurobehavioral activity	
				Diminished infarction volume	
C. sativus	Crocetin	50 mg/kg, orally	Weight-drop model	Inhibition of neuronal apoptosis	[36]
				↑Expression levels of VEGFR-2, expression levels of SRF	
	Crocin	50 and 80 mg/kg, i.p.	MCAO	Improved neurologic outcome	[44]
				↓The infarct size in both cortex and striatum, percentage of tissue swelling	
	Crocin	40 mg/kg, orally	Four-vessel occlusion	↓OSI, possible ischemic complications	[45]
				Inhibiting the protein expression of HIF-1α, TUNEL, and caspase-3 after cerebral ischemia	
	Crocin	5, 10, 20 mg/kg, orally	BCCAO	↓MDA content, GRK2 expression	[46]
				↑total antioxidant capacity, cytosol GRK2 expression	
				Inhibited ERK1/2 phosphorylation and MMP-9 expression	
	Crocin	15, 30, 60, and 120 mg/kg, i.p.	MCAO	Improved neurologic outcome	[43]
				↓MDA content	
				↑SOD, total antioxidant capacity	
	Aqueous extract	100 mg/kg, orally	MCAO	↓MDA content, neuronal cell death	[47]
				↑GSH level, CAT activity	
C. cyminum	Aqueous extract	25, 50, or 100 mg/kg, orally	MCAO	↓Permeability of the BBB and BBB damage	[51]

(continued)

Table 1 (continued)

Plant	Ext./Cons.	Dose	Exp. model	Effect	Ref.
Curcumin		150 mg/kg/day, orally	HIE	↑ MBP expression, the quantity of neuronal cells, ↑Nrf2 and HO-1 expression	[62]
				Inhibited the caspase-3 activity	
F. limonia	Methanolic extract	250 and 500 mg/kg, orally	I/R	Improved the neurobehavioral parameters	[67]
				↓Total nitrite, lipid peroxidation	
				↑CAT and SOD	
G. jasminoides	Ethanolic extract	50, 100, 150 mg/kg, orally	Chronic cerebral ischemia model	Improved learning and memory ability	[70]
				↑SOD	
			* Morris	↓The activity of AChE	
			water maze test	Protected the neurons in brain cortex and hippocampus CA1	
G. biloba	Aqueous extract	100 mg/kg, i.p.	MCAO	↓Infarct volume, cleaved caspase-3 levels	[74]
				Prevented injury-induced downregulation of pAkt	
				↑Anti-apoptotic signals: Akt, Bad, and FKHR	
	Aqueous extract	100 mg/kg, i.p.	MCAO	↓Overall activity, sensitivity to light, the extent of brain swelling	[75]
K. parviflora	Ethanolic extract	100, 200, 300 mg kg, orally	MCAO	Improved the neurological performances	[81]
				↓Brain infarct volume, Nissl bodies	
M. longifolia	Ethanol extract	50, 100, and 200 mg/kg/day, i.p.	MCAO	Median NDS, brain water content, MDA	[86]
				↑Antioxidant capacity	
N. sativa	Oil	2 ml/kg, i.p.	Transient focal cerebral ischemia	↓The infarct volume, ischemic brain injury	[89]
				Improved the motor functions	
Olive	Aqueous extract	250, 500, 1000 mg/kg, orally	Lead poisoning-induced brain injury	↑SOD, CAT, alkaline phosphatase, and acid phosphatase	[95]
				↓MDA, Bax protein expression	
Orientin		1.62, 3.24, and 6.48μmol/kg, i.p.	MCAO	↓Neurological deficits, infarct volumes, MDA	[98]
				Glu and Asp contents, levels of NF-κB, and TLR4, brain edema, AQP-4 expression	
				Improved pathomorphology and shrink of neurons	
P. granatum	Aqueous extract	250, 500 mg/kg, orally	I/R	↓MDA, NO, TNF-α, NF-κB p65 content, caspase-3, percentage of untailed brain cells	[107]
				↑ SOD, GPX, GRD, IL-10, brain ATP level	
Quercetin		30 mg/kg, i.p.	MCAO	↓Infarct size, neurological deficits, TBARS level	[112]
				Upregulating the antioxidant status	

(continued)

Table 1 (continued)

Plant	Ext./Cons.	Dose	Exp. model	Effect	Ref.
Rice bran	Aqueous extract	28, 57, 115, 200 mg/kg, i.p.	MCAO	↓Total infarct volume, TUNEL-positive cells	[116]
				↑Expression disulfide isomerase, Nrf2, BDNF, NGF, GDNF	
R. damascena	Ethanolic extract	0.5, 1, 2 mg/ml, i.p.	I/R	↓Number of dark neurons	[120]
				↑NGF, NT3, and BDNF mRNA expression	
T. vulgaris	Ethanolic extract	50, 100, and 200 mg/kg, i.p.	I/R	↑Latency time, antioxidant capacity, ↑MDA levels of cortex	[126]
				↓MDA levels of serum, NO levels,	
V. odorata	Hydroethanolic extract	25, 50, and 75 mg/kg, orally	MCAO	infarct volume, neurological deficit scores	[133]
W. coagulans	Hydroethanolic extract	500, 1000 mg/kg, orally	I/R	↓Pycnotic neurons in brain cortex, TUNEL-positive cells, apoptosis, and histopathological alterations	[139]
Z. officinale	Ethanolic extract	100, 200, and 300 mg/kg, orally	MCAO	↓Scape latency, the neuronal density only in CA3, MDA	[144]
				↑SOD, CAT, GSH-Px	
Z. spina-christi	Ethanolic extract	50, 100, and 200 mg/kg/day, orally	Transient global cerebral ischemia	↑The frequency of passing, the shortened step-through latency, antioxidant capacity	[147]
				↓MDA, NO	

Abbreviations: *Exp.* Experimental, *Ref.* Reference, *Ext.* Extract, *Conc.* Concentration, *MCAO* middle cerebral artery occlusion, *NO* Nitric oxide, *MDA* Malondialdehyde, *OFT* Open field test, *FST* Forced swimming test, *mNSS* Modified Neurological Severity Scores, *VEGFR-2* Vascular endothelial growth factor receptor-2, *TUNEL* Transferase biotin-dUTP nick end labeling, *SRF* Serum response factor, *OSI* Oxidative stress index, *BCCAO* Bilateral common-carotid artery occlusion, *MMP-9* Matrix metalloproteinase-9, *ERK1/2* Extracellular signal-regulated kinase 1/2, *GRK2* G Protein-coupled receptor kinase-2, *SOD* Superoxide dismutase, *GSH* Glutathione, *CAT* Catalase, *Glu* Glutamate, *Asp* Aspartate, *4VO* Four vessel occluding, *MBP* Myelin basic protein, *HIE* Hypoxic-ischemic brain injury, *AchE* Acetylcholinesterase, *EGb* 761 Standardized extract of *Ginkgo biloba*, *NDS* Neurologic deficit scores, *NF-κB* Nuclear factor kappa B, *TLR4* Toll-like receptor 4, *TNF-α* Tumor necrosis factor alpha, *AQP-4* Aquaporin-4, *GRD* Glutathione reductase, *GPX* Glutathione peroxidase, *TBARS* Thiobarbituric acid reactive substances, *NGF* Nerve growth factor, *BDNF* Brain-derived neurotrophic factor, *Nrf2* Nuclear factor-E2-related factor 2, *GDNF* glial neurotrophic factor, *TUNEL* terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling, *GSH-Px* Glutathione peroxidase, *I/R* Induction of cerebral ischemia/reperfusion, *FKHR* Forkhead transcription factor

ily (α-, β-tubulin subunits), and 14-3-3 protein family (14-3-3£, ε) and extracted from irradiated mice prefrontal cortex, suggesting that *A. senticosus* could cross through the "blood-brain barrier" (BBB) for repairing damage induced by radiation [7].

3.2 *Bacopa monnieri*

Bacopa monnieri (*B. monnieri*) is a creeping herb belonging to the Scrophulariaceae family and is commonly used in Ayurvedic medicine [8]. Studies have shown that this plant extract possesses antioxidant property [9]. The effect of bacopaside (3, 10, and 30 mg/kg), in a rat model of transient focal ischemia induced by "middle cerebral artery occlusion" (MCAO), showed that orally administration of bacopaside for 6 days significantly reduced neurological deficits, cerebral infarct edema, and volume. It increased the brain ATP content, total adenine nucleotides, energy charge, and Na + K + ATPase activity. It also improved GPx, malondialdehyde (MDA) content, CAT, and SOD in the brain [10].

3.3 Carnosol

Carnosol is a phenolic diterpene derived from the Lamiaceae family [11]. Various pharmacological effects, such as anti-inflammatory and antioxidant activities, are attributed to carnosol [12, 13]. One study evaluated the impact of carnosol on the restraint stress-induced brain injury by keeping animals in restrainers. Treatment with carnosol (1, 5, 10 mg/kg, i.p.) for 21 days significantly decreased the immobility time in the forced swimming test (FST) and increased the number of crossing in the open field test (OFT). Besides, carnosol improved the level of CAT, MDA, SOD, GSH, GR, and GPx [14].

3.4 *Cassia mimosoides*

Cassia mimosoides (C. mimosoides) is a short-lived perennial herb, belonging to the Leguminosae family, and is found in Korea, Japan, and China [15]. *C. mimosoides* and its components have several pharmacological effects. For example, luteolin isolated from this plant have high antioxidant activity [16, 17]. The effect of methanol extract and ethyl acetate fraction of *C. mimosoides* was evaluated using the MCAO rat model with ischemia-reperfusion. They examined the effect of the whole extract, water fraction (WA), butanol fraction (BU), hexane fraction (HX), and ethyl acetate fraction (EA) (10, 100, and 1000μg/ml) on human hepatocellular carcinoma cells (HepG2) under hypoxic condition. Their results showed that the EA fraction improved HepG2 cell viability. Besides, the ethyl acetate fraction and methanol extract significantly reduced infarct size [18].

3.5 *Centella asiatica*

Centella asiatica (*C. asiatica*) is a perennial herbaceous plant belonging to the Umbelliferae family [19]. Several pharmacological effects have been attributed to this plant including wound healing, sedative and anxiolytic, antidepressant, and antioxidant [20]. Also, it has potent antioxidant [21] and anti-inflammatory properties [22] and has a protective role in Parkinson disease and Alzheimer's disease [23, 24]. The bioactive triterpenes of this plant are asiatic acid, asiaticoside, madecassic acid, and madecassoside [25]. The effects of *C. asiatica* ethanolic extract in MCAO on male rats was evaluated by Tabassum et al. Oral administration of *C. asiatica* for 21 days greatly improved neurobehavioral disorders in flexion test, rotarod test, and grip strength, decreased infarction volume, and improved histological morphology of the brain. Additionally, it reduced the "thiobarbituric acid reactive species" (TBARS) level, restored glutathione content, and increased the activities of GSH, SOD, GPx, and GR. The possible mechanism for protective effects of *C. asiatica* could be associated to the free radical scavenging, reduction of oxidative stress, and antioxidant activity of bioactive triterpenes [26].

3.6 *Crocus sativus*

Crocus sativus (*C. sativus*) is a medicinal plant with a long reputation in traditional medicine [27]. Various pharmacological effects have been described for *C. sativus* including antioxidant, anti-inflammatory [28], free radical scavenger [29], hypolipidemic [30], and anticonvulsant effects and improved activities on memory and learning [31, 32]. Crocin, crocetin, safranal, and picrocrocin were the main constituents of *C. sativus* [33]. Crocetin is a carotenoid dicarboxylic acid with a multi-unsaturated conjugate olefin acid structure. It has many physiological properties including antioxidant and anti-atherosclerotic effects [29, 34]. Also, it has neuroprotective activities on Parkinson's disease, memory impairment, and cerebral ischemia [35]. The effect of crocetin (50 mg/kg, orally) was evaluated for 15 days after the induction brain injury by a weight-drop model in rat. The results indicated that crocetin significantly recovered neurological function and inhibited neuronal apoptosis 72 h following treatment by reducing the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells. Besides, crocetin increased the expression of "vascular endothelial growth factor receptor-2" (VEGFR-2)

and "serum response factor" (SRF) in microvessel endothelial cells [36].

Crocin is another carotenoid from saffron and has antioxidant [37], anti-apoptotic [38], anti-inflammation [39], and antihypertensive activities [40]. Crocin can also reduce ischemia-reperfusion injury [41–43]. The effect of crocin in a rat model of transient focal ischemia induced by MCAO was examined. The results showed that crocin significantly reduced the cortical infarct and striatal infarct volume, the number of pre-necrotic neurons, axonal damage in ischemic regions, and the fiber demyelination and improved the neurological deficit score (NDS). Their results showed crocin reduced the cortical and striatal infarct volume and improved the neurological deficit score of ischemic rats. Their results also showed that crocin reduced the tissue swelling percentage of the ischemic hemisphere [44].

In another study, the effect of crocin on the global cerebral ischemia reperfusion induced by four-vessel occlusion was examined on the female rat. Administration of crocin decreased oxidative stress index (OSI) and total oxidant status (TOS) and increased total antioxidant capacity (TAS) in serum and brain of rats. Additionally, crocin improved histopathological parameters in the CA1 region of the brain and reduced the expression of caspase-3, HIF-1α, and the number of TUNEL-positive cells in the hippocampal region [45].

In another study, the effects of crocin on ischemia/reperfusion-induced brain damage using bilateral common carotid artery occlusion (BCCAO) in male mice was investigated. Their results showed that crocin pretreatment decreased the level of MDA, NO, and nitric oxide synthase (NOs) activities and increased the activities of SOD and glutathione peroxidase (GSH-px) in cortical microvascular homogenates. Additionally, crocin repaired serous edema with substantial microvilli loss, perivascular edema, vacuolation and membrane damage, mitochondria, and rough endoplasmic reticulum (RER), inhibited the expression of "extracellular signal-regulated kinase" (ERK) phosphorylation and "matrix metalloproteinase-9" (MMP-9) and membrane "G protein-coupled receptor kinase 2" (GRK2), and increased cytosol GRK2 in cortical microvascular homogenates [46].

The effects of crocin on brain injuries and cerebral edema after the induction brain injury by MCOA model in the rat were studied. Crocin significantly reduced brain edema and infarct volume and increased total antioxidant capacity (TAC) and activities of SOD and GPx in the ischemic cortex and improved NDS [43]. Treatment with *C. sativus* aqueous extract 7 days before the induction brain injury by MCOA model in rat increased the activities of Na + K + -ATPase, GSH, GR, GST, GPx SOD, and CAT, reduced MDA, and improved the neurobehavioral functions and histopathological parameters [47].

3.7 *Cuminum cyminum*

Cuminum cyminum (*C. cyminum*) belonging to the Apaiaceae family has been used for the treatment of diarrhea, toothache, and epilepsy in Chinese traditional medicine [48]. Also, in recent years antidiabetic and estrogenic activities of this plant are reported [49]. Main compounds of this plant are cuminol, carvone, apigenin, and luteolin [50]. The effects of *C. cyminum* before the induction brain injury by MCOA model in the rat were evaluated. In this study, the strength of the BBB was investigated. Results indicated that *C. cyminum* significantly decreased the permeability of the BBB, as the concentration of Evans Blue reduced in the right cerebral hemisphere [51].

3.8 Curcumin

Curcumin is a dietary polyphenol from turmeric, with an acceptable profile of safety and manifold salutary effects [52–61]. The effects of curcumin on hypoxic-ischemic brain injury were investigated. Treatment with curcumin inhibited nitric oxide synthase (iNOS) protein expression and the caspase-3 activity and increased the expression of Nrf2. Curcumin reversed the changes in MDA levels and SOD activities in neonatal rats with ischemic brain injury [62].

3.9 *Feronia limonia*

Feronia limonia (*F. Limonia*) belonging to the Rutaceae family has had different therapeutic effects such as antimicrobial, laxative, purgative, antihypertensive, astringent, diuretic, and cardiotonic properties [63, 64]. Since this plant is rich in beta-carotene, riboflavin, citric acid, oxalic acid, and malic acid [65], it could be potentially used as a neuroprotective agent against ischemia-reperfusion injury [66]. In one study, neuroprotective effect of *F. limonia* methanolic extract on brain injury was evaluated. Their results showed treatment with *F. limonia* after the induction of 30 min ischemia and reperfusion attenuated the neurological deficit, motor performance, and the total nitrite and MDA levels and increased the CAT and SOD enzyme activities [67].

3.10 *Gardenia jasminoides*

Gardenia jasminoides (*G. jasminoides*) belongs to the family of Rubiaceae. The fruit of *G. jasminoides* has been used to manage inflammation, jaundice, hepatic disorders, hypertension, edema, headache, and fever [68]. The chemical compositions of *G. jasminoides* are iridoid glycosides, flavonoids, volatile oil, saponins, and polysaccharides. The iridoid glycoside of *G. jasminoides* is a strong anti-inflammatory agent of *G. jasminoides* [69]. The effect of *G. jasminoides* was evaluated with chronic cerebral ischemia in rats. Their results showed that *G. jasminoides* shortened the escape latency, reduced the apoptosis and necrosis of the cortex and hippocampus, improved the content of SOD, and inhibited AchE and NOS activities in brain tissue [70].

3.11 *Ginkgo biloba*

Ginkgo biloba (G. biloba) belongs to the Ginkgoaceae family and includes flavonoids, proanthocyanidins, and terpenoids. *G. biloba* prevents the neuronal cell death and ischemic brain injury [71]. It showed neuroprotective properties in hypoxia and ischemia and increases the cerebral blood flow and reduces ischemic brain damage [72, 73]. Researchers found that *G. biloba* extract on MCAO model in rat modulates the neuroprotective effects, reduced infarct volume, and prevented the injury-induced increase of cleaved caspase-3 levels [74]. In a similar study, the effect of *G. biloba* extract 1 h before the onset of MCAO prevented the injury-induced decrease of pAkt and its downstream targets, pFKHR and pBad [75].

3.12 *Kaempferia parviflora*

Kaempferia parviflora (K. parviflora) belongs to the Zingiberaceae family and has been used for the treatment of hypertension [76]. It has also anti-inflammatory and antioxidant activities [77–79]. Moreover, it has been shown to reduce the brain damage and improve memory impairment [80]. In a study, the effect of *K. parviflora* ethanolic extract 14 days before and 7 days after induction of brain injury by MCAO model in rats was studied. Their results showed that treatment with *K. parviflora* reduced the brain infarct volume, mitigated the reduction of Nissl bodies in the hippocampus, and prevented the development of ischemic injury [81].

3.13 *Mentha longifolia*

Mentha longifolia (*M. longifolia*) is a perennial herb belonging to the family of Lamiaceae. It is widely used in herbal medicine for the treatment of coughs, colds, and influenza [82]. It has been used in Iranian traditional medicine for treating digestive disorders and a carminative agent and as an antispasmodic agent [83]. Aerial parts of this plant possess therapeutic effects such as fungicidal, anti-inflammatory, antimicrobial, and antioxidant activities [84, 85]. The effect of *M. longifolia* hydroethanolic extract before the induction brain injury by MCAD model in rat reduced total infarct volume, Evans Blue extravasation in the ischemic hemisphere, MDA level in serum, and BBB permeability. It also reduced

lipid peroxidation and increased the antioxidant capability of the brain [86].

3.14 *Nigella sativa*

Nigella sativa (*N. sativa*) belongs to the Ranunculaceae family. It is widely used throughout the world [87]. The seeds of *N. sativa* contain thymoquinone and monoterpenes which have been used in folk medicine for headache, back pain, and gastrointestinal diseases [88]. The effects of *N. sativa* oil on transient focal cerebral ischemia on an ischemic-reperfusion model reduced the infarct volume and the water content in the ischemic lesioned hemisphere and improved the motor functions [89].

3.15 Olive

Olive is a species of small tree belonging to the family of Oleaceae. Olive leaf extract scavenges free radicals, and it is used in traditional medicine for the management of heart diseases and diabetes mellitus [90–92]. Olive leaf contains oleuropein, ligustroside, oleuroside, triterpenes, luteolin, apigenin, rutin, and diosmetin [93, 94]. The intragastric administration of olive leaf extract in a model of lead poisoning-induced brain injury in mice reduced neuronal and capillary injury and damage to organelles and matrix around the capillaries in the frontal lobe, Bax protein expression in the cerebral cortex, and malondialdehyde content and increased the activities of catalase, SOD, and alkaline and acid phosphatase [95].

3.16 Orientin

Orientin is one of the active flavonoid glycosides in the Ranunculaceae genera such as *Trollius* and *Ranunculus* [96]. Various pharmacological effects have been attributed for orientin such as its antioxidant, anti-inflammatory, and radioprotection activities [97].

The protective effects of orientin 24 and 72 h after induction cerebral ischemia-reperfusion (I/R) injury by MCAO method in rats were examined. The results showed that orientin reduced oxidative damage, neurological deficits, cerebral edema, and neurotoxicity of excitatory amino acids, downregulated AQP-4 expression, and improved cell structure and morphology [98].

3.17 *Punica granatum*

Punica granatum (*P. granatum*) belongs to the Punicaceae family and has antibacterial, antidiarrheal, anti-ulcer, antioxidant, and anti-lipoperoxidative activities [99–103]. Its active compounds are ascorbic acid, flavonoids, proanthocyanidins, vitamin E, polyphenols, punicalin, and punicalagin [104–106]. The effect of *P. granatum* extract 15 days before I/R brain injury reduced brain levels of MDA, NO, caspase-3, NF-κB p65, and TNF-α and increased activities of GRD, SOD, and GPX. It also increased brain levels of cerebral ATP and IL-10. It decreased brain levels of caspase-3, NF-κB p65, and TNF-α. In addition, comet assay showed that treatment with *P. granatum* reduced brain DNA damage in rats [107].

3.18 Quercetin

Quercetin is a natural flavonoid. It is found in many plants and foods, such as red wine, onions, green tea, apples, and berries. Several pharmacological effects have been described for quercetin including antioxidant, anti-inflammatory, anti-blood coagulation, anti-ischemic, and neuroprotective effects [108–110]. Quercetin provided protective effects in the treatment of different types of brain injury and cerebral edema [109, 111], which is mediated by inhibition of neurological deficit, lipid peroxidation, polymerase (PARP) activity, caspase-3 activity, p53 expression, and increase in endogenous antioxidant defense enzymes [108–112]. Treatment with quercetin before and after the induction ischemia by MCAO model in rat significantly reduced infarct

size, TBARS level, and the neurological deficits, suppressed neuronal loss, diminished the p53 expression, and elevated the activity of poly (ADP-ribose) polymerase (PARP) and caspase-3 [112].

3.19 Rice Bran

Rice bran is a byproduct of the rice milling process and has been used as a feedstock and food ingredient. Different pharmacological effects such as anti-inflammatory, antioxidant, lipid-lowering, and anti-hyperglycemic effects of this plant have been reported [113]. The main components of rice bran are phytic acid, tocopherols, ferulic acid, oligosaccharides, oryzanols, phenolic acids, tocotrienols, peptides, and antioxidants [114, 115]. The effect of rice bran aqueous extract supplemented with ferulic acid on ischemic brain injury-induced MCAO model in the rat was examined. Treatment with rice bran combined with ferulic acid daily for 3 days after induction of MCAO significantly improved neurological function and enhanced the anti-apoptotic effect in the cortex and neural cell densities in DG and CA1 of the hippocampus. It stimulated the expression of antioxidant genes and neurotrophic factor and synaptophysin, neuronal nuclei (NeuN), and glutamic acid decarboxylase 67 proteins [116].

3.20 *Rosa damascena*

Rosa damascena (*R. damascena*) belongs to the family of Rosaceae [117]. It is used for the treatment of premenstrual breast tenderness. *R. damascena* also have various pharmacological effects such as bacteriostatic and antispasmodic effects [118]. The chemical constituents of *R. damascena* are fats, volatile essential oils, malic, resins, tannic acids, tartaric acids, and flavonoids [119]. The neuroprotective effect of *R. damascena* extract on adult rat following ischemic brain injury was studied by Moniri et al. Their results showed that *R. damascena* significantly decreased NT3 mRNA expression, BDNF, and NGF in neurons of the hippocampus [120].

3.21 *Thymus vulgaris*

Thymus vulgaris (T. vulgaris) (Lamiaceae family) has been used in the folk medicine and found to have antispasmodic, antitussive, diuretic, and carminative properties [121]. Thymus species also have antimicrobial and antioxidant activities [122–124]. The main components of *T. vulgaris* are thymol, carvacrol, borneol, and linalool [125]. The effect of *T. vulgaris* extract in rats showed that *T. vulgaris* ethanolic extract significantly increased second latency time in passive avoidance test and reduced MDA levels of the brain cortex [126].

3.22 *Viola odorata*

Viola odorata (*V. odorata*) (Violaceae family) is used in traditional medicine for the management of insomnia, anxiety, and hypertension [127–129]. The main components of the plant include alkaloid, saponins, methyl salicylate, mucilage, and glycoside [130]. The plant possesses antioxidant and diuretic activities [131, 132]. The effect of *V. odorata* administered by gastric gavage on reducing infarct volume and neurological defects was evaluated by MCAO method. The results showed a reduction in total infarct volume and neurological deficit scores treated with *V. odorata* extract [133].

3.23 *Withania coagulans*

Withania coagulans (W. coagulans) belongs to the family of Solanaceae. The main compounds of this plant are withanolides, free amino acids, essential oils, and fatty oils [134, 135]. Withanolides are steroidal lactones with an ergostane skeleton that has hepatoprotective and anti-inflammatory activities [136]. Also, withanolides from *W. coagulans* have neuroprotective effects [137] and have protective effects against myocardial I/R injury [138]. In one study, the neuroprotective effects of *W. coagulans* extract on the brain cortex in a rat model of ischemia and reperfusion were studied. The results showed the ethanolic extract of *W. coagu-*

lans significantly increased pycnotic (dying) neurons and reduced pycnotic and TUNEL-positive neurons in the ischemic brain [139].

3.24 *Zingiber officinale*

Zingiber officinale (Z. officinale) (belonging to Zingiberaceae family) has been traditionally used to treat several disorders such as catarrh, rheumatism, constipation, gingivitis, toothache, nausea, and diarrhea [140–143]. In a study, the effect of ethanolic extract of *Z. officinale* rhizome before and after MCAO was evaluated. The results showed that *Z. officinale* improved cognitive function and neuron density in the hippocampus of rats, decreased the brain infarct volume, and increased the activities of GSH-Px and SOD in the hippocampus and cerebral cortex [144].

3.25 *Ziziphus spina-christi*

Ziziphus spina-christi (*Z. spina-christi*) (Rhamnaceae family) has also been found in traditional medicine as a remedy for sores, pneumonia, and dysentery [145]. Chemical studies have shown the presence of cyclopeptide alkaloids, saponins, C-glucosylflavones, and betulic and ceanothic acid from this plant [146]. In one study, the neuroprotective effect of *Z. spina-christi* on brain injury following transient global cerebral ischemia and reperfusion was studied. Their results indicated that the hydroethanolic extract of *Z. spina-christi* leaf significantly improved motor coordination and balance, prolonged the shortened step-through latency, reduced the MDA level of serum and brain, and improved brain and serum antioxidant capacity [147].

4 Neuroprotective Mechanisms of Medicinal Plants on Brain Injury

We have reviewed various potential mechanisms that have been postulated for the neuroprotective effects of medicinal plants on brain injury in this section (Fig. 1).

The possible mechanism for the therapeutic effects of *A. senticosus* could be due to regulating different action pathways, such as phagosome,

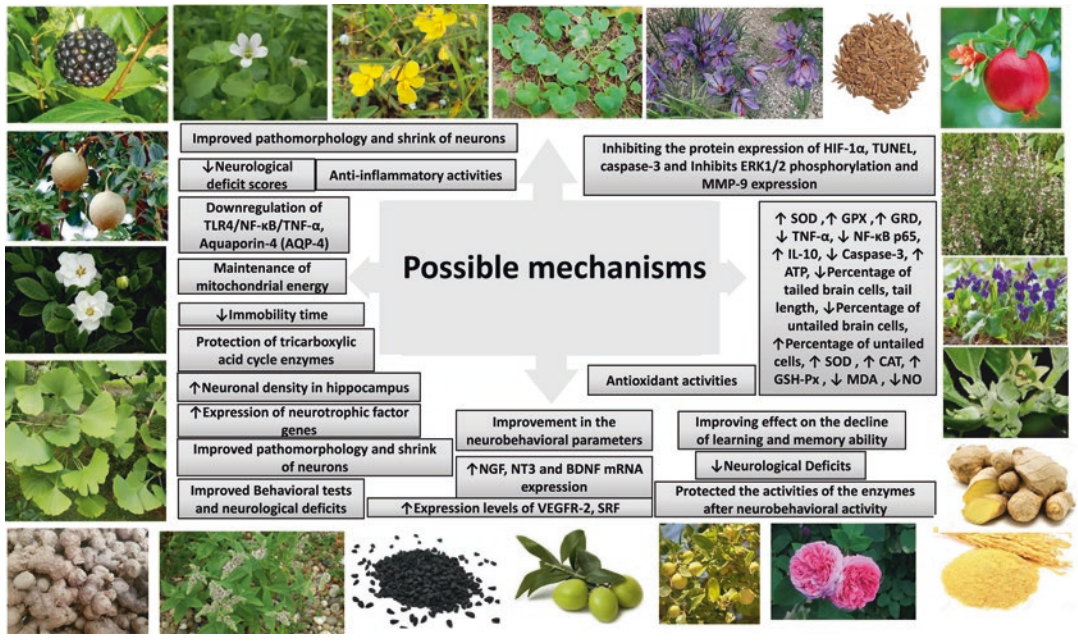

Fig. 1 Potential mechanisms for the neuroprotective effects of medicinal plants against brain injury.

Hippo, PI3K/Akt, neurotrophin, gap junction, glycolysis/gluconeogenesis, HIF-1 (hypoxia-inducible factor 1), and Rap1 (Ras-related protein RAP-1A) signaling pathways to maintain normal neurological activity [7]. The antiradiation effects of *A. senticosus* polysaccharides include eliminating toxic and harmful free radicals, maintaining the hematopoietic function, enhancing immune cell phagocytosis, preventing the growth inhibition of cells caused by radiation, and inhibiting the infiltration of inflammatory factors or cancerous cells in a murine model. HIF-1 signaling pathways regulated by *A. senticosus* extract in mouse brain showed that *A. senticosus* had positive effects on improving the energy supply of mouse brain cells, which improved the tolerance of mice to radiation [7].

The possible mechanism for therapeutic effects of bacopaside could be due to inhibition of lipid peroxidation, improvement of brain energy metabolism, and increase in the GSH-Px, CAT, and SOD activities and NO production in the brain tissue [10]. Results showed that carnosol by enhanced antioxidant defenses and decreased oxidative injury could be for the potential neuroprotective drug in cases of brain injury [14]. The possible mechanism for the protective effects of *C. mimosoides* could be associated with the inhibition of brain cell apoptosis [18].

C. asiatica prevented neuronal injury by its free radical scavenging properties [26]. *C. asiatica* hasantioxidant properties and has been shown to scavenge free radicals. Thus, the anti-ischemic activity of *C. asiatica* could be attributed to its antioxidant compounds [21]. The protective effect of crocetin on brain injury could be associated to the inhibiting apoptosis and enhancing vessel angiogenesis at the sub-acute stage of cerebral injury [36]. The protective effect of crocin on brain injury could be related to increased antioxidant enzyme activity and the suppression of the production of free radicals [43].

The protective effect of crocin on apoptosis after cerebral ischemia may be associated with its mechanism of decreased OSI induced by ROS generation and inhibited the protein expression of HIF-1α, TUNEL, and caspase-3 [45]. The possible mechanism for neuroprotective effect of crocin on mice model of transient global ischemia could be due to the reduction of oxidative stress, GRK translocation in the ischemic brain, and the activation of ERK pathway [46].

The possible mechanism for therapeutic effects of *C. sativus* could be due to the inhibition of lipid peroxidation, enhancing GSH, and improving energy metabolism [47]. The protective effect of *C. cyminum* on ischemic stroke may be related to anti-inflammatory and antioxidant activities of flavonoids and phenolic compounds of this plant [51]. The protective effect of curcumin on brain injury could be associated with the reduction of hypoxic-ischemic brain injury in neonatal rats through the induction of HO-1 and Nrf2 [62].

The protective effect of *F. limonia* on neurobehavioral disorders after the induction of ischemia and reperfusion may be due to its inhibition of oxidative stress, enhancement of the catalase and superoxide dismutase enzyme activities, reduction of the total nitrite and MDA which is the marker of lipid peroxidation [67].

Possible therapeutic mechanisms of *G. jasminoides* in attenuating cerebral ischemia injury mainly include preventing the apoptosis of neurons and antioxidant activities. *G. jasminoides* has a protective effect on brain injury caused by chronic cerebral ischemia. The mechanisms were found to be correlated with the reduction of free radicals, NO toxicity, and AChE activity [70].

The possible mechanism of *G. biloba* extract on MCAO model in rat could be through Akt and downstream targets, Bad and forkhead transcription factor (FKHR), and prevention of the injury-induced decrease of Akt phosphorylation [75]. The possible mechanism for the neuroprotective effect of *K. parviflora* extract on brain injury could be due to free radical scavenger and antioxidant activities. Other potential mechanisms include upregulation of the expressions of eNOS and inhibition of iNOS expression [81].

The possible mechanisms of *M. longifolia* on brain injury could be due to a reduction of oxidant markers and an increase of antioxidant markers [86]. The potential mechanism for the therapeutic effects of *N. sativa* could be due to the inhibition of lipid peroxidation and ROS pro-

duction [89]. Administration of olive leaf extract which relieves neurons and capillaries from a lead-induced brain injury was by reducing apoptosis and increasing antioxidant capacity [95].

The attenuation of oxidative stress could be part of the protective mechanism by utilizing orientin in brain ischemia-reperfusion injury rats. Moreover, the inflammatory response has a close relationship with oxidative stress in the brain I/R injury. Orientin could provide neuroprotection against inflammatory response in I/R rats through the TLR4/ NF-κB/TNF-α signaling pathway. The molecular mechanism might involve the down-regulation of the AQP-4 and TLR4/NF-κB/ TNF-α signaling pathway [98].

The protective effect of *P. granatum* extract on I/R-induced brain damage is due to its ability to reduce the brain MDA and NO levels. Also, the neuroprotective effects of pomegranate polyphenols can be due to the protection of TCA cycle enzymes from the attack of free radicals and maintenance of mitochondrial energy production [107].

The possible mechanism for protective effects of quercetin could be associated with the inhibition of lipid peroxidation, caspase-3 activity, p53 expression, and PARP activity and increase in endogenous antioxidant defense enzymes [112].

The effects of rice bran on functional recovery are related to the antioxidant genes and increased expression of neurotrophic factor gene stimulation of the SYP, brain-derived neurotrophic factor NeuN, nuclear factor-E2-related factor 2, and GAD-67 expressions [116]. The protective effect of *R. damascena* following cerebral ischemia may be related to these neurotrophic factor expression increases [120].

In the Steorki (2017) study, *T. vulgaris* extract was found to exert a neuroprotective action. Mechanisms underlying the neuroprotective activity might involve inhibition of oxidative stress and promoting antioxidant activity in the rat brain [126].

The mechanism of *W. coagulans* extract in neuroprotective on brain is very sensitive to oxidative damages caused by ischemia/reperfusion [139].

The cognitive-enhancing effect of *Z. officinale* is due to increased neuronal density in the hippocampus and antioxidant activity of the extract that could enhance cerebral blood flow [144].

The mechanism involved in the neuroprotective activity of *Z. spina-christi* extract may be associated with antioxidant activity by its flavonoid compounds and inhibition of oxidative stress in the brain [147].

5 Conclusion

In this review, we have described the protective effects of various herbal medicines on brain injury. Herbal medicines demonstrated a significant decrease in brain injury by different mechanisms. The present work summarized several studies reporting the protective effects of herbal medicines and their mechanisms on brain injury. However, their exact mechanisms have not been fully elucidated. Among these plants, *A. senticosus, G. jasminoides*, and *G. biloba* had the best mechanisms of actions than other plants mentioned in this review. However, further investigations are required to reveal the clinical effects of herbal medicines on brain injury.

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The Effects of Ginsenosides on the Nrf2 Signaling Pathway

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Abstract

Nuclear factor erythroid-2 related factor 2 (Nrf2) is a major signaling pathway for the maintenance of homeostasis and redox balance. This pathway also plays a significant role in proteostasis, xenobiotic/drug metabolism, apoptosis, and lipid and carbohydrate metabolism. Conversely, the Nrf2 signaling pathway is impaired in several pathological conditions including cancer. Although various drugs have been developed to target the Nrf2 pathway, plant-derived chemicals than can potentially impact this pathway and are particularly attractive due to their minimal side effects. Ginsenosides are active components of ginseng and have been shown to exert pharmacological effects including antioxidant, anti-inflammatory, antitumor, antidiabetes, neuroprotective, and hepatoprotective activities. In this article, we have reviewed the effects of ginsenosides on Nrf2 signaling pathway.

Keywords

Ginsenosides · Ginseng · Nrf2 signaling pathway · Herbal medicine · Protective effects

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1 Introduction

In many countries, medicinal herbs have been extensively used as traditional remedies for the treatment of various diseases and infections [1–4]. Many drugs developed in recent years have a similar base and structure to plant-derived chemicals [5–7]. Nevertheless, there is growing interest to develop naturally occurring nutraceutical compounds as potential novel therapeutic agents due to their therapeutic potential and limited side effects [8–14]. The Food and Drug Administration (FDA) has characterized several plant-derived chemicals as safe compounds, and it seems that unlikely to synthetic medications, they are under less strict regulatory conditions [15].

Ginseng, a member of the Araliaceae family, has attracted much interest due to its potentially beneficial biological and therapeutic actions [16]. Historically, ginseng has been recommended for the management of various disorders [17]. Due to its antioxidant activity, it can be used for enhancing the antioxidant defense system and has great potential to prevent the harmful impact of oxidative stress caused by exposure to potentially toxic chemicals such as heavy metals [18–20]. Besides, ginseng has anti-inflammatory [21], hepatoprotective [22], cardioprotective [23], antidiabetes [24], and neuroprotective [25] activities. It has been demonstrated that ginseng exhibits antitumor activity so that it reduces the viability, proliferation, and epithelial-to-mesenchymal transition (EMT) of tumor cells [26–28]. Its antitumor effects are further mediated through stimulation of apoptotic and autophagic cell death [29, 30]. Importantly, compounds derived from ginseng have been shown to exhibit similar features and properties, at least as favorable as those exhibited by ginseng [31].

Ginsenosides are steroidal saponins isolated from the root of ginseng and used as medicinal herbs [31]. Structurally, ginsenosides have a hydrophobic backbone with connections to sugar moieties. Ginsenosides are divided into three characteristic categories including (a) panaxadiol, (b) panaxatriol, and (c) oleanolic classes. Three major steps are involved in the synthesis of ginsenosides [32]. Initially, the mevalonate pathway (MVP) leads to the production of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These two substances form 2, 3-oxidosqualene, which further undergoes several processes (i.e., cyclization, hydroxylation, and glycosylation) to finally form ginsenosides [32, 33].

Similar to ginseng, ginsenosides appear to exert favorable antioxidant [34], anti-inflammatory [35], cardioprotective [36], hepatoprotective [37], and antidiabetes effects [38] (Fig. 1). Ginsenosides has low bioavailability, and it has been demonstrated that nanostructures can be considered as efficient tools to improve their bioavailability [39, 40]. In the current study, we have reviewed the evidence on the therapeutic and biological activities of ginsenosides mediated by nuclear factor erythroid 2-related factor 2 (Nrf2).

2 The Physiological Importance of the Nrf2 Signaling Pathway

Specific mechanisms and pathways ensure the homeostasis and survival at a cellular and organism level. These pathways play a significant role during conditions of stress, when intrinsic or extrinsic factors impair the homeostasis of cells. Oxidative stress is one of the most common conditions during cell life and several systems responsible for encountering its harmful effects [41, 42]. The antioxidant defense system is stimulated during oxidative conditions [43–45]. There are also a number of signaling pathways accounting for improvement in antioxidant defense system [45]. It has been demonstrated that Nrf2 signaling pathway plays a critical role in this aspect [46]. At physiological conditions, Nrf2 signaling pathway is at the dormant form [46]. Exposure to stress conditions such as higher levels of free radical species leads to the induction of Nrf2 signaling pathway [46].

During conditions of oxidative stress, a high concentration of reactive oxygen species (ROS) is produced [47]. ROS generation also occurs during normal metabolism of living organisms

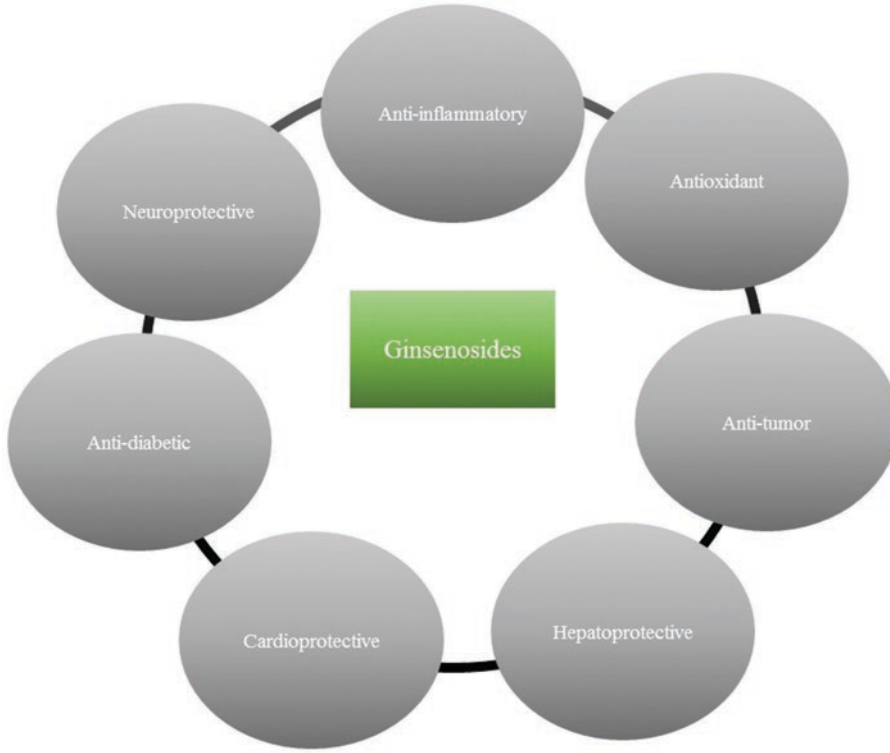

Fig. 1 Favorable therapeutic and biological activities of ginsenosides

[48]. It appears, however, that ROS produced during metabolism are not more than the capacity of the antioxidant defense system and this system will be able to deal with it [48]. High production of ROS during conditions of stress exceeds the capability of the antioxidant defense system leading to the stimulation of complementary pathways such as Nrf2 signaling pathway [48]. Nrf2 pathway exerts a stimulatory effect on the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) to improve the antioxidant capacity of cells thereby reducing the toxic impacts of oxidative stress [49].

3 Underlying Molecular Pathways of Nrf2 Signaling

The Nrf2 signaling pathway facilitates redox balance and protects cells against oxidative stress (Fig. 2) [50, 51]. During physiological conditions, when the level of oxidative stress is low, the Nrf2 signaling pathway is inactivated [52]. This inactivation is mediated by a cytosolic inhibitor, known as kelch-like ECH-associated protein 1 (keap1) [53–56]. This is a zinc metalloprotein, located near the plasma membrane, which suppresses the activity of the Nrf2 signaling pathway via the formation of a complex with Cullin 3 (Cul3) and Ring-box 1 [57]. This complex results in Nrf2 ubiquitination and its subsequent by the 26 s proteasome [58–60].

In contrast, oxidative stress causes the induction of Nrf2 signaling pathway [61–63]. Upon oxidative stress, an alteration occurs in the keap1-Cul3-Ring box 1 complex, and this complex is no longer able to degrade Nrf2 [46], resulting in accumulation of Nrf2 in the cytoplasm [46]. The Nrf2 then translocates into the nucleus where it activates the transcription of genes containing antioxidant-response elements (AREs). These genes which can reinforce the antioxidant defense system undergo upregulation under the function

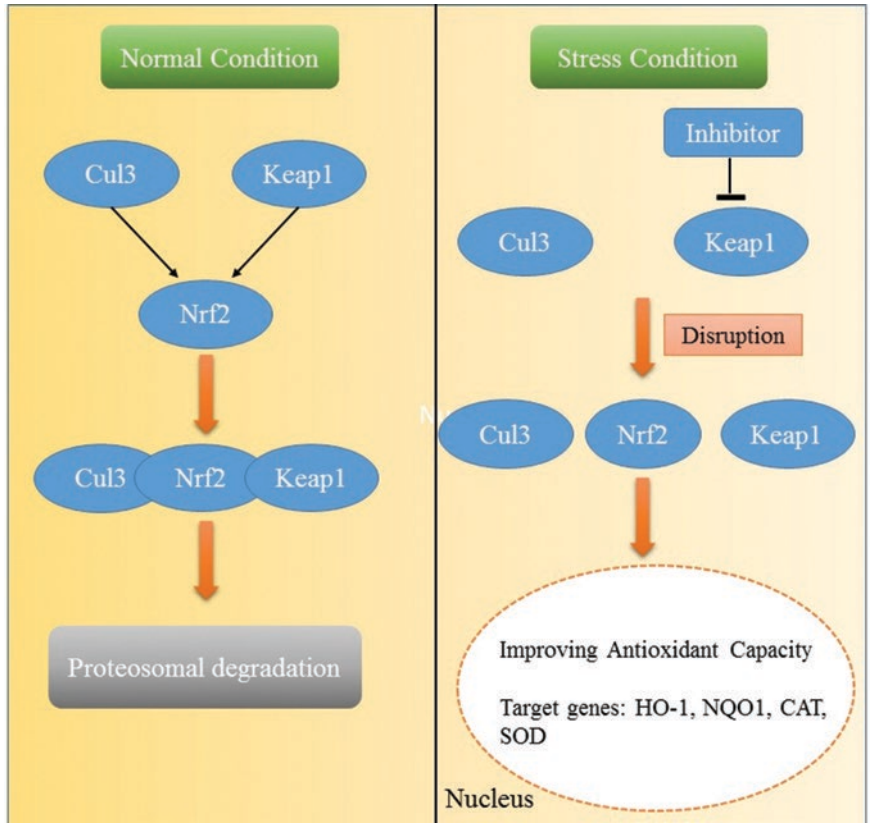

Fig. 2 Inactivation (left) and induction (right) of the Nrf2 signaling pathway during normal and stress conditions

of Nrf2 that include CAT, SOD, heme-oxygenase 1 (HO-1), NADPH oxidoreductase 1 (NQO-1), and glutathione S-transferase (GST) [64–66].

4 Nrf2 Signaling Pathway in Pathological Conditions

The Nrf2 signaling pathway has potential roles in maintaining cell survival and is involved in a number of processes such as proteostasis, xenobiotic/drug metabolism, apoptosis, and carbohydrate and lipid metabolism [67]. It has been demonstrated that any impairment in the Nrf2 signaling pathway is associated with the development of various pathological conditions [67] including cancer [68]. Enhanced expression of the Nrf2 is associated with drug-resistant tumor cells [69]. Su and colleagues demonstrated that osthole, as a naturally occurring nutraceutical compound, inhibits the progression of drug-resistant cervical tumor cells via diminishing the expression profile of Nrf2 [69].

It has been demonstrated that three genetic variants of the Nrf2 pathway, rs3124761, rs17458086, and rs1630747, can enhance the risk of pancreatic cancer [70]. Beinse and colleagues demonstrated that in TP53-mutated endometrial carcinomas, the expression level of one of the downstream mediators of Nrf2, namely, NQO1, is low, and it is considered as a potential target in the treatment of this cancer [49]. These results show that the modulation of the Nrf2 signaling pathway using plant-derived chemicals or synthetic drugs can be a potential therapeutic option for the treatment of cancer [68].

The disorders which are related to the higher levels of oxidative damages can be potentially

managed by targeting the Nrf2 pathway. The neurological disorders are one of the conditions that can be induced by perturbation in the Nrf2 signaling pathway and enhanced level of oxidative stress [71–73]. It has been shown that Parkinson's disease (PD) and Alzheimer's disease (AD) can be stimulated by the impairment in Nrf2 signaling pathway and consequently increased the level of oxidative stress suggesting a potential to target Nrf2 signaling pathway in the treatment of neurological disorders [74]. These are just some examples of the potential role of Nrf2 in the management of various pathological conditions. Elucidating the role of Nrf2 in various pathological states is out of the scope of this review.

5 Therapeutic and Biological Activities of Ginsenosides Mediated by Nrf2 Signaling Pathway

5.1 Nephroprotective Activity

In 2019, Liu and colleagues investigated whether the ginsenoside F2 can ameliorate hydrogen peroxide (H_2O_2)-mediated cell injury in kidney cells [75]. H_2O_2 remarkably elevated the levels of ROS and malondialdehyde (MDA), while it enhanced the activities of CAT, SOD, and glutathione peroxidase (GPx) [75]. It was found that treating human embryonic kidney cells (HEK-213 cells) with ginsenoside F2 (doses of 1.25, 5 and 20 μmol/l) enhanced the viability of cells by upregulating the Nrf2 signaling pathway and, consequently, by decreasing the level of ROS in a dose-dependent manner [75].

5.2 Protection Against Irradiation

Liu and colleagues examined the potential protective effects of vina-ginsenoside R7 against the harmful impact of ultraviolet B in normal human dermal fibroblasts [76]. Treatment with vina-ginsenoside R7 inhibited the oxidative stress-induced damages by enhancing antioxidant capacity through upregulation of the Nrf2 signaling pathway. The ginsenoside C-Y was also effective in ameliorating irradiation-mediated damages by improving the Nrf2 pathway [77].

5.3 Protection Against Ischemia/Reperfusion (I/R) Injury

In a recent study, Chen and coworkers evaluated the protective impact of ginsenoside Rb1 against intestinal I/R injury [78]. Administration of ginsenoside Rb1 was associated with an amelioration of intestinal I/R injury, so that ginsenoside Rb1 reduced the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and MDA, while it enhanced SOD activity [78]. Mechanistically, it was found that these protective effects were mediated by stimulation of phosphatidylinositide 3-kinase (PI3K)/protein kinase-B (Akt)/Nrf2 signaling pathway. It is well established that oxidative stress occurs after I/R injury [79]. It has been suggested that ginsenoside Rb3 is a potential tool in suppressing the I/R-mediated oxidative stress. Ginsenoside Rb3 was able to diminish oxidative stress by reinforcing and stimulating an antioxidant signaling pathway, known as protein kinase R-like endoplasmic reticulum kinase (PERK)/Nrf2/HMOX-1 [80]. Importantly, Chu and coworkers provided a novel pathway for decreasing the adverse effects of I/R injury [81]. It was shown that ginsenoside Rg1 effectively reduces the toxic impacts of I/R injury by improving antioxidant defense system through the miR-144/Nrf2/ARE signaling pathway.

5.4 Antidiabetic Activity

Dong and colleagues examined the potential ameliorative impact of ginsenoside Rb1 on diabetic retinopathy which is a common complication of diabetes [82]. Ginsenoside Rb1 reduced the diameter of retinal blood vessels and MDA

levels and enhanced the activity of glutathione (GSH). These ameliorative effects were a result of the upregulation of the Nrf2 signaling pathway [82]. However, more investigations need to be done in this area.

5.5 Hepatoprotective Activity

Ginsenoside Rg1 potentially protects hepatocytes against oxidative stress and apoptotic cell death through stimulation of the Nrf2 signaling pathway [83]. Ning and colleagues examined the impact of ginsenoside Rg1 against acetaminophen (APAP)-mediated liver injury [84]. Before the injection of APAP, C57BL/6 mice were treated with ginsenoside Rg1 (15, 30, and 60 mg/kg) for 3 days. In a dose-dependent manner, ginsenoside Rg1 enhanced the expression of Nrf2 and its downstream targets such as NQO1, HO-1, GCLC, and GCL and, therefore, reduced the APAP-induced oxidative stress M [84]. In the same study, Rg1 reduced the concentration of the toxic metabolite of APAP, N-acetyl-p-benzoquinone, imine by inhibiting the activities of Cyp2e1, Cyp3a11, and Cyp1a2 transporters and enzymes [84]. In order to investigate the role of Nrf2 signaling pathway in these protective activities, Nrf2 was inactivated by siRNA (in vitro) and all-trans retinoic acid (in vivo). It was found that the abrogation of the Nrf2 signaling pathway was associated with reduced hepatoprotective effects of ginsenoside Rg1 [84].

5.6 Neuroprotective Properties

Fan and colleagues evaluated the effects of ginsenoside Rg1 for the amelioration of depression-like behaviors in rats [85]. Ginsenoside Rg1 reduced neuronal apoptosis by upregulation of Bcl-2 and downregulation of caspase-3 and caspase-9 [85]. Furthermore, ginsenoside Rg1 administration prevented synaptic deficits and depression-like behaviors [85]. These protective impacts were partially mediated through enhancing the expression of the Nrf2 pathway [85]. As a determining factor in AD, amyloid-β (Aβ) can be modulated by natural compounds [86]. Ginsenoside compound K can improve memory capacity by regulating Aβ concentrations. Yang et al. in 2019 found that reduction in Aβ levels, inhibition of neuronal cell death, and improvement of the antioxidant defense system upon ginsenoside compound K administration were induced by the activation of Nrf2 signaling pathway [86].

Spinal cord injury (SCI) is associated with an enhanced level of oxidative stress, and various attempts have been performed to target oxidative stress. Ginsenoside Rb1 was administered to an animal model of SCI [87]. Treating animals with ginsenoside Rb1 significantly improved antioxidant defense system through increasing the activities of SOD, CAT, and GSH, while it reduced lipid peroxidation and MDA [87]. In addition, ginsenoside Rb1 decreased inflammatory cell infiltration and degeneration of spinal cord neurons. Mechanistically, these ameliorative impacts were mediated by stimulation of endothelial nitric oxide synthase (eNOS)/Nrf2/HO-1 signaling pathway [87].

The anti-seizure activity of ginsenoside Rb1 can be a result of modulation of Nrf2 signaling pathway [88]. Ginsenoside Rb1 is able to decrease the duration of seizure and enhance its latency [89, 90]. Its administration was associated with reduced cognitive deficits, MDA level, and neuronal apoptosis in a dose-dependent manner [90]. Ginsenoside Rb1 enhanced the level of Bcl-2, while it diminished the level of LC3 and inducible nitric oxide synthase (iNOS) [89]. These ameliorative impacts were a result of the stimulation of the Nrf2/HO-1 signaling pathway [89]. During oxidative stress, the phosphorylation of mitogen-activated protein kinase (MAPK) occurs, resulting in improvement in the antioxidant defense system by stimulation of the Nrf2 signaling pathway [89]. It has been suggested that ginsenoside Rh1 follows a similar route to improve the antioxidant capability, so that ginsenoside Rh1 induces the phosphorylation of MAPK and subsequently activates the Nrf2/HO-1 signaling pathway leading to the protection against H_2O_2-mediated oxidative stress and neuronal cell death [91].

5.7 Lung-Protective Activities

In a study, Ji and colleagues in 2018 evaluated the ameliorative impact of ginsenoside Rg1 against the lipopolysaccharide (LPS)-mediated damages [92]. This study provided a novel pathway which ginsenoside Rg1 follows to protect the lung epithelial cells against LPS-induced damage [92]. Exposing MLE-12 cells into LPS was associated with stimulation of apoptotic cell death, and it was found that autophagy induction diminishes the number of apoptotic cells [92]. Autophagy stimulates the Nrf2 signaling pathway to reduce apoptosis [93]. This pathway is beneficial in understanding the mechanism of action of ginsenoside Rg1 [93]. This demonstrates a novel molecular pathway whereby ginsenoside Rg1 stimulates autophagy and thereby enhances the expression of Nrf2 resulting in decreased LPS-mediated damages [92].

5.8 Cardioprotective Potencies

It has suggested by Wang et al. in 2018 that treating rats with ginsenoside reduces the harmful effects of isoproterenol on the heart [94]. Ginsenoside diminished the levels of MDA, troponin T, and activity of creatine kinase-MB (CK-MB) [94]. Also, ginsenoside treatment was related to the reduction in inflammatory cells infiltration and necrosis [94]. Mechanistically, these ameliorative activities were mediated by stimulation of the Nrf2 signaling pathway and various target genes such as GCLC and GCLM [94].

There are a variety of antitumor drugs which are extensively used for managing cancer, and adriamycin [95] is one of them. These various chemotherapeutic agents may negatively affect the various systems and organs of body, and heart is one of the most sensitive organs [95]. Wang and colleagues in 2015 evaluated the effects of ginsenoside Rg3 for reducing the adverse impacts of ADM on the heart [96]. They conducted in vitro (cardiac microvascular endothelial cells) and in vivo (rats) experiments [96]. Notably, ginsenoside Rg3 inhibited the reduction in the ejection fraction (EF) and fractional shortening (FS) and enhanced left ventricular outflow [96]. In addition, inhibition of endothelial dysfunction was observed in the rats exposed to the ginsenoside Rg3 [96]. Furthermore, in vitro experiment demonstrated that ginsenoside Rg3 reduces oxidative stress and apoptotic cell death [96]. It was found that these ameliorative impacts were mediated by induction of Nrf2/ARE signaling pathway through Akt [96].

Drug	Effect	In vitro	In vivo	Dose	Administration period	Major outcomes	Refs
Ginsenoside F2	Nephroprotective	Human embryonic kidney cells (HEK-293 cells)	–	1.25, 5, 20 μmol/L	24 h	Improving the viability by decreasing ROS production through upregulation of Nrf2 signaling pathway	[75]
Vina-ginsenoside R7	Protection against irradiation	Normal human dermal fibroblasts	–	1, 10 and 20 μM	72 h	Inhibition of oxidative stress-mediated damage by improving antioxidant capacity through upregulation of Nrf2 signaling pathway	[76]
Ginsenoside C-Y	Protection against irradiation	Normal human dermal fibroblasts	–	1-250 μM	24 h	Decreasing the irradiation-mediated damages by improving Nrf2 signaling pathway	[77]
Ginsenoside Rb1	Protection against I/R	–	Sprague Dawley rats	15 mg/kg	1 h before induction of I/R	Reducing the levels of MDA, IL-1b, and TNF-a and enhancing SOD activity by PI3K/Akt/Nrf2 signaling pathway	[78]
Ginsenoside Rb1	Antidiabetic	–	Rats	20 and 40 mg/kg	4 weeks	Decreasing the diameter of retinal blood vessels and MDA levels and enhancing GSH activity by upregulation of Nrf2 signaling pathway	[82]
Ginsenoside Rg1	Hepatoprotective		–			Inhibition of oxidative stress and apoptotic cell death by Nrf2 pathway upregulation	[83]
Ginsenoside Rg1	Neuroprotective	–	Rat	40 mg/kg	5 weeks	Decreasing neuronal apoptosis, depression-like behaviors, and synaptic deficits by upregulation of Nrf2 signaling pathway	[85]
Ginsenoside compound k	Neuroprotective	–	Mice			Reducing amyloid β (Aβ) expression, suppressing neuronal cell death, and enhancing antioxidant capacity by upregulation of Nrf2 signaling pathway	[86]
Ginsenoside Rg1	Lung-protective	–	Mice	30 mg/kg	8 h after LPS treatment	Induction of Nrf2 signaling pathway by autophagy activation, resulting in decreased LPS-mediated damages	[92]
Ginsenoside Rb3	Protection against I/R injury	H9C2 cells	–	0, 2, 5 and 8 μM	5 days	Decreasing I/R-mediated oxidative stress through PERK/Nrf2/HMOX1 signaling pathway	[80]
Ginsenoside Rg1	Protection against I/R injury	PC12 cells	Rats	0-10 μmol/l 20 mg/kg	–	Improving antioxidant defense system through activation miR-144/Nrf2/ARE signaling pathway	[81]

Drug	Effect	In vitro	In vivo	Dose	Administration period	Major outcomes	Refs
Ginsenoside Rh2	Lung-protective	–	Lung-injury animal model	5, 10, and 20 mg/kg	1 h before LPS administration	Amelioration LPS-induced lung injury by activation of Nrf2/HO-1 signaling pathway	[97]
Ginsenoside Rb1	Neuroprotective	–	Animal model of SCI	10 mg/kg	7 days	Enhancing antioxidant defense system, decreasing inflammatory infiltration and inhibition of neuron degeneration by activation of eNOS/Nrf2/HO-1 pathway	[87]
Ginsenoside Rg1	Hepatoprotective	–	C57BL/6 mice exposed to APAP	15, 30, and 60 mg/kg	3 days	Enhancing antioxidant and detoxification capacity through elevating the expression of Nrf2 signaling pathway	[84]
Ginsenoside Rg1	Hepatoprotective	–	Carbon tetrachloride-mediated liver injury	15, 30, and 60 mg/kg	7 days	Promotion of liver repair, decreasing the serum levels of ALT, AST, and ALP, reducing the level of MDA, and improving detoxification capability	[98]
Ginsenoside re	Cardioprotective	–	Isoproterenol-induced myocardial injury	5 and 20 mg/kg	7 days	Decreasing troponin T and MDA levels, reducing CK-MB activities, and diminishing the necrosis and inflammatory cell infiltration through improving Nrf2 signaling pathway	[94]
Ginsenoside C-mx	Protection against irradiation	Human dermal fibroblasts	–	1, 10 and 20 μ M	24 h	Improving cytoprotective antioxidant by stimulation of Nrf2/HO-1 signaling pathway	[99]
Ginsenoside Rg1	Hepatoprotective	HSC-T6 cells	Animal model of liver fibrosis			Amelioration of liver fibrosis by decreasing ROS levels through induction of Nrf2 signaling pathway	[100]
Ginsenoside Rh3	Protection against irradiation	Retinal pigment epithelium cells and retinal ganglion cells	–	1, 3, 10, and 30 μ M	6 h	Inhibition of keap1 by miR-141 and, subsequently, stimulation of Nrf2 signaling pathway, leading to the activation of Nrf2 signaling pathway and improving antioxidant capacity	[101]
Ginsenoside Rb1	Neuroprotective	–	Epilepsy kindled rats	15, 30, and 60 mg/kg	26 days	Decreasing apoptotic cell death and MDA level and improving seizure latency by induction of Nrf2 signaling pathway	[88]
Ginsenoside Rg1	Protection against I/R injury	H9c2 cells	–	10, 20, 40, and 60 μM	–	Suppressing I/R injury by reducing oxidative stress through activation of Nrf2 signaling pathway	[102]
Ginsenoside Rg1	Protection against irradiation	Human keratinocytes (HaCaT cells)	–	50 μM	1 h	Amelioration of ultraviolet-mediated glucocortiside resistance through Nrf2/HDAC2 signaling pathway	[103]

Drug	Effect	In vitro	In vivo	Dose	Administration period	Major outcomes	Refs
Ginsenoside Rg1	Hepatoprotective	–	Male mice	20, 40, 80, 160, and 320 mg/kg	5 days	Inhibition of hepatic injury by improving antioxidant capacity through enhancing the accumulation of p62, activation of JNK, and, consequently, stimulation of Nrf2 signaling pathway	[104]
Ginsenoside Rh1	Neuroprotective	Rat primary astrocytes	–	30, 100, and 300 μM	30 min	Improving antioxidant defense system and protection against H2O2-mediated oxidative stress by activation of Nrf2/HO-1 signaling pathway through MAPK phosphorylation	[91]
Ginsenoside Rd	Protection against I/R injury	–	Rats	50 mg/kg	30 min before induction of I/R injury	Improving cardiac function, decreasing infarct size, and reducing serum levels of troponin I and lactate dehydrogenase (LDH) by induction of Nrf2/HO-1 signaling pathway	[91]
Ginsenoside Rg3	Cardioprotective	Cardiac microvascular endothelial cells	Rats	10, 20, and 40 mg/kg	14 days	Inhibition of endothelial dysfunction, decreasing oxidative stress and apoptotic cell death and improving left ventricular outflow by induction of Nrf2 signaling pathway	[96]
Ginsenoside Rb1	Protection against I/R injury	–	Mice	30 and 60 mg/kg	10 min before reperfusion	Reducing TNF-a, MDA, and IL-6 levels and increasing SOD activity by induction of Nrf2/HO-1 signaling pathway	[105]
Ginsenoside Rg1	Hepatoprotective	–	Rat model of hepatic fibrosis	10, 20, and 40 mg/kg	2 weeks	Diminishing the levels of ALT, AST, ALP, and LDH levels and enhancing SOD, CAT, and GPx activities by stimulation of Nrf2 signaling pathway	[106]
Ginsenoside Rb1	Neuroprotective	Neural progenitor cells	–	10 μM	24 h	Protection against oxidative damage by activation of Nrf2 signaling pathway	[107]

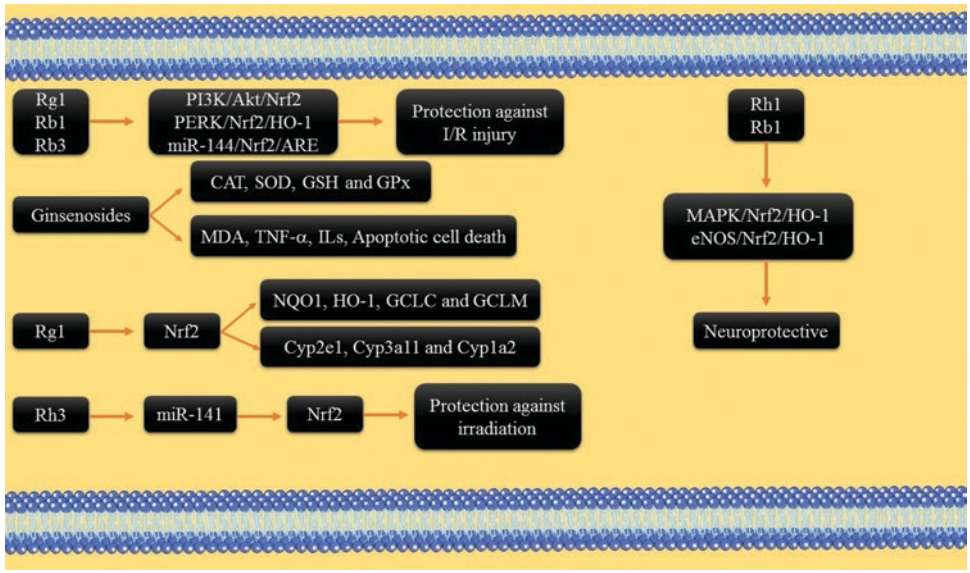

Fig. 3 Protective effects of ginsenosides mediated by Nrf2 signaling pathway. PI3K, phosphatidylinositide 3-kinase; Akt, protein kinase-B; miR, microRNA; HO-1, heme oxygenase-1; ARE, antioxidant response element; PERK, protein kinase R-like endoplasmic reticulum kinase; CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; GPx, glutathione peroxidase; TNF, tumor necrosis factor; ILs, interleukins; NQO1, NADPH quinone oxidoreductase 1; GCLC, glutamate cysteine ligase catalytic; GCLM, glutamate cysteine ligase modifier; Cyp, cytochrome p450; I/R, ischemic/reperfusion; MAPK, mitogen-activated protein kinase; eNOS, endothelial nitric oxide synthase

6 Conclusion

Targeting the Nrf2 signaling pathway is a novel strategy in the management of various pathological conditions, and it has been reported that naturally occurring nutraceutical compounds are of importance due to their low side effects and valuable biological and therapeutic activities. Ginsenosides are medicinal herbs derived from the root of ginseng and have shown great pharmacological effects. At the present review, we have shown that ginsenosides target Nrf2 signaling pathway to exert their protective effects (Fig. 3). The Nrf2 pathway is induced by ginsenosides to improve the antioxidant defense system by enhancing the activities of CAT, SOD, GSH, and GP resulting in reduced levels of MDA and lipid peroxidation. In addition, inflammatory cytokines such as TNF-α and ILs are inhibited under the activation of the Nrf2 pathway by ginsenosides.

Among the impacts of ginsenosides on Nrf2 pathway, some pathways are novel such as shown in Fig. 2. In order to exert its protective impact against I/R injury, ginsenosides induce PI3K/Akt/Nrf2, PERK/Nrf2/HO-1, and miR-144/Nrf2/ARE pathways leading to the inhibition of adverse effects of I/R. In terms of neuroprotective activity, ginsenosides Rh1 and Rb1 stimulate MAPK/Nrf2/HO-1 and eNOS/Nrf2/HO-1 pathways to improve antioxidant capacity. Besides, it has been shown that miR-141, as a target of ginsenoside Rh3, stimulates the Nrf2 signaling pathway and protect cells against irradiation.

Conflict of Interest The authors declare no conflict of interest.

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The Effect of Green Coffee Bean Extract on Cardiovascular Risk Factors: A Systematic Review and Meta-analysis

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Abstract

Background and aim: Cardiovascular disease remains the primary cause of noncommunicable disease- related death. The present systematic review and meta-analysis was performed to assess the possible benefit of the green coffee bean extract on cardio-metabolic markers.

Methods: PubMed, Scopus, Web of Science, and Cochrane Library were systematically searched to identify clinical trials that examined the effect of green coffee bean extract on cardio-metabolic risk factors including serum lipid profiles, glycemic status-related markers, blood pressure, and anthropometric indices. Since the included RCTs were carried out in different settings, random effect models were used to conduct all meta-analyses.

Results: Fifteen studies (19 arms) consisting of 637 participants were included. The results indicated that green coffee bean extract significantly reduced levels of total cholesterol (−5.93 mg/dl; 95% CI: −9.21, −2.65; I^2: 0%), fasting plasma glucose (−2.21 mg/dl; 95% CI: −3.94, −0.48; I^2: 32%), systolic blood pressure (−3.08 mmHg; 95% CI: −4.41, −1.75; I^2: 26%), diastolic blood pressure (−2.27 mmHg; 95% CI: −3.82, −0.72; I^2: 61%), body weight (−1.24 kg; 95% CI: −1.82, −0.66; I^2: 15%), and BMI (−0.55 kg/m^2; 95% CI: −0.88, −0.22; I^2: 73%). Although the pooled effect size of

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LDL-C, fasting insulin, and waist circumstance were significant, the results were significantly influenced by individual studies. No significant effect was detected for triglycerides, HDL-C, HbA1C, and HOMA-IR. However, the nonsignificant pooled effect size for triglyceride levels was influenced by one individual study.

Conclusion: The present study suggests that green coffee been extract consumption can improve total cholesterol, triglycerides, body weight, blood pressure, and fasting plasma glucose.

Keywords

Cardiovascular disease · CVD · Green coffee · Chlorogenic acid

Abbreviations

BMI	Body mass index
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
FPG	Fasting plasma glucose
HbA1C	Hemoglobin A1C
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
LDL-C	Low-density lipoprotein cholesterol
RCT	Randomized clinical trial
SBP	Systolic blood pressure
SD	Standard deviation
TC	Total cholesterol
WC	Waist circumstance

1 Introduction

Cardiovascular disease (CVD) is the major cause of mortality and morbidity worldwide. In 2016, approximately 31% of all global deaths were due to CVD, with over 75% of these CVD deaths occurring in low- and middle-income countries [1]. Main risk factors associated with CVD are sedentary lifestyle leading to overweight/obesity, unhealthy diet [2], raised blood glucose levels, hypertension [3], dyslipidemia [4], psychosocial factors [5], and smoking [6]. Modifiable lifestyle factors such as diet and physical activity can play an important role in alleviating CVD risk [7]. Several epidemiological and interventional studies have shown that bioactive compounds present in fruits and vegetables, such as polyphenols, carotenoids, flavonoids, and anthocyanins, may have a beneficial effect against the development of CVD [8–11]. Furthermore, there is a growing research interest in the potential beneficial cardioprotective properties of polyphenol-rich beverages such as tea [12–14], wine, beer [15], and coffee [16].

Among these beverages, coffee is one of the most popular drinks in the world [17]. Coffee plants, native to Africa, belong to the genus *Coffea* (family Rubiaceae) and are grown for their seeds (beans) which are roasted, ground, and sold for brewing coffee [18]. Coffee contains bioactive phenolic compound chlorogenic acid, methylxanthines, flavonoids, hydroxycinnamic acid, melanoidin, diterpenes, trigonelline, lignans, and minerals [19–21]. Ample evidence suggests that green coffee beans have anti-inflammatory and antioxidant properties, which are mainly attributed to bioactive compounds including chlorogenic acid, caffeine, diterpene, and trigonelline [22]. Chlorogenic acid has been inversely associated with metabolic syndrome, obesity [23], and chronic liver diseases [24]. Consumption of green coffee bean extract has been shown in both preclinical and emerging clinical trials to ameliorate the risk of diabetes mellitus type 2, ischemic stroke, and CVD [25] through reduction in high serum lipid concentrations [26], appetite level [27], abdominal obesity [28], oxidative damage [29], as well as high fasting blood sugar levels, fasting glucagon, insulin sensitivity [30], high blood pressure [31], arterial elasticity [32], and endothelial dysfunction [33].

The cardioprotective properties of green coffee bean extract have been investigated in human studies [32, 34–37]; however, the results of individual studies have not been consistent. To the authors' knowledge, a systematic review and meta-analysis of these studies has not been previously conducted. Therefore, the present system-

atic review and meta-analysis of clinical trials was designed to assess the overall effect of the green coffee bean extract on cardio-metabolic markers including anthropometric indices, BP, blood glucose, and lipid profile within the adult population.

2 Methods

The present investigation was designed and reported in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [38].

2.1 Search Strategy

Systematic literature searches were conducted using the data sources PubMed, Scopus, ISI Web of Science, and Cochrane Library, from their inception until January 2020. The search strategy texts which were applied for exploring into databases were constituted from two main concepts including "Green coffee" and relevant cardiovascular risk factors. Another search keyword forming from "Green coffee" and "clinical trial" terms was also used to cover those eligible studies in which the outcomes of interest were reported as secondary outcomes and were not mentioned in abstract. The search strategy which was applied based on each database is presented in Supplemental Table 1. An additional manual search was followed by reference lists of selected studies to detect other relevant papers. Two authors (A.H and M.P) separately searched the electronic databases, and disagreements were resolved by group discussion.

2.2 Study Selection

After excluding duplicate publications, studies were independently screened by two reviewers (A.H and A.N) based on their titles, abstracts, and full texts. Articles were eligible for inclusion if they fulfilled the following criteria: (1) the study design was a controlled clinical trial, (2) the population of interest was adults (aged >18 years), (3) the intervention was green coffee supplementation, (4) the outcomes of interest were body weight, body mass index (BMI), waist circumference (WC), glycosylated hemoglobin (HbA1C), fasting plasma glucose (FPG), homeostasis model assessment-estimated insulin resistance (HOMA-IR) index, serum insulin, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP), and diastolic blood pressure (DBP). We excluded studies if they lacked a control group (single-arm studies) or with no proper control group (i.e., active agent supplemented as control group), duration of intervention was <2 weeks, green coffee was administrated as complex with other active substances, and age of participants was <18 years. All discrepancies were addressed by consensus or by discussion with a third author (M.P).

2.3 Data Extraction

The following data were extracted from the full text of included studies using a predesigned abstraction form: first author's last name, publication year, location of the study, study design, gender, mean age and BMI of participants, total sample size, study duration, dose and type of green coffee bean extract, and reported outcomes. When the data were reported at multiple measurements, only the outcomes at the end of the intervention were included in the analysis. Data extraction was conducted by two authors, independently (A.N and A.H). Subsequently, full-text studies were assessed, and discrepancies were resolved through discussion with a third author (M.P).

2.4 Risk of Bias Assessment and Credibility of Evidence

The risk of bias of the included studies was performed by two reviewers (A.H and M.P) using the Cochrane Collaboration Risk of Bias tool [39]. The main categories consisted of the following six items: (1) sequence generation sufficiency (selection bias), (2) allocation concealment

(selection bias), (3) blinding (performance bias), (4) clarification of failures and incomplete outcome data (attrition bias), (5) selective reporting of the results (reporting bias), and (6) other possible sources of bias. Each domain was assessed as "high risk, " "low risk, " or "unclear." Finally, the overall quality of the studies was categorized into weak or fair if ≥3 or < 3 domains were rated as unclear/high risk, respectively.

The credibility of the present study was evaluated based on GRADE handbook for grading quality of evidence and strength of recommendations [40] by using GRADEpro online software [41]. It assesses the quality of evidence in accordance with several criteria which explore risk of bias, inconsistency, indirectness, impression, and publication bias in each outcome of interests. The rigorous quality of evidences is categorized as very low, low, moderate, and high quality.

2.5 Statistical Analysis

All analyses were performed using STATA software version 12 (StataCorp, College Station, TX, USA). The mean difference and the standard deviation (SD) of intervention and control groups for all the outcomes of interest were extracted to calculate overall effect size. In studies in which mean change was not directly reported in the intervention and control groups, it was calculated by subtracting the post-intervention data from the baseline value. Furthermore, if the SD of change was not provided directly, SD for net changes were imputed according to the method of Follmann et al. [42].

The correlation coefficient used for SD of change calculation was also assessed by studies which provided sufficient data using the following formula: $[R = (SD^2_{Baseline} + SD^2_{Final}\text{-}SD_{Change}) / (2\times SD_{Baseline} \times SD_{Final})]$ [43]. The correlation coefficient (R) for each was the following: triglyceride, 0.74; total cholesterol, 0.68; LDL, 0.70; HDL-C, 0.78; FPG, 0.70; fasting insulin, 0.6; HOMA-IR, 0.61; HbA1C, 0.50; SBP, 0.78; DBP, 0.75; body weight, 0.97; BMI, 0.98; and WC, 0.95. Because of high correlation coefficient calculated for anthropometric indices (body weight, BMI, and WC), the correlation coefficient was assumed 0.9 for these parameters, and sensitivity analysis was also performed to assess whether the results of anthropometric indices are sensitive to different levels of correlation coefficient (0.8 and 0.6).

Since the included RCTs were carried out in different settings, random effect models were used to conduct all meta-analyses. The heterogeneity between studies was examined by the I-squared (I^2) index. The level of heterogeneity across studies was rated as low, moderate, or high corresponding to I^2 value of 0–30%, more than 30–60%, and more than 60%, respectively [39].

We conducted subgroup analysis according to dose of green coffee, duration of study BMI of participants, and/or health condition where possible to assess the impact of heterogeneity on outcomes. Sensitivity analyses were also performed to explore the extent to which inferences might depend on a particular study or group of studies as well as the impact of studies with a high risk of bias. Meta-regression was conducted to detect the effect of potential confounders on changes in outcomes of interest including dose of intervention, duration of study, and baseline measures of outcome of interest. We also assessed publication bias by visual inspection of funnel plot and two formal tests, the Begg-adjusted rank correlation test [44] and the Egger's regression asymmetry test [45]. A P-value <0.05 was accepted as statistically significant, unless otherwise specified.

3 Results

The study selection process, number of removed articles in each steps, and reason for excluding studies are illustrated in Fig. 1. In brief, after primary assessment and discarding irrelevant articles, 21 studies were selected for full-text screening. Of those, six studies were excluded due to a lack of a proper control group (n = 2), use of a combination intervention (n = 1), only reporting postprandial parameters (n = 1), the outcomes which were measured before-after an exercise intervention (n = 1), or the study which

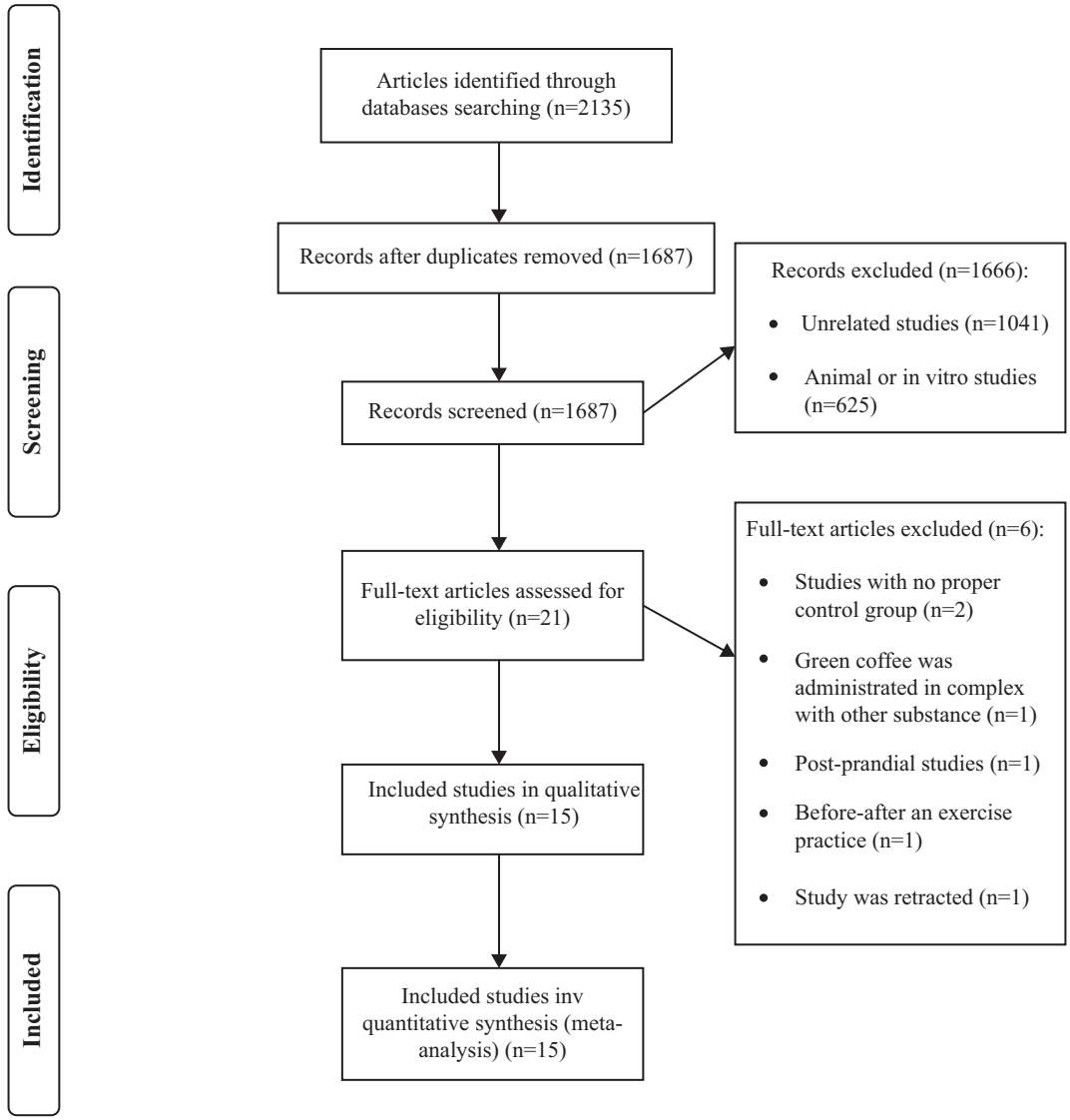

Fig. 1 Flow chart of the process of the study selection

was retracted (n = 1). Finally, 15 studies met eligibility and were included to systematic review. Kozuma et al. [46] administrated three different doses of green coffee bean extract and were considered as three separate active arms. Martínez-Lopez et al. [47] recruited normocholestrolemic and hypercholestrolemic patients and reported the outcomes for each condition independently. In this case, each condition was regarded as separate arm for pooling analysis. In addition, in a study conducted by Naderi et al. [37], participants were divided to four groups, in which green coffee bean extract was administrated to two of the four groups. Therefore, 15 studies including 19 active arms were selected for quantitative analysis.

The main characteristics of included studies are presented in Table 1. Fifteen clinical trials [31, 32, 34, 36, 37, 46–55] comprising a total of 637 participants were included to meta-analysis. The mean age of included participants was 38, and the average BMI was 27.5. Studies were conducted in different countries including Japan [31, 46, 48, 51, 53], Iran [32, 36, 37, 49], South Korea

Table 1 Study characteristics

First author (publication year)	Country	Number and gender	Mean age (Years)	BMI (kg/m^2)	Clinical trial design/ randomized/ blinding	Duration (days)	Comparison group	Type and amount of green coffee/CGA intake	Notes about participants	Outcomes
Suzuki et al. (2019)	Japan	Intervention: 8	Range: 35–56	Intervention: 21.9 ± 1.7	Parallel/ NR/yes	14	Beverage	Beverage containing CGA	Healthy	TG, TC, HDL, LDL, FPG, SBP, DBP
		Control: 8	Intervention: 44.6 ± 6.2	Control: 21.8 ± 2.2				300 mg/day		
		Males	Control:							
Zuniga et a. (2017)	Mexico	Intervention: 14	Range: 30–60	Intervention: 32.6 ± 24	Parallel/ yes/yes	84	Capsule	Capsule containing CGA	Impaired glucose tolerance	TG, TC, HDL, LDL, FPG, HbA1C, SBP, DBP, body weight, BMI, WC
		Control: 12	Intervention: 43 ± 11	Control: 32.1 ± 25				1200 mg/day		
		Both gender	Control: 45 ± 9							
Ochiai et al. (2004)	Japan	Intervention: 10	Range: NR	Intervention: 24.7 ± 1.6	Parallel/ NR/yes	120	Beverage	Beverage containing green coffee bean	Healthy	TG, TC, HD, LDL, FPG, fasting insulin, SBP, DBP
		Control: 10	Intervention: 37.2 ± 1.6	Control: 23.8 ± 0.6				140 mg/day		
		Males	Control: 34.8 ± 2.3							
Haidari et al. (2017)	Iran	Intervention: 30	Range: 20–45	Intervention: 31.58 ± 4.37	Parallel/ yes/yes	56	Capsule	Capsule containing green coffee bean extract	Obesity	TG, TC, HDL, LDL, FPG, fasting insulin, HOMA-IR, body weight, BMI
		Control: 34	Intervention: 36.1	Control: 32.07 ± 4.96				400 mg/day		
		Female	Control: 35.7	Intervention: 31.58 ± 4.37						

First author (publication year)	Country	Number and gender	Mean age (Years)	BMI (kg/m^2)	Clinical trial design/ randomized/ blinding	Duration (days)	Comparison group	Type and amount of green coffee/CGA intake	Notes about participants	Outcomes
Kim et al. (2012)	South Korea	Intervention: 10 Control: 10 Female	Range: NR Intervention: 44.7 ± 10.10 Control: 46.2 ± 10.91	Intervention: 25.6 ± 0.73 Control: 25.1 ± 0.61	Parallel/ yes/yes	56	Capsule	Capsule containing green coffee bean extract 100 mg/day	Overweight/ obese adults	TC, FPG, SBP, DBP, body weight, BMI WC
Fukagawa et al. (2017)	Japan	Intervention: 23 Control: 26 Female	Range: 25–40 NR	Range: 18–25 NR	Parallel/ yes/yes	56	Beverage	Beverage containing green coffee been extract 270 mg/day	Healthy	TG, TC, HDL, LDL, FPG, fasting insulin, HbA1c
Roshan et al. (2017)	Iran	Intervention: 21 Control: 22 Both gender	Range: 18–70 Intervention: 52.76 ± 9.83 Control: 51.95 ± 8.67	Intervention: 31.60 ± 3.58 Control: 31.16 ± 4.88	Parallel/ yes/yes	56	Capsule	Capsule containing green coffee bean extract 400 mg/day	Metabolic syndrome	TG, TC, HDL, LDL, FPG, fasting insulin, HOMA-IR, HbA1C, SBP, DBP, WC, body weight, BMI
Shahmohammadi et al. (2017)	Iran	Intervention: 22 Control: 22 Both gender	Rnge:20–70 Intervention: 41.36 ± 7.69 Control: 44.50 ± 5.24	Intervention: 31.27 ± 2.58 Control: 31.45 ± 2.18	Parallel/ yes/yes	56	Capsule	Capsule containing green coffee bean extract 1000 mg/day	Nonalco-holic fatty liver disease	TG, TC, HDL, LDL, FPG, fasting insulin, HOMA-IR, body weight, BMI, WC

(Continued)

Table 1 (continued)

First author (publication year)	Country	Number and gender	Mean age (Years)	BMI (kg/m^2)	Clinical trial design/ randomized/ blinding	Duration (days)	Comparison group	Type and amount of green coffee/CGA intake	Notes about participants	Outcomes
Kozuma et al. (2005)	Japan	Intervention: Group I: 29 Group II: 28 Group III: 31 Control: 29 Male	Range: 30–50 Intervention: Group I: 42.9 ± 8.2 Group II: 43.3 ± 8.3 Group III: 43.4 ± 8.4 Control: 43.1 ± 9.1	Intervention: Group I: 25.2 ± 4.0 Group II: 24.4 ± 2.6 Group III: 25.1 ± 3.6 Control: 24.0 ± 3.1	Parallel/ yes/yes	28	Low-sodium soy sauce plus soup without green coffee	Low-sodium soy sauce plus soup containing green coffee bean extract Group I: 46 mg/day Group II: 93 mg/day Group III: 185 mg/day	Mild hypertension	TG, TC, HDL, LDL, body weight, BMI
Martínez-López et al. (2018)	Spain	Normocholesterolemics: 25 Hypercholesterolemics: 27 Both gender	Range: 18–45 Normocholesterolemics: F: 26.6 ± 7.7 M: 24.7 ± 5.8 Hypercholesterolemics F: 33.3 ± 10.2 M: 34.8 ± 9.2	Normocholesterolemics F: 21.9 ± 2.5 M: 24.2 ± 2.8 Hypercholesterolemics F: 21.4 ± 2.5 M: 24.9 ± 2.3	Crossover/ yes/ yes	56	Beverage	Beverage containing green/roasted coffee 6 g/ day	Normocholesterolemic/ hypercholesterolemic	TG, TC, HDL, LDL, SBP, DBP, body weight
Watanabe et al. (2006)	Japan	Intervention: 14 Control: 14 Both gender	Range: NR Intervention: 52 ± 11 Control: 51 ± 8	Intervention: 23.8 ± 3.3 Control: 25.0 ± 3.5	Parallel/ yes/yes	84	Beverage	Beverage containing green coffee bean extract 140 mg/day	Mild hypertension	TG, TC, LDL, HDL, BMI
Hasani et al. (2017)	Iran	Intervention: 7 Control: 10 Female	Range: NR Intervention: 24.5 ± 3.06 Control: 24.57 ± 2.98	Intervention: 28.89 ± 2.95 Control: 29.10 ± 4.05	Parallel/ yes/no	42	Exercise	Green coffee plus exercise 250 mg/day	Overweight/ obese women	Body weight, BMI, WC

First author (publication year)	Country	Number and gender	Mean age (Years)	BMI (kg/m^2)	Clinical trial design/ randomized/ blinding	Duration (days)	Comparison group	Type and amount of green coffee/CGA intake	Notes about participants	Outcomes
Naderi et al. (2017)	Iran	Intervention: 12 Control: 12	Range: NR Intervention: 32.23 ± 5.44 Control: 32.25 ± 7.03	Intervention: 33.2 ± 1.37 Control: 32.56 ± 1.5	Parallel/ yes/no	56	Aerobic and resistance trainings	Green coffee capsule plus aerobic And resistance trainings 400 mg/day	Obese women	FPG, insulin, HOMA-IR, BMI
		Intervention: 12 Control: 12 Female	Range: NR Intervention: 30.15 ± 5.58 Control: 31 ± 5.27	Intervention: 31.58 ± 1.67 Control: 32.71 ± 1.68			No training/ supplementation	Green coffee capsule 400 mg/day		
Park et al. (2010)	South Korea	Intervention: 23 Control: 20 Female	Range: NR Intervention: 33.1 ± 1.92 Control: 33.1 ± 2.19	Intervention: 26 ± 0.45 Control: 26.3 ± 0.77	Parallel/ yes/yes	56	Capsule	Green coffee capsule 50 mg/day	Overweight/ obese women	TG, TC, HDL, LDL, FPG, fasting insulin, body weight, BMI, WC, SBP, DBP
Dellalibera et al. (2006)	Italy	Intervention: 30 Control: 20 Both gender	Range: 19–75 NR	NR	Parallel/ yes/yes	60	Capsule	Capsule containing green coffee bean extract 200 mg/day	Overweight	Body weight, BMI

Abbreviations: *CGA* chlorogenic acid, *TG* triacylglycerol, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *FPG* fasting plasma glucose, *HbA1C* hemoglobin A1C, *HOMA-IR* homeostasis model assessment of insulin resistance, *BMI* body mass index, *WC* waist circumstance, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *NR* not reported

[50, 52], Mexico [54], Italy [55], and Spain [47]. Three studies recruited only male participants [46, 51, 53], six studies enrolled only female participants [36, 37, 48–50, 52], and six remaining studies included both sexes [31, 32, 34, 47, 54, 55]. Except for Martínez-Lopez et al. [47], which had crossover design, all studies were parallel studies. The duration of included interventions spanned from 14 to 120 days, with an average of 56 days. Six studies recruited obese/overweight adults [36, 37, 49, 50, 52, 55], three trials enrolled healthy participants [48, 51, 53], two studies involved patients with mild hypertension [31, 46], one study enrolled participants with normocholesterolemic and hypercholesterolemic conditions separately [47], one study included nonalcoholic fatty liver disease-diagnosed patients [34], one study included participants with metabolic syndrome [32], and one study included participants with impaired glucose tolerance [54]. The dose of green coffee bean extract ranged from a minimum of 100 mg to a maximum of 6 g. Nine studies provided an encapsulated green coffee bean extract [32, 34, 36, 37, 49, 50, 52, 54, 55], fives trials provided a beverage containing green coffee [31, 47, 48, 51, 53], and one study administrated green coffee as part of a soup [46]. All studies were published between 2004 and 2019.

4 Risk of Bias Assessment and Credibility of Evidence

Thirteen studies were randomized [31, 32, 34, 36, 37, 46–50, 52, 55]; however, the method of randomization and allocation concealment was sufficiently addressed in six trials [32, 34, 36, 50, 52, 54]. Eleven studies were blinded [31, 32, 34, 36, 46, 48, 50, 51, 53–55], and 13 trials provided sufficient information around attrition bias [31, 32, 34, 46–55]. Nine studies acknowledged public, commercial, or industry financial support as well as any relation of authors with external agency which might influence the results [32, 34, 36, 47–49, 52–54]. The risk of bias summary is presented in Table 2.

The credibility of evidence for some outcomes of interest including triglyceride, TC, LDL-C, HDL-C, FPG, SBP, and body weight was high and reliable. However, quality of evidences for the rest of them was moderate in fasting insulin, DBP, and BMI, low in HbA1C and WC, and very low in HOMA-IR. Overall, there is a moderate confidence in estimated effects (Supplemental Table 2).

5 Meta-analysis

5.1 Effect of Green Coffee Bean Extract on Lipid Profiles

The results of the included meta-analysis demonstrated a significant reduction in total cholesterol (−5.93 mg/dl; 95% CI: −9.21, −2.65; I^2 = 0%) and LDL-C (−4.41 mg/dl; 95% CI: −7.55, −1.27; I^2 = 7%) levels after green coffee bean extract consumption. No significant effect was detected on triglycerides (−6.25 mg/dl; 95% CI: −13.34, 0.84; I^2 = 20%) and HDL-C (0.95 mg/dl; 95% CI: −0.46, 2.37; I^2 = 31%) serum levels (Fig. 2a–d).

Subgroup analysis based on the duration of intervention indicated that triglyceride levels were significantly reduced in studies with ≥84-day duration (−26.93 mg/dl; 95% CI: −53.11, −0.76; I^2 = 0%), while no significant difference was observed in studies with <84-day follow-up (−4.57 mg/dl; 95% CI: −11.35, 2.21; I^2 = 15%). No favorable effect was detected for triglycerides in further stratified analyses. When studies were stratified according to BMI of participants (>25 or ≤ 25), type of intervention (capsule or beverage), duration of administration, or dosage of administration, a greater reduction on total cholesterol levels was observed in subgroups including studies with mean BMI > 25 (−7.11 mg/dl; 95% CI: −12.69, −1.52; I^2 = 18%), green coffee bean extract administration as capsule (−8.65 mg/dl; 95% CI: −14.96, −2.34; I^2 = 20%), duration ≥84 day (−17.25 mg/dl; 95% CI: −29.63, −4.86; I^2 = 0%), and dosage ≥400 mg/days (−7.56 mg/dl; 95% CI: −13.38, −1.73; I^2 = 38%) (Table 3). Subgroup analyses for LDL-C and HDL-C were skewed by one study with a large weighting [36].

Table 2 The summary of review authors' judgments about each risk of bias item for included studies

Study	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Other bias
Suzuki et al. (2019)	U	U	L	L	L	U
Zuniga et a. (2017)	L	L	L	L	L	L
Ochiai et al. (2004)	U	U	L	L	U	U
Haidari et al. (2017)	L	L	L	U	L	U
Kim et al. (2012)	L	L	L	L	U	U
Hasani et al. (2017)	L	U	H	L	L	U
Fukagawa et al. (2017)	L	U	L	L	L	U
Roshan et al. (2017)	L	L	L	L	L	L
Shahmohammadi et al. (2017)	L	L	L	L	L	L
Kozuma et al. (2005)	L	U	L	L	U	L
Martínez-López et al. (2018)	L	U	H	L	L	L
Watanabe et al. (2006)	L	U	L	L	U	U
Naderi et al. (2017)	L	U	H	U	U	H
Park et al. (2010)	L	L	U	L	L	L
Dellalibera et al. (2006)	L	U	L	L	U	U

H high risk of bias, *L* low risk of bias, *U* unclear or unrevealed risk of bias. Criteria defined for risk of bias assessment are according to the Cochrane guidelines

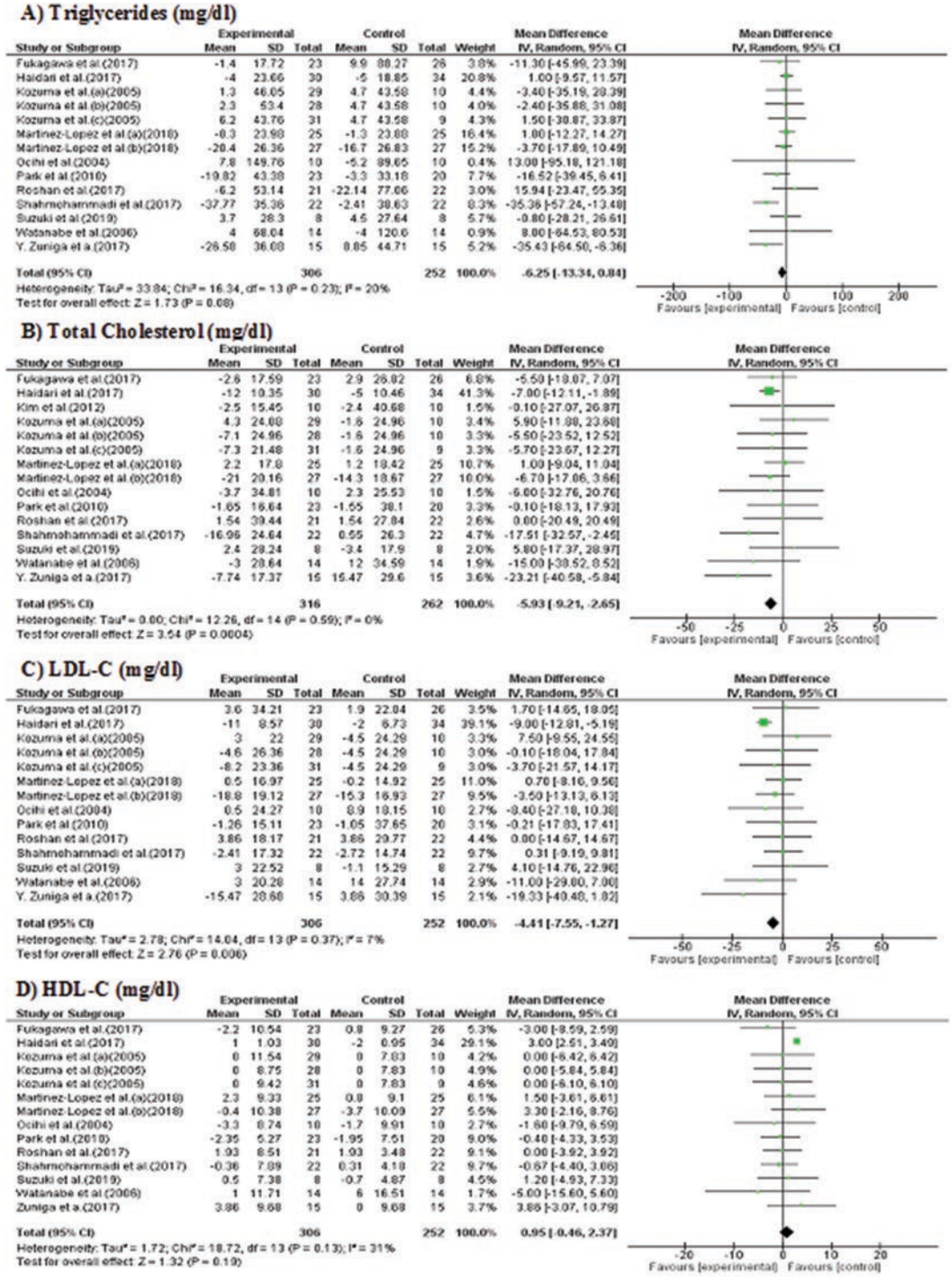

Fig. 2 The meta-analysis results of the effect of green coffee administration on lipid profiles

Table 3 Subgroup analyses

Variables	Subgroup analysis based on		Number of trials	Mean difference (95%CI)	Within study heterogeneity I^2	Between study heterogeneity (P-value)
Triglycerides (mg/dl)	Participants' condition	BMI > 25	7	−6.26 (−13.35, 0.84)	58%	0.29
		BMI ≤ 25	7	−1.62 (−10.08, 6.84)	0%	
	Duration	≥84	3	**−26.93 (−53.11, −0.76)**	0%	0.09
		<84	11	−4.57 (−11.35, 2.21)	15%	
	Dose (mg/day)	≥400	6	−8.52 (−21.10, 4.07)	66%	0.84
		<400	8	−6.04 (−17.84, 5.76)	0%	
Total cholesterol (mg/dl)	Participants' condition	**BMI ≥ 25**	8	**−7.11 (−12.69, −1.52)**	18%	0.34
		BMI < 25	7	−3.83 (−9.27, 1.62)	0%	
	Duration (day)	≥84	3	**−17.25 (−29.63, −4.86)**	0%	0.06
		<84	12	**−5.07 (−8.48, −1.67)**	0%	
	Dose (mg/day)	≥400	6	**−7.56 (−13.38, −1.73)**	38%	0.28
		<400	9	−3.01 (−9.31, 3.29)	0%	
Fasting plasma glucose (mg/dl)	Participants' condition	BMI ≥ 25	8	**−3.03 (−5.67, −0.39)**	54%	0.37
		BMI < 25	4	−0.93 (−3.36, 1.50)	0%	
	Duration (day)	≥84	3	**−4.02 (−7.21, −0.84)**	0%	0.15
		<84	9	−1.87 (−3.84, 0.10)	36%	
	Dose (mg/day)	≥400	6	−3.36 (−6.86, 0.14)	56%	0.66
		<400	6	−1.57 (−3.46, 0.32)	0%	
Systolic blood pressure (mmHg)	Participants' condition	**Elevated blood pressure**	5	**−4.19 (−5.95, −2.43)**	43%	0.02
		Other conditions	7	−1.23 (−3.23, 0.77)	0%	
	Duration (day)	≥84	3	−3.79 (−8.24, 0.65)	64%	0.28
		<84	9	**−2.89 (−4.10, −1.68)**	1%	
	Dose (mg/day)	≥400	4	−2.65 (−5.38, 0.08)	29%	0.56
		<400	8	**−3.23 (−4.84, −1.61)**	32%	
Diastolic blood pressure (mmHg)	Participants' condition	**Elevated blood pressure**	5	**−3.09 (−4.94, −1.25)**	58%	0.10
		Other conditions	7	−1.53 (−4.11, 1.05)	60%	
	Duration (day)	≥84	3	**−5.90 (−7.80, −4.01)**	0%	<0.001
		<84	9	**−1.42 (−2.76, −0.08)**	33%	
	Dose (mg/day)	≥400	4	**−2.08 (−3.85, −0.31)**	0%	0.47
		<400	8	**−2.35 (−4.49, −0.22)**	71%	

Table 3 (continued)

Variables	Subgroup analysis based on		Number of trials	Mean difference (95%CI)	Within study heterogeneity I^2	Between study heterogeneity (P-value)
Body weight (kg)	Participants' condition	**BMI ≥ 25**	10	**−1.32 (−2.01, −0.63)**	30%	0.32
		BMI < 25	3	−0.34 (−2.23, 1.55)	0%	
	Duration (day)	≥60	2	**−2.52 (−3.43, −1.60)**	0%	0.002
		<60	11	**−0.78 (−1.34, −0.22)**	0%	
	Dose (mg/day)	≥400	6	**−1.34 (−2.11, −0.56)**	0%	0.79
		<400	7	−0.92 (−2.09, 0.25)	51%	
BMI (kg/m^2)	Participants' condition	**BMI ≥ 25**	12	**−0.63 (−0.98, −0.28)**	75%	0.05
		BMI < 25	2	0.10 (−0.63, 0.83)	0%	
	Duration (day)	≥60	3	**−0.85 (−1.37, −0.33)**	43%	0.001
		<60	11	**−0.48 (−0.86, −0.11)**	69%	
	Dose (mg/day)	≥400	6	**−0.91 (−0.88, −0.22)**	80%	0.002
		<400	8	−0.27 (−0.70, 0.16)	67%	

-Due to substantial heterogeneity in some subgroups, all analysis was performed based on random effect methods

5.2 Effect of Green Coffee Bean Extract on Glycemic Status-Related Markers

Green coffee bean extract significantly improved FPG (−2.21 mg/dl; 95% CI: −3.94, −0.48; I^2 = 32%) and fasting insulin (−0.33 μU/ml; 95% CI: −0.62, −0.04; I^2 = 0%) concentration. However, no significant influence was observed in either HbA1C (−0.02%; 95% CI: −0.19, 0.16; I^2 = 27%) or HOMA-IR (−0.22 mg/dl; 95% CI: −0.69, 0.24; I^2 = 57%) (Fig. 3a–d). Subgroup analysis based on participants' mean BMI demonstrated a significant reduction in FPG levels in a subset of studies with an average participant BMI >25 (−3.03 mg/dl; 95% CI: −5.67, −0.39; I^2 = 54%) but not in studies with an average BMI ≤25 subgroup (−0.93 mg/dl; 95% CI: −3.36, 1.50; I^2 = 0). When studies were stratified according to study duration, FPG levels had a greater decrease in studies with ≥84-day follow-up (−4.02 mg/dl; 95% CI: −7.21, −0.84; I^2 = 0%) (Table 3). Due to low number of included studies, subgroup analysis was not performed for HbA1C, fasting insulin, and HOMA-IR.

5.3 Effect of Green Coffee Bean Extract on Blood Pressure

The results indicated a significant effect of green coffee bean extract on SBP (−3.08 mg/dl; 95% CI: −4.41, −1.75; I^2 = 26%) and DBP (−2.27 mg/dl; 95% CI: −3.82, −0.72; I^2 = 61%). This reduction was more pronounced in studies that included patients with elevated blood pressure for both SBP (−4.19 mmHg; 95% CI: −5.95, −2.43; I^2 = 43%) and DBP (−3.09 mmHg; 95% CI: −4.94, −1.25; I^2 = 58%) (Fig. 4a, b). Stratified analysis indicated SBP lowering effect of green coffee bean extract is greater in subgroups with a duration <84 days (−2.89 mmHg; 95% CI: −4.10, −1.68; I^2 = 1%) or dosage of administration <400 mg/day (−3.23 mmHg; 95% CI: −4.84, −1.61; I^2 = 32%) than the subset with ≥84-day follow-up (−3.23 mmHg; 95% CI: −4.84, 0.65; I^2 = 32%) or ≥ 400 mg/day green coffee intervention

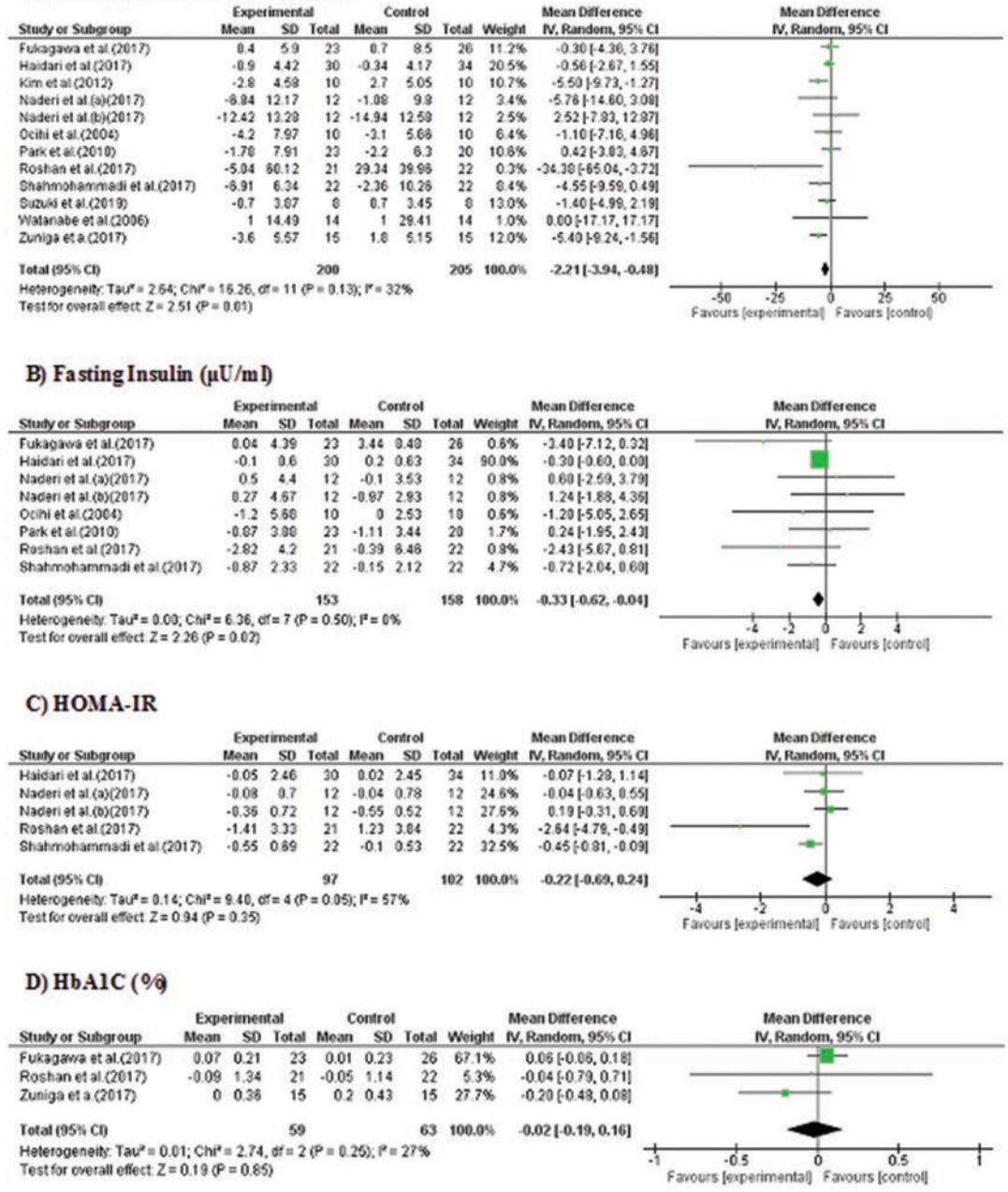

Fig. 3 The meta-analysis results of the effect of green coffee administration on glycemic-related factors

(−3.79 mg/dl; 95% CI: −8.24, 0.08; I^2 = 29%). Furthermore, the effect of green coffee bean extract on DBP levels was more robust in studies that were ≥ 84 days in duration (−5.90 mmHg; 95% CI: −7.80, −4.01; I^2 = 0%). No remarkable difference was detected in other subgroups for DBP (Table 3).

5.4 Effect of Green Coffee Bean Extract on Anthropometric Indices

The pooled results demonstrated that green coffee bean extract significantly decreased body

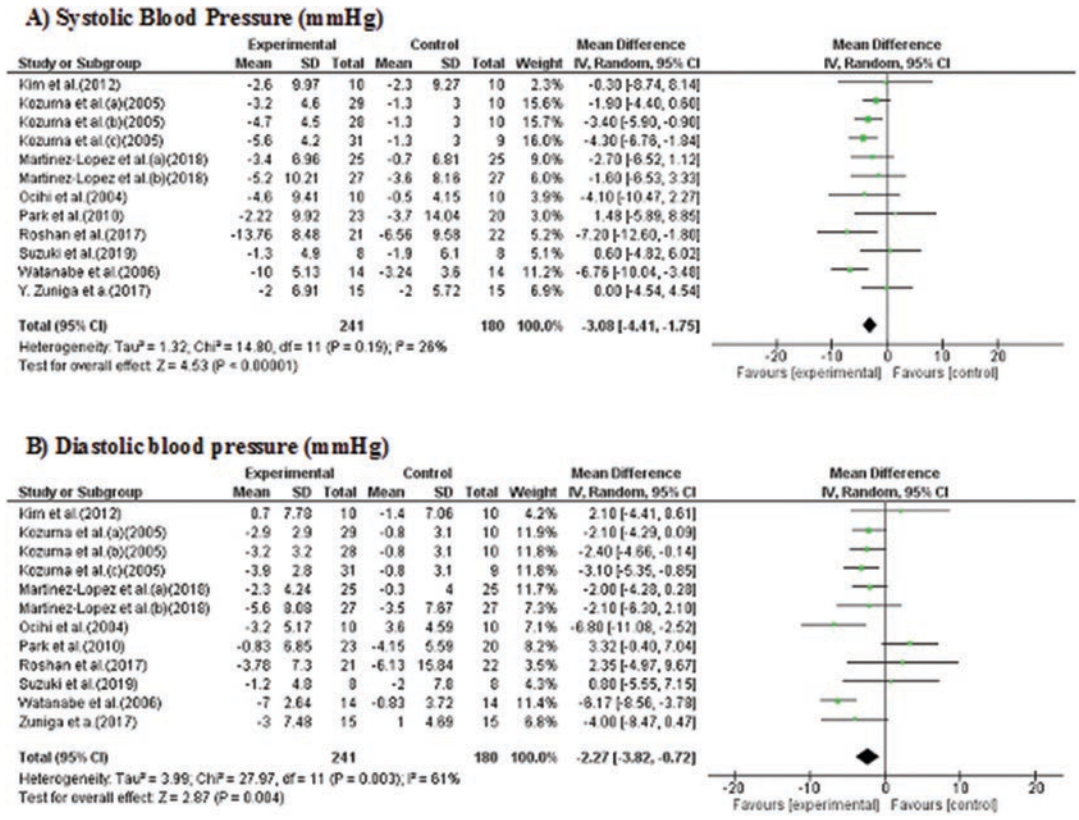

Fig. 4 The meta-analysis results of the effect of green coffee administration on blood pressure

weight (−1.24 kg; 95% CI: −1.82, −0.66; I^2 = 15%), BMI (−0.55 kg/m^2; 95% CI: −0.88, −0.22; I^2 = 73%), and WC (−1.01 cm; 95% CI: −1.78, −0.23; I^2 = 0%) (Fig. 5a–c). A higher level of body weight loss by green coffee bean extract consumption was found in subgroups with mean BMI ≥25 (−1.32 kg; 95% CI: −2.01, −0.63) and ≥ 400 dose of intervention (−1.34 kg; 95% CI: −2.11, −0.56), while no favorable effect on body weight was found in subgroup with mean BMI < 25 (−0.34 kg; 95% CI: −2.23, 1.55) or < 400 dose of intervention (−0.92 kg; 95% CI: −2.09, 0.25). Similarly, a greater BMI reduction was observed in subgroups with a mean BMI ≥25 (−0.63 kg/m^2; 95% CI: −0.98, −0.28) and ≥ 400 dose of intervention (−0.91 kg/m^2; 95% CI: −0.88, −0.22) (Table 3). Due to the low number of studies that reported waist circumference as outcomes, subgroup analysis was not conducted.

6 Sensitivity Analysis

In a sensitivity analysis that removed individual studies at a time, the removal of Haidari et al. [36] from the triglyceride pooled effect size changed the result to significant (−8.19 mg/dl; 95% CI: −16.5, −0.13; I^2 = 18%). The LDL-C overall effect size became nonsignificant after removing Haidari et al. [36] (−1.42 mg/dl; 95% CI: −5.29, 2.44; I^2 = 0%). Similarly, fasting insulin pooled effect size was also sensitive to Haidari et al. [36] with the removal of this study resulting in a non-significant pooled effect (−0.60 µU/ml; 95% CI: −1.51, 0.30; I^2 = 0%). When Haidari et al. [36] was discarded from the BMI pooled effect size, the heterogeneity changed from 73% to 48%, while the results remained significant (−0.44 kg/m^2; 95% CI: −0.69, −0.19). In addition, by excluding of Roshan et al. [32] from WC result, the pooled effect size became nonsignificant (−0.47 cm; 95% CI: −1.49, 0.54; I^2 = 0%). Pooled

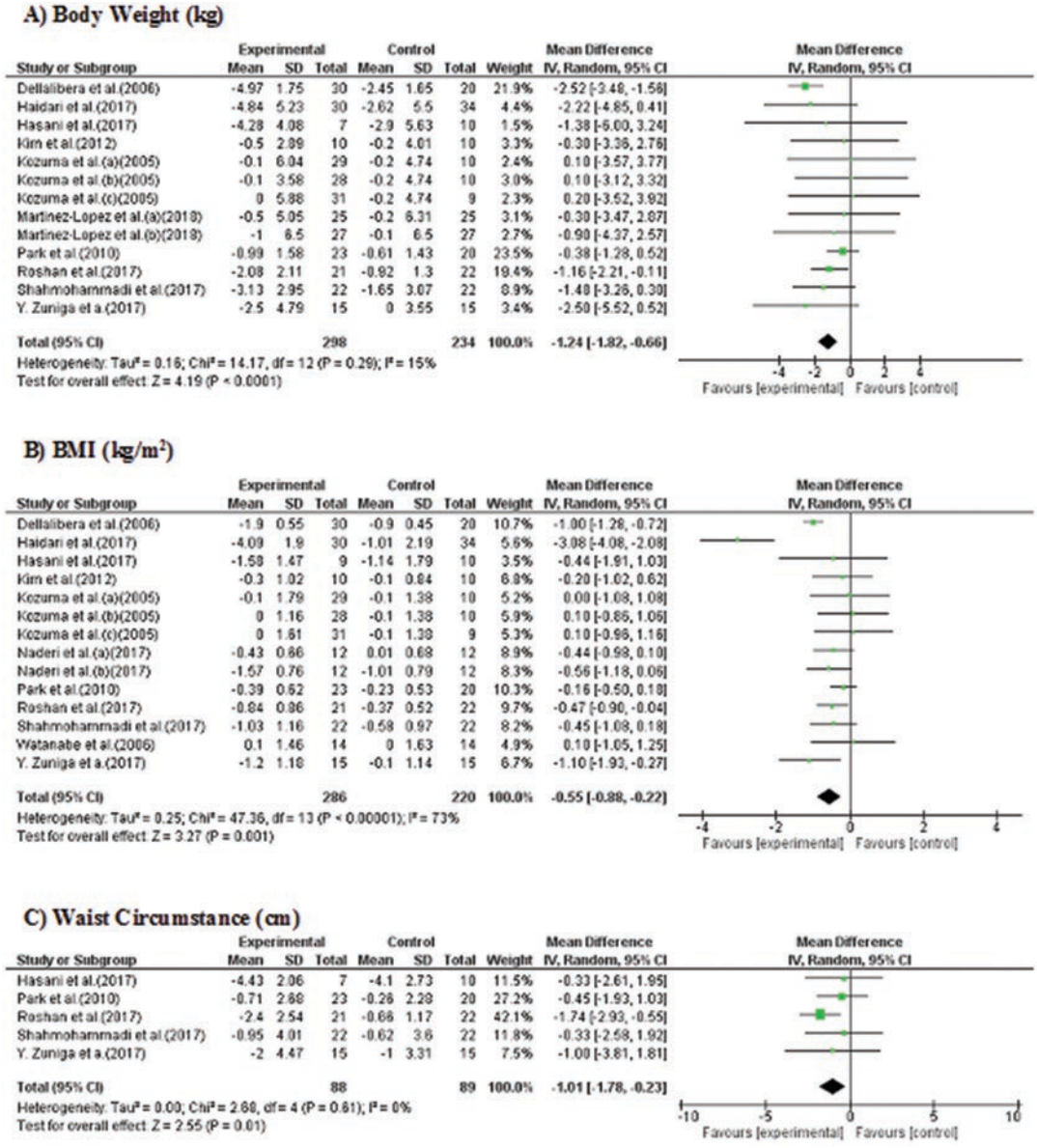

Fig. 5 The meta-analysis results of the effect of green coffee administrations on anthropometric measures

results did not reveal any sensitivity to individual study in other variables including total cholesterol, FPG, SBP, DBP, BMI, and body weight.

Sensitivity analysis by excluding high risk of bias studies [31, 37, 49, 51, 53, 55] indicated that removing studies with high risk of bias did not alter the overall effect size for triglycerides (−5.30 mg/dl; 95% CI: −11.17, 0.56; I^2 = 38%), total cholesterol (−5.99 mg/dl; 95% CI: −9.37, −2.62; I^2 = 0%), LDL-C (−5.27 mg/dl; 95% CI: −8.07, −2.46; I^2 = 21%), HDL-C (1.11 mg/dl; 95% CI: −0.39, 2.61; I^2 = 35%), FPG (−1.99 mg/dl; 95% CI: −3.40, −0.59; I^2 = 59%), SBP (−2.86 mmHg; 95% CI: −4.05, −1.68; I^2 = 0.6%), DBP (−1.86 mmHg; 95% CI: −2.85, −0.87; I^2 = 35%), body weight (−0.83 kg; 95% CI: −1.39, −0.23; I^2 = 0%), and BMI (−0.42 kg/m^2; 95% CI: −0.62, −0.21; I^2 = 77%). Because of the high correlation coefficient (r) calculated for body weight and BMI (0.9), we also performed

sensitivity analysis for alternative levels of correlation coefficient for imputing SD of change. The pooled effect sizes of both BMI (r = 0.8; −0.61 kg/m^2; 95% CI: −0.78, −0.44; I^2 = 58%; r = 0.6; −0.62 kg/m^2; 95% CI: −0.80, −0.44; I^2 = 43%) and body weight (r = 0.8; −1.28 kg; 95% CI: −1.80, −0.77; I^2 = 2%; r = 0.6; −1.30 kg; 95% CI: −1.83, −0.77; I^2 = 0%) were not sensitive to different levels of correlation coefficient.

7 Publication Bias

Visual inspection of funnel plot suggested a mild-to-moderate asymmetry in estimating the influence of green coffee bean extract on nearly all outcomes of interest (Supplemental Fig. 1). However, except for HDL-C, no potential evidence of publication bias was detected by Egger's regression asymmetry test and Begg's rank-correlation methods test for any outcome [triglycerides (Begg's test P = 0.69; Egger's test P = 0.73), total cholesterol (Begg's test P = 0.11; Egger's test P = 0.13), LDL-C (Begg's test P = 0.20; Egger's test P = 0.06), FPG (Begg's test P = 0.33; Egger's test P = 0.19), SBP (Begg's test P = 0.30; Egger's test P = 0.31), DBP (Begg's test P = 0.45; Egger's test P = 0.27), BMI (Begg's test P = 0.78; Egger's test P = 0.70), and body weight (Begg's test P = 0.32; Egger's test P = 0.53)]. Although Begg's rank correlation method test did not show any significant evidence of publication bias (P = 0.82), Egger's regression asymmetry test was significant for HDL-C (P < 0.001). After excluding Haidari et al. (2017), which the HDL-C pooled effect size was sensitive to, the Egger test changed to nonsignificant (P = 0.88). Due to the low number of study (<10) included in the meta-analysis for waist circumference, fasting insulin, HbA1C, and HOMA-IR, the publication bias test was not applicable.

8 Meta-regression

Meta-regression was performed to evaluate the influence of potential covariates, including dose of intervention, duration of study, and baseline measures of outcome of interest, on changes of CVD risk factors in response to green coffee bean extract. The results indicated that changes in FPG serum concentrations were associated with its baseline value (FPG: coefficient = −0.21; P = 0.01). Furthermore, a trend toward a significant association was detected for BMI changes following green coffee bean extract consumption and BMI baseline measures (BMI: coefficient = −0.11; P = 0.05). No other relation was observed between change in BMI and FGP and dose of green coffee bean extract as well as duration of intervention. Furthermore, the effect of green coffee bean extract on all other included CVD risk factors was independent of the potential covariates. Supplemental Table 3 shows the meta-regression results in detail.

9 Discussion

The present systematic review and meta-analysis suggests that green coffee bean extract consumption can be beneficial for controlling total cholesterol, FPG, blood pressure, and body weight. Although the results indicated a significant effect of green coffee bean extract on LDL-C, fasting insulin, and WC, these variables were sensitive to one study and should be interpreted with caution. Similarly, the pooled effect size for triglyceride levels became significant after one study was excluded. Furthermore, while the results of the included meta-analyses reported green coffee bean extract to provide significant improvements in a number of CVD-related outcomes, many of the improvements were relatively small (e.g., a significant body weight decrease of −0.84 kg). However, a relatively minor decrease in CVD risk factors can provide a marked reduced in overall risk of CVD. For example, a 2-mmHg reduction in SBP results in a reduced incidence of mortality related to stroke and ischemic heart by 10% and 7%, respectively [56–58].

The meta-analysis also indicated that the results of all outcomes of interest were robust after excluding studies with high risk of bias methodology. However, the credibility of evidence was only sufficient for some outcomes,

which implied that more studies are needed to make evidence-based conclusion. In addition, meta-regression analysis showed an association between changes in FPG concentration in response to green coffee consumption and FPG baseline measures, but not in case of dose or duration of study. This finding implied that the FPG-lowering effect of green coffee might be more visible in subjects with higher blood glucose levels and could be promising for controlling diseases with impaired glucose metabolism especially diabetes. Furthermore, we conducted a series of subgroup analyses to explore factors that may influence treatment response. While the subgroup analyses were not consistent for each outcome, the subgroup analyses generally suggest that chronic administration of green coffee bean extract (>60 or 84 days) in doses >400 mg may be more effective. In addition, green coffee bean extract may be more effective in populations with cardiovascular risk factors (e.g., BMI >25 or elevated blood pressure).

The mechanisms of action by which green coffee bean extract may improve CVD risk factors require further exploration in human intervention studies. Preclinical animal and cell-culture studies suggest that chlorogenic acid, the primary polyphenol compound within green coffee extract, may improve blood pressure via the stimulation of nitric oxide, antioxidant activity, and the inhibition of angiotensin-converting enzymes [59, 60]. Furthermore, chlorogenic acid may improve glycemic control and lipid profile via its effect on expression of peroxisome proliferator-activated receptor-ɣ and AMP-activated protein kinase [22]. Additional compounds such as caffeine and other polyphenol compounds may also exert a beneficial effect. Several reports have shown a beneficial impact of polyphenols on health condition, and its cardioprotective effect have been frequently suggested [61, 62]. Further studies that utilize techniques such as metabolomic analysis may provide further information regarding the relevant pathways in humans.

Furthermore, interindividual differences in the absorption and bioavailability of green coffee bean extract has not been well-explored in humans. Polyphenol absorption is highly dependent on gut microbiota composition. Monteiro et al. [63] explored the absorption of chlorogenic acid isomers and metabolites in humans and reported significant interindividual differences. This has also been reported for other polyphenol compounds such as Urothillin A, a polyphenol present in pomegranates, which appears to be differentially absorbed based on microbiota composition [64]. Further exploration regarding factors that influence absorption may inform future trials and reduce the possible influence of these factors on treatment response.

In this area, a few meta-analyses have investigated the effect of green coffee on only some of CVD risk factors such as lipid profiles [65] and anthropometric measures [66] and reported a positive influence on these outcomes. Although they supported the hypotheses behind the beneficial effect of green coffee, they only reported a simple influence without any investigation on quality of evidence, and these studies could be considered as primary outcomes. In this case, we put one step forward to clarify the quality of evidences across outcomes by performing adjustments for multiple confounders and exploring risk of bias for each finding. It made our results more reliable to decide whether using green coffee could be practical in prevention/treatment of CVD or there is still a lack of sufficient evidence to make any final conclusion. On the other hand, as these risk factors are known as the surrogate factors for CVD and are not hard outcomes of interest, there is a need to consider all risk factors as far as possible to make an evidence-based decision.

The comparative efficacy of green coffee bean extract compared to standard pharmacotherapy has not yet been evaluated. However, due to the demonstrated benefit of current pharmacological interventions for CVD management and prevention, green coffee bean extract may be of greater benefit as an adjunctive intervention by providing additional improvements in CVD markers. Furthermore, the use of an effective adjuvant intervention may allow for a reduction in the dose of pharmacological interventions in participants experiencing side effects. However, the efficacy

of green coffee bean extract as an adjuvant intervention to standard pharmacotherapy has not yet been evaluated.

While no serious adverse events were reported in the included studies, the safety of green coffee bean extract was only reported in seven studies. Zuniga et al. [54] reported that abdominal pain and distention, headache, diarrhea, and polyuria were experienced by participants in both groups. These side effects disappeared at the end of the first week of the intervention. Two participants in a study conducted by Roshan et al. [32] experienced discomfort by green coffee bean extract. One participant with a history of stomach irritation reported stomachaches, and another participant experienced dizziness. Furthermore, there is a lack of sufficient safety data in chronic conditions such as autoimmune conditions and conditions related to the liver and kidney. Further studies in this area are needed to enhance our knowledge about the safety of green coffee bean extract and possible interaction with pharmacological agents.

The current study has the following limitations which should be considered. The number of included studies per outcome was relatively low, especially in subgroups. The amount of the main active component of green coffee bean extract was not reported in all trials, which limited the ability of meta-regression analyses to adjust for dosage. Therefore, the included subgroup analyses that explored dose of intervention should be interpreted with caution. Due to the wide variation in bioactive compounds in herbal and plant-based interventions, future studies are recommended to report the level of key bioactive compounds and/or use of standard extracts to ensure a consistent dose is provided [67]. Several included studies did not control for participant's diet or physical activity, which may have influenced the study results. Finally, the participant population varied across the studies (i.e., participants with chronic diseases vs healthy participants) which might be as source of heterogeneity.

10 Conclusion

The present systematic review and meta-analysis suggests that green coffee bean extract consumption can have beneficial effect on improving triglycerides, total cholesterol, FPG, blood pressure, and body weight. Due to the promising cardioprotective effect and safety profile, further studies should explore the use of standardized green coffee bean extract as adjuvant therapy to conventional medical treatment. Further studies are also required to explore the relevant mechanisms of action and interindividual responses.

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Competing Interests None.

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Availability of Data and Material This is a review article and there is no raw data.

Authors' Contributions M.P., A.H., W.M., S.K, and A.S. carried out the conceptualization, design, and drafting of this study. A.N., A.H. and M.P. searched databases, screened articles, and extracted data. M.P. and A.H. performed the acquisition, analysis, and interpretation of data. W.M. critically revised the manuscript. All authors approved the final version of the manuscript. A.S. and A.H are the guarantors of this study.

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Nanomicellar Curcumin Supplementation Improves the Clinical Manifestations of HAM/TSP Patients

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Abstract

Background: HTLV-1 infection causes a chronic, progressive, demyelinating, neuroinflammatory disease called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Treatment of HAM/TSP patients which have high levels of proviral load and pro-inflammatory markers is a challenge for clinicians. Therefore, we aimed to investigate the immunomodulatory, anti-inflammatory, and antiviral effects of curcumin in HAM/TSP patients.

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Methods: In this study, 20 newly diagnosed HAM/TSP patients (2 men and 18 women) were enrolled and evaluated for clinical symptoms, HTLV-1 proviral load, Tax and HBZ expression, neopterin serum concentration, and complete blood count (CBC) before and 12 weeks after treatment with nanomicellar curcumin (80 mg/day, orally).

Results: Clinical symptoms such as the mean Osame Motor Disability Score and Ashworth Spasticity Scale Score were significantly improved after the treatment (P = 0.001 and P = 0.001). Sensory symptoms such as pain and paresthesia were significantly decreased in all of the patients (P = 0.001). Furthermore, urinary disorders, including urinary frequency, incontinence, and the feeling of incomplete bladder emptying, were significantly improved (P = 0.001, P = 0.003, and P = 0.03). However, the mean HTLV-1 proviral load (P = 0.97) and CBC were similar, whereas Tax, HBZ, and neopterin levels tend to increase after the treatment (P = 0.004, P = 0.08, and P = 0.04).

Conclusion: Results suggest that curcumin can safely improve the clinical symptoms of HAM/TSP patients but has no observable positive effects on the HTLV-1 proviral load, Tax, and HBZ expression. Therefore, prolonged use or the use of curcumin with antiviral agents in addition to clinical signs and symptoms can reduce the HTLV-1 proviral load and the expression of functional viral factors such as Tax and HBZ.

Keywords

HTLV-1 · Neuroinflammation · HAM/TSP · Curcumin therapy

1 Introduction

Human T-lymphotropic virus type 1 (HTLV-1) infection causes several diseases such as adult T-cell leukemia/lymphoma (ATL/ATLL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and other inflammatory disorders [1, 2]. It is estimated that HTLV-1 infects between 10 and 20 million people worldwide, mainly in large endemic areas such as the southern part of Japan, the Caribbean, Melanesia, south of America, central and west of Africa, the Middle East, and Mashhad in northeast of Iran [1–3].

HAM/TSP is a chronic progressive neuroinflammatory disease associated with demyelination and axonal damage in the brain and spinal cord [4–6]. Progressive weakness, paralysis of the lower limbs, sphincter dysfunction, and mild sensory disturbance are the most signs and symptoms of HAM/TSP patients [4–6]. HAM/TSP patients compared to HTLV-1-infected asymptomatic carriers significantly have higher proviral load and higher levels of inflammatory markers in peripheral blood [4–6]. Although the exact underlying mechanism is still unknown, it seems that immunological and virological factors and inflammatory responses influence the proviral load and play a pivotal role in the disease pathogenesis [4–6].

HTLV-1 provirus encodes regulatory proteins such as Tax and HTLV-1 basic leucine zipper factor (HBZ) which are playing critical roles in HTLV-1 pathogenesis [7–10]. Tax and HBZ activate several cellular genes through interaction with NF-κB and induce cell proliferation and leukemogenesis [7–10]. The interaction between HTLV-1 Tax- and HBZ-specific cytotoxic $CD8^+$ T lymphocytes (CTLs) with HTLV-1-infected $CD4^+$ T cells in HAM/TSP patients induces inflammatory responses and thus contributes to immunopathogenesis [7–10].

Neopterin is a nonspecific valuable biomarker for evaluation of the cell-mediated immune responses, the severity of inflammation, and the degree of T-cell activation [11, 12]. Its concentration in biological fluids such as serum, urine, and cerebrospinal fluid (CSF) could be used for the evaluation of cellular immune responses in infectious and inflammatory diseases. Change in neopterin serum levels has been reported in several neuroinflammatory and infectious diseases such as multiple sclerosis (MS) [13–15], human immunodeficiency virus (HIV) [16–18], and HTLV-1 infection [19, 20].

Curcumin, the phytochemical extract of turmeric, has been used extensively for the treatment of several chronic neuroinflammatory diseases [21–23]. Curcumin has several pharmacological properties such as anti-inflammatory, immunomodulatory, antiviral, and antioxidant activities [21–30]. Curcumin through interaction with several pro-inflammatory mediator signaling pathways effectively suppresses inflammatory processes and then the production of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8 by the immune cells [21–23] (Fig. 1). Curcumin also effectively induces the production of anti-inflammatory mediators such as IL-10 and transforming growth factor-β (TGF-β) [21–23]. So far no toxicity or side effects have been reported for curcumin usage even at very high doses and in a long-time use either in animals or in humans [31].

Since therapeutic strategies for HAM/TSP patients remain unsatisfactory and due to the beneficial anti-inflammatory and therapeutic effects of curcumin in other clinical trials, the current study was aimed to investigate the therapeutic effects of curcumin supplementation on the HTLV-1 DNA proviral load and Tax and HBZ mRNA expression as an indicator of viral activity, peripheral blood parameters as indicators of curcumin safety, serum neopterin concentration as a biomarker of the cellular immune responses, and clinical manifestations of HAM/TSP patients.

2 Materials and Methods

2.1 Ethical Approval of the Study Protocol

This clinical trial study was registered at Mashhad University of Medical Sciences (MUMS, Mashhad, Iran) (no: 922212), and the study protocol was reviewed and approved by the Biomedical Research Ethics Committee. Written informed consent was obtained from all patients enrolled before the study starting for blood donating and also for the inclusion of personal data.

2.2 Study Population

This clinical trial study was performed at HTLV-1 and Neurology Clinic of the Ghaem Hospital (MUMS, Mashhad, Iran). In this study, we enrolled 20 newly diagnosed Iranian HAM/TSP patients consisting of 18 women and 2 men, which resided in Mashhad, an HTLV-1 endemic area in the northeast of Iran. Patients were adults and between 21 and 65 years with a mean age of 46.95 ± 12.86 years. The diagnosis of HAM/TSP was made by two neurologists based on modified diagnostic guidelines for HAM/TSP [32]. HTLV-1 seropositivity was determined using an enzyme-linked immunosorbent assay (ELISA) (Diapro, Italy) and confirmed by polymerase chain reaction (PCR) or Western blotting (WB) (Diagnostic Biotechnology HTLV WB 2.4, Genelabs Technologies, Singapore). Patients with other viral infections such as HIV, hepatitis B and C (HBV and HCV) infections, any previous history of heart failure, diabetes, liver and thyroid disorders, chronic renal failure, autoimmune diseases, pregnant or nursing women, and any history of previous treatment for HAM/TSP were excluded from the study [33–35].

2.3 Treatment Protocol

Concerning the low oral bioavailability of curcumin, we used a nanomicellar curcumin preparation (SinaCurcumin, Exir Nano Sina, Tehran, Iran). Each patient was administered one soft gel of nanomicellar curcumin containing 80 mg of curcuminoids per day for 12 weeks [34, 35]. During the study, all patients received their treatment, and they were not deprived of their usual routine treatment.

2.4 Clinical Evaluation

Standardized checklists were completed by two neurologists including demographic information and the clinical symptoms of the HAM/TSP disease as shown in Table 1. Neurological evaluation including Osame Motor Disability Score

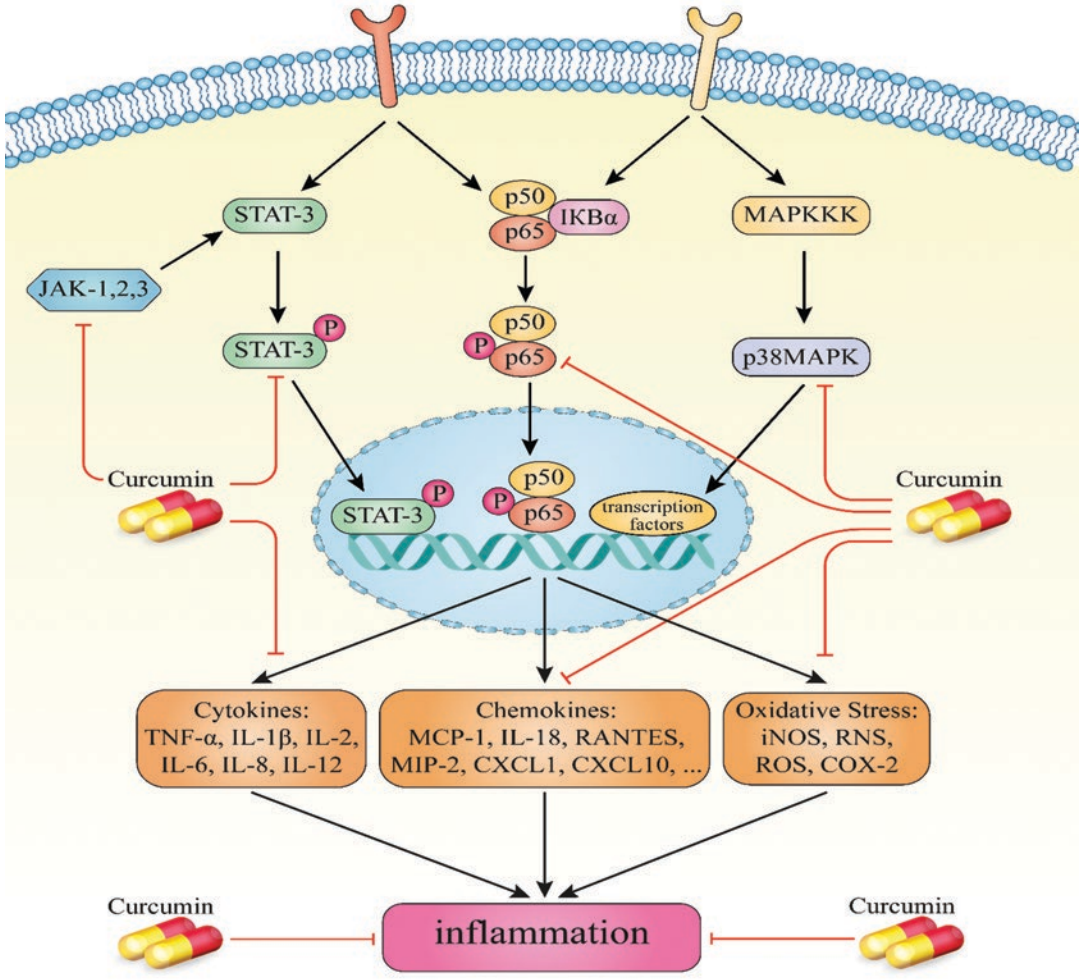

Fig. 1 Antioxidant and anti-inflammatory mechanisms of curcumin. Curcumin has known to possess potent antioxidant and anti-inflammatory activities. Curcumin so far is the most extensively studied spice-derived component for the treatment of several chronic inflammatory diseases in both preclinical and clinical studies. Approximately, more than 120 clinical trials have proven the safety and efficacy of curcumin to treat different chronic diseases without showing any adverse side effects. Thus, this nutraceutical is a potentially safe and effective agent against several chronic inflammatory diseases. Overactivation of immune cells mostly microglial cells prompt the central nervous system disorders. It has been shown that curcumin through suppressing the different signaling pathway molecules such as JAK-STAT, NF-κB, MAPK, etc. effectively suppresses the production of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, and other pro-inflammatory mediators in various preclinical and clinical settings. Curcumin interestingly attenuates JAK-STAT phosphorylation, NF-κB activation, and iNOS in inflammatory responses in monocytes/macrophages and microglial cells and then inhibits the inflammatory processes

(OMDS) ranges from 0 (normal walking and running) to 13 (completely bedridden), and the Ashworth Spasticity Scale (ASS) were performed before and after the treatment [36]. Urinary disorders including urinary frequency, incontinence, and feeling of incomplete emptying were evaluated based on the Incontinence Questionnaire-Urinary Incontinence Short Form before and after the treatment. Moreover, sensory symptoms such as pain and paresthesia were measured via the Visual Analogue Scale (VAS) [36] (Table 1).

Table 1 Clinical profile of HAM/TSP patients enrolled before and 12 weeks after curcumin treatment

Patient no.	Age (years)	Sex	Disease duration (years)	OMDS		ASS		Frequent urination		Urinary incontinence		The feeling of incomplete emptying		Paresthesia	
				Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	41	Female		2	1	1	0	3	1	0	0	0	0	Positive	Negative
2	21	Female		3	2	1	1	3	1	0	0	0	0	Positive	Negative
3	53	Female		2	1	1	0	3	1	1	0	0	0	Negative	Negative
4	33	Female		5	4	3	2	3	3	3	3	2	1	Positive	Negative
5	52	Female		4	3	2	1	3	1	3	1	0	0	Positive	Negative
6	61	Male		4	4	3	2	3	1	3	1	1	1	Negative	Negative
7	23	Female		5	4	3	1	3	1	3	1	0	0	Positive	Negative
8	47	Female		4	3	3	2	3	1	0	0	0	0	Positive	Negative
9	63	Female		7	7	4	3	3	1	3	1	1	1	Positive	Negative
10	58	Male		2	1	1	1	1	0	0	0	0	0	Positive	Negative
11	44	Female		4	3	2	1	3	1	3	1	1	0	Positive	Negative
12	54	Female		5	5	3	2	3	1	3	3	0	0	Positive	Negative
13	65	Female		4	4	2	1	3	1	3	1	1	1	Positive	Negative
14	45	Female		6	6	3	2	3	1	3	1	3	1	Positive	Negative
15	43	Female		5	4	2	1	3	1	3	1	3	1	Positive	Negative
16	56	Female		6	5	3	2	3	3	3	3	0	0	Positive	Negative
17	58	Female		2	1	1	1	1	0	1	0	0	0	Positive	Negative
18	54	Female		2	2	1	1	1	0	0	0	1	0	Positive	Negative
19	38	Female		2	2	1	1	1	0	0	0	0	0	Negative	Negative
20	30	Female		2	2	1	1	3	1	0	0	0	0	Negative	Negative

OMDS Osame Motor Disability Score, *ASS* Ashworth Spasticity Scale

2.5 Blood Sample Collection, PBMCs, and Serum Separation

Serum, peripheral blood, and peripheral blood mononuclear cells (PBMCs) were collected before and after treatment. For serum separation, 3 mL of peripheral blood samples were drawn in serum separator tubes (SST). Then samples were allowed to clot for 30 min at room temperature and then centrifugated for 15 min at 1000 g. Serum samples were then collected and stored at −70 °C until analysis as described previously [33–35].

For complete blood count (CBC) analysis and PBMC separation, 3 mL and 5 mL of peripheral blood samples were collected before and after treatment in CBC tubes and Venoject/Vacutainer vials containing EDTA as an anticoagulant. CBCs were performed as soon as possible by an automated hematology analyzer (Sysmex-KX-21, Sysmex Corporation, Kobe, Japan). PBMCs were separated over Ficoll-Hypaque (Lympholyte®-H from Cedarlane, Burlington, Ontario, Canada) by density gradient centrifugation. The PBMCs were then harvested, washed twice with cold PBS, and preserved in TriPure reagent (Roche Diagnostic GmbH, Mannheim, Germany) in −70 °C until RNA extraction as described previously [33–35].

2.6 RNA Extraction, Tax, and HBZ mRNA Quantification

Total cellular RNA from PBMCs of HAM/TSP patients was extracted using the TriPure reagent (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's protocols. Then 5 µg of total RNA samples were converted into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA USA) according to the manufacturer's instruction. Tax- or HBZ-specific primers and probes were added to cDNA samples and amplified on a Rotor-Gene 6000 instrument (QIAGEN, Hilden, Germany) as previously described [33–35] (Table 2). The level of Tax and HBZ mRNA expression was then calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control by the relative quantification method [33–35].

2.7 DNA Isolation and HTLV-1 DNA Proviral Load Quantification

Total DNA was isolated from PBMCs and purified in columns using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. HTLV-1 DNA proviral load was determined from DNA by the TaqMan method using a commercially available absolute quantification HTLV-1 RG kit (Novin Gene, Tehran, Iran) and Rotor-Gene 6000 Real-Time PCR machine (QIAGEN, Hilden, Germany) [35, 37]. Albumin DNA was used as an endogenous reference gene. HTLV-1 and albumin DNA concentrations were calculated from two five-point standard curves. The normalized HTLV-1 DNA proviral load values were measured as the ratio of (HTLV-1 DNA copy number/albumin DNA copy number/2) × 10.4 The value of the proviral load was reported as the number of HTLV-1 copies/10^4 cells as mentioned previously [35, 37].

Table. 2 The oligonucleotide sequences of specific primers and probes

Characteristics	Before treatment	After treatment	Wilcoxon signed rank test	
			P-value	Z
OMDS	2.4±1.9	1.2±1.8	0.001	3.46
ASS	2.05±1	1.30±0.73	0.001	3.63
Urinary frequency	2.45±1	0.95±0.83	0.001	3.70
Urinary incontinence	1.75±1.44	0.85±1.04	0.003	2.97
Feeling of incomplete emptying	0.65±0.98	0.30±0.47	0.03	2.07
VAS	4.6±2.5	3.1±2	0.001	3.34

Table 3 Clinical findings of the HAM/TSP patients before and 12 weeks after curcumin treatment

Characteristics	Before treatment	After treatment	Wilcoxon signed rank test	
			P-value	Z
OMDS	2.4±1.9	1.2±1.8	0.001	3.46
ASS	2.05±1	1.30±0.73	0.001	3.63
Urinary frequency	2.45±1	0.95±0.83	0.001	3.70
Urinary incontinence	1.75±1.44	0.85±1.04	0.003	2.97
Feeling of incomplete emptying	0.65±0.98	0.30±0.47	0.03	2.07
VAS	4.6±2.5	3.1±2	0.001	3.34

Data were shown by the mean and standard error of the mean (SEM) and analyzed using the Wilcoxon signed rank test. *OMDS* Osame Motor Disability Score, *ASS* Ashworth Spasticity Scale, *VAS* Visual Analogue Scale

2.8 Serum Neopterin Concentration Measurement

The serum neopterin level was assayed by using an ELISA kit for the quantitative determination of neopterin in human serum (IBL International GmbH, Hamburg, Germany) according to the manufacturer's protocol. Optical densities were obtained at 450 nm as the wavelength using an automated ELISA microplate reader processing system (MRP4, Hiperion) and values were calculated according to the manufacturer's protocol.

3 Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., New York, NY, USA). The paired data before and after treatment were compared using Wilcoxon's signed rank test. Data were expressed as the mean ± standard error of the mean (mean ± SEM), and p-values less than 0.05 were considered statistically as significant.

4 Results

4.1 Patient Characteristics and Clinical Improvements

In this clinical trial study, 20 patients with HAM/TSP enrolled, in which 18 individuals were female and only 2 patients were male with the mean age of 46.95 ± 12.86 years. The mean OMDS before and after treatment were 2.4 ± 1.9 and 1.2 ± 1.8, respectively, which shows significant improvement after taking curcumin treatment (P = 0.001) (Table 3). Also, the ASS score after the treatment was significantly improved (P = 0.001). The mean ASS before the treatment was 2.05 ± 1 and after the treatment was 1.30 ± 0.73, respectively. Furthermore, there was a significant improvement in urinary disorders after the treatment; the mean urinary frequency such as incontinence and feeling of incomplete emptying was significantly decreased (P = 0.001, P = 0.003, and P = 0.03, respectively) (Table 3). Paresthesia was reported in 16 patients before the treatment; however, it was improved after the treatment in all of the patients (Table 1). A total of 4 patients had no pain before the treatment. VAS scores (the intensity of the pain) before and after the treatment were 4.6 ± 2.5 and 3.1 ± 2, respectively, which represents a significant decrease in VAS score after the treatment. Tables 1 and 3 show the clinical findings of HAM/TSP patients before and after the treatment.

4.2 HTLV-1 DNA Proviral Load, Tax, and HBZ mRNA Expression and Serum Neopterin Concentration

Since HTLV-1 DNA proviral load is associated with the risk of HAM/TSP, we monitored changes in the HTLV-1 copy numbers in PBMCs before and after treatment. The mean amount of HTLV-1 DNA proviral load before treatment compared with after the treatment was almost similar and

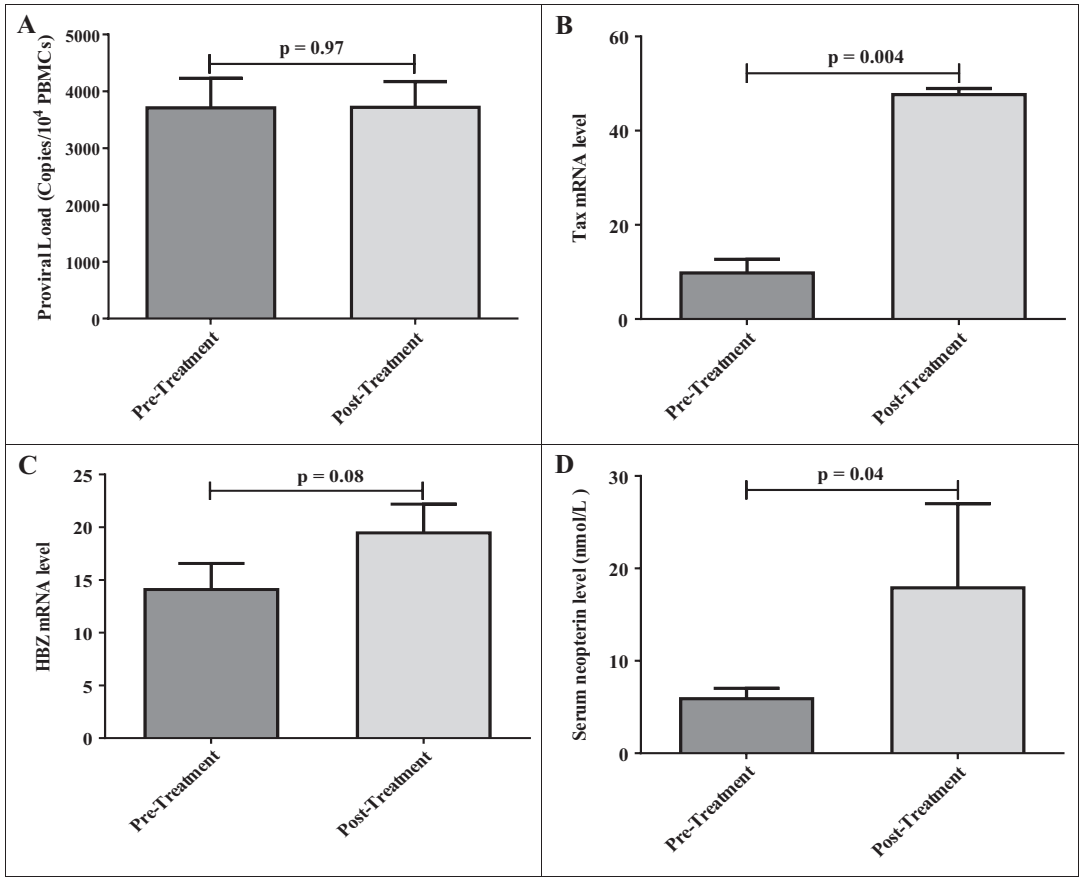

Fig. 2 Effect of curcumin treatment on the proviral load, Tax and HBZ mRNA expression, and serum neopterin level in HAM/TSP patients. HAM/TSP patients were treated with 80 mg curcumin for 12 weeks, and then freshly isolated peripheral blood mononuclear cells (PBMCs) and serum were then prepared and analyzed for the amount of proviral load (A), mRNA expression of Tax (B), and HBZ (C) by TaqMan probe quantitative real-time PCR and serum neopterin level (D) by enzyme-linked immunosorbent assay (ELISA). (Mean ± SEM)

showed no significant changes (Fig. 2a). Tax mRNA expression in HAM/TSP patients tends to increase after the treatment (Fig. 2b). The mRNA level of HTLV-1 HBZ also tended to be increased after the treatment but failed to achieve statistical significance (Fig. 2c). As shown in Fig. 2d, the serum levels of neopterin significantly increased after the treatment.

4.3 Peripheral Blood Parameters

The CBC is a crucial test that may be ordered by a healthcare provider to monitor overall health as part of a routine checkup, to screen a variety of hematological disturbances, malignancies, infections, inflammations, and cancers. The test may also be ordered when a person has had abnormal CBC results in the past and to monitor changes in the peoples receiving treatments that can affect whole blood cell counts such as chemotherapy and other medications.

In this study, hematological tests were performed on whole blood samples collected from HAM/TSP patients before and after the treatment for monitoring the safety of curcumin supplementation or its adverse effects on the peripheral blood parameters. The results of this study showed that curcumin supplementation had no adverse effect on the peripheral blood parameters

Table 4 The results of peripheral blood parameters analysis in HAM/TSP patients before and after treatment by an automated hematology analyzer

Laboratory findings	Mean (before treatment)	Mean (after treatment)	P-value
WBCs	5.9190	5.7476	0.675
RBCs	4.5538	4.5752	0.873
Hb	12.7333	12.8619	0.706
HCT	38.8810	39.0429	0.886
MCV	85.6025	85.5178	0.949
MCH	28.0615	28.2088	0.799
MCHC	32.7775	32.9746	0.596
PLT	251.0000	245.0000	0.782
RDW	13.0048	12.8238	0.609
Lymph	35.0476	34.6143	0.794
Neutrophil	55.6824	57.0846	0.916
Mixed	9.3000	9.8417	0.918

WBCs white blood cells, *RBCs* red blood cells, *Hb* hemoglobin concentration, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelet count, *RDW* red blood cell distribution width, *Lymph* lymphocyte count, *Neutrophil* neutrophil count, *mixed* mixed cell population count containing monocytes, asophils, and eosinophils

of HAM/TSP patients after 12 weeks of treatment. The results of hematological test (CBC) analysis and the P-values are shown in Table 4.

4.4 Adverse Effects

There were no observable serious and significant adverse effects in HAM/TSP patients responsible for withdrawal from the study.

5 Discussion

As far as we know, the present study was the first clinical trial aimed to investigate the effects of curcumin supplementation on the HTLV-1 DNA proviral load as an indicator of viral activity in the host, Tax and HBZ mRNA expression, peripheral blood parameters, serum neopterin concentration as a biomarker of the cellular immune response, and clinical manifestations of HAM/TSP patients. HAM/TSP is the most well-known progressive myelopathy with spastic paraparesis, sphincter dysfunction, and mild sensory impairment in the lower limbs due to the inflammation caused by changes in the immune responses against the virus [7]. Therapeutic strategies for HAM/TSP patients include symptomatic and etiologic regimen. In symptomatic treatment, anti-spastic and anticholinergic agents, analgesics, and physiotherapy could be used, and the emotional and social problems of the patients are generally managed. In etiological treatment, corticosteroids, cytotoxic agents, interferon-alpha, plasma exchange, and other immunomodulatory agents could be prescribed. These drugs have been partially effective in the improvement of clinical manifestations; however, side effects such as fever, chills, weakness, malaise, alopecia, and depression have been reported. Therefore, it seems that the use of safe and effective anti-inflammatory and immunomodulatory agents could be one of the best strategies for the improvement of the clinical signs and symptoms in HAM/TSP patients.

Curcumin is a potent antioxidant, immunomodulatory, and anti-inflammatory agent that has been used extensively for the prevention and treatment of several chronic inflammatory diseases [21–23]. Different studies have shown that curcumin inhibits inflammation in the stimulated monocytes, macrophages, and DCs through suppressing the expression of pro-inflammatory mediators such as cytokines and chemokines [21–23] (Fig. 1). Curcumin prevents axonal degeneration which suggests a potential therapeutic role for curcumin in MS, Alzheimer's, and Parkinson's disease [38]. Furthermore, in the animal model of MS, treatment with polymerized nano-curcumin resulted in decreased disease severity and was associated with a decrease in demyelination, inflammation, and blood-brain barrier damage [39]. All of these findings emphasize the protective role of curcumin supplementation in neuroinflammatory and neurodegenerative disorders which are in line with the results of our study.

The mean OMDS revealed a significant difference before and after the treatment in which OMDS before the treatment was twofold greater than after the treatment. In a study conducted on traumatic spinal cord injury (SCI) in Sprague-Dawley rats, results showed that following cur-

cumin treatment and after spinal cord hemisection, the Basso, Beattie, and Bresnahan (BBB) scoring that is used to assess the motor function of rats was significantly improved. Immunohistochemistry of NeuN, a neuron-specific marker, revealed a remarkable neuronal loss in the vehicle group after hemisection. In comparison, curcumin significantly protected neurons after SCI. Furthermore, curcumin significantly attenuated apoptosis after SCI. Moreover, the expression of the glial fibrillary acidic protein (GFAP) was significantly inhibited by curcumin hemisection [40]. It seems that curcumin reduces the effects of injury to the spinal cord, which may be beneficial for neuronal survival [41]. These findings suggest that curcumin provides neuroprotection and improves neurological function after SCI which could confirm the results of our study.

In the present study, the mean ASS showed a significant reduction at 63% after treatment. Furthermore, after the treatment, urinary disorders, including urinary frequency, incontinence, and the feeling of incomplete bladder emptying, were significantly decreased as 38%, 48%, and 46%, respectively, which shows that urinary disorders in less than half of the study population were significantly decreased. Also, our results indicated the improvement of urinary symptoms in HAM/TSP patients. Hishikawa et al. [42] demonstrated that turmeric significantly reduces neural and sensory symptoms and urinary incontinence in Alzheimer patients. In general, it seems that curcumin increases the effectiveness of the urinary tract.

Regarding the sensory symptoms, paresthesia was not observed in all of the patients and completely improved after the treatment. Besides, the VAS score showed a 68% decrease of radicular and non-radicular pain with paresthesia after the treatment. Our finding is in line with the results of previous studies which showed that curcumin supplementation could be effective in reducing pain and may increase the recovery of muscle performance with no major side effects in osteoarthritis [43–45].

Curcumin was reported to be safe and well-tolerated in clinical trials performed to date, and none of the studies reported a higher frequency of adverse events in the curcumin treatment group compared to the control group [43–45]. There is also evidence from a systematic review and meta-analysis of randomized controlled trials favoring the analgesic efficacy of curcumin [46]. In this study, for monitoring the safety or adverse effects of curcumin supplementations, the peripheral blood parameters of HAM/TSP patients before and after the treatment were compared. The results of this study showed that curcumin supplementation had no adverse effect on the peripheral blood parameters after 12 weeks of treatment. Also, we could not find any observable, undesirable, and unwanted side effects in HAM/TSP patients during the study. Therefore, curcumin could be considered as a safe and effective adjunct and alternative agent that could be used in the treatment of HAM/TSP disease.

In the present study, although clinical outcomes of the patients after treatment were significantly improved, we could not observe any decrease in HTLV-1 DNA proviral load, Tax, and HBZ expression. Also, after the treatment serum level of neopterin, a nonspecific valuable biomarker of the cellular immune responses elevated. Increased neopterin concentrations are established in patients with an activated Th1-mediated immune response which includes viral infection, allograft rejection, as well as various neuroinflammatory and autoimmune disorders. Neopterin is released in large amounts from human monocyte-derived macrophages and DCs and a little amount from other cell types like human endothelial cells and B lymphocytes preferentially following interferon-gamma (IFN-γ) stimulation. Thus, the measurement of neopterin concentrations as a laboratory diagnostic tool, reflecting the immune activation status, allows studying the immunological network and its interaction with the pathogenesis of the disease [11, 12]. It seems that curcumin supplementation does not have a direct effect on the HTLV-1 DNA proviral load and the expression of viral functional proteins such as Tax and HBZ, but may show its antiviral properties through upregulation of cell-mediated and cytotoxicity-related molecules such as neopterin, granzyme A, and granu-

lysin rather than a direct effect on HTLV-1 itself [33]. On the other hand, curcumin shows an immunomodulatory function on effector cells which act against the viral infections. It reduces the killing activity of macrophages and decreases the activity of NK cells via inhibition of the JAK-STAT signaling pathway [21–23] (Fig. 1). These reasons and findings could explain the lack of HTLV-1 viral load change and a slight increase in Tax and HBZ expression after the curcumin treatment. Also, in this study, we administered curcumin for 3 months; prolonged use of curcumin or the use of curcumin with antiviral agents may be helpful to reduce the burden of the virus and its functional proteins Tax and HBZ. This study was conducted as a pilot trial with a small number of patients and a relatively short treatment duration. The present study had some limitations which deserve mentioning. Besides, the single-arm design of this study necessitates confirmation of findings in the context of future randomized controlled trials.

6 Conclusion

In conclusion, the present study, being the first of its kind, showed that curcumin could safely improve the clinical manifestations of HAM/TSP patients, though the HTLV-1 viral load was unchanged. Further evidence from large-scale randomized double-blind placebo-controlled trials is required to confirm the findings of this pilot study. Finally, the value of combination therapy with curcumin and anti-retroviral agents in improving clinical manifestations and HTLV-1 proviral load in HAM/TSP patients merits further investigation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest to declare.

Ethical Standards This clinical trial study was registered and approved by the Biomedical Research Ethics Committee at Mashhad University of Medical Sciences (MUMS, Mashhad-Iran) (no: 922212). All HAM/TSP patients gave informed consent for blood donating and also for the inclusion of personal data before the study started.

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The Effects of Ivy (*Hedera helix*) on Respiratory Problems and Cough in Humans: A Review

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Abstract

Hedera helix (ivy) belongs to the genus *Hedera* of the Araliaceae family. The leaf of this plant has several active ingredients with medicinal uses. The active constituents of *H. helix* include monodesmoside α-hederin, hederacoside B, hederacoside C, and hederacoside D.

H. helix leave have been used for the treatment of cough and respiratory problems, and now, other uses have emerged. As a medicinal plant, *H. helix* has been approved by the German Commission E due to its antispasmodic, spasmolytic, antimicrobial, anti-inflammatory, anthelmintic, antioxidative, antitumor, and antileishmanial activities. It comes with several formulations, including tablets, liquids, and topical ointments. In this review, we focus on the respiratory effects of tablet and liquid forms of *H. helix*.

Keywords

Hedera helix · English ivy · Araliaceae · Cough · Respiratory problem · Bronchitis

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1 Introduction

Cough is a common health problem and is one of the most common consultation reasons in general practice. The most common cause of a cough is an acute viral infection of the upper respiratory tract. In addition, asthma and chronic obstructive pulmonary disease (COPD) characterized by airway obstruction and mucus hypersecretion are critical causes of chronic cough. Inappropriate antibiotic use for the treatment of viral respiratory tract infections can be a significant problem leading to substantial healthcare costs and pathogen drug resistance without relieving the cough. Thus, non-antibiotic alternatives for the treatment are required.

Many factors, including age, socioeconomic status, and environmental factors, may affect chronic airflow obstruction (CAO) prevalence. In adolescents and children, bronchial asthma symptoms may significantly affect daily activity and quality of life. In both chronic and acute bronchitis, excessive viscous mucus production triggers a coughing reflex in order to expectorate the excessive mucus. In addition to expectoration treatments, alternative treatments include secretolytic and mucolytic therapy. Also, herbal cough medicines based on thyme or ivy leaf may be used [1].

Hedera helix L. (English ivy, common ivy) leaves have been approved by the German Commission E for its efficacy against respiratory tract catarrhs and chronic inflammatory bronchial conditions. *H. helix* is an evergreen dioecious woody liana and belongs to the genus *Hedera* of the Araliaceae family. Coriaceous leaves of this plant are 4–10 cm both in length and width and are cordate at the base with 3–5 lobed palmate lamina (Fig. 1). The lower surface is grayish green with a distinct raised venation, while the upper surface is dark green with a paler radiate venation. Small greenish yellow flowers arise from summer until late autumn and come in umbels of 3–5 cm in diameter. The fruits are small blackberries, which ripen in winter.

Common ivy is native to Southern, Central, and Western Europe but has also been cultivated in Asia and North America. In numerous countries, it is considered as a common ornamental plant [2].

Fig. 1 Hedera helix. (Photo by H. Baharara)

The plant is known to produce bioactive compounds such as triterpene saponins, and hederagenin bidesmosidic glycoside with medical uses, including monodesmoside α-hederin, hederacoside B, hederacoside C, and hederacoside D. Other bioactive classes of compounds produced by these plants are phenols (e.g., phenolic acid, anthocyanins, flavonoids, and coumarins), steroids, volatile and fixed oils, vitamins, amino acids, β-lactins, and polyacetylenes [2].

Many of the bioactive compounds of *H. helix* translates to several possible medicinal effects, including antispasmodic, spasmolytic, antimicrobial, anti-inflammatory, anthelmintic, antioxidative, antitumor, and antileishmanial activities [2, 3]. Topical use of the complex of hedera-saponin (hederacoside B, C, and α-hederin) has been found to be beneficial as a liposclerosis treatment and may help weight

loss. Lotion, cream, and shampoo formulations have been applied in cosmetic use and treatment of skin diseases, due to the anti-inflammatory effects [1, 2].

Mucolytic agents and antitussives are among the most popular over-the-counter drugs to treat acute cough in adults and children and *H. helix* liquid has been found to be effective in this regard (4). This review aims to catalogue the effects of the *H. helix* plant on coughing and upper respiratory tract disorders.

2 Method

As previously mentioned, the aim of the current review is to collect articles on ivy and its medicinal effects on the respiratory system. For this purpose, the researchers searched the Internet-based databases, including PubMed, Embase, Scopus, and Google Scholar databases for keywords including asthma, *Hedera helix*, Araliaceae, English ivy, respiratory problems, bronchitis, and cough, and collected the relevant articles to review the topic. There was no language restriction.

3 *Hedera helix* Active Compounds and Their Bronchodilator Mechanisms

Ivy leaf contains saponins, which have spasmolytic, bronchodilator, mucolytic, and antibacterial effects [4]. Aforementioned, hederacoside C and α-hederin, in addition to hederagenin, phenolic compound: quercetin and kaempferol, and 3,5-O-dicaffeoyl-quinic acid, play notable roles in the pharmacological activities of ivy. This saponins' activity is calculated in the form of papaverine equivalent value (PE is defined as the activity of 1 g test substance equivalent to the activity of x mg of papaverine). Evaluations suggested significant activity for hederagenin and α-hederin (PE = 49 and 55) and phenolic compounds: kaempferol and quercetin (PE = 143 and 54), yet hederacoside C PE value only reaches 6. These compounds show antispasmodic and bronchodilator effects [2]. Table 1 illustrates the papaverine equivalent value.

Table 1 Papaverine equivalent value of saponins

Name of saponin	Papaverine equivalent value
Hederagenin	49
A-hederin	55
Kaempferol	143
Quercetin	54
Hederacoside C	6

The β2-adrenergic receptor is a transmembrane receptor that is linked with G protein (GPCR, G protein-coupled receptors). The G protein initiates a chain of reactions. Saponins exert their effects by inhibiting the internalization of β2-adrenergic receptors on the surface of alveolar type II cells and bronchial smooth muscle cells. Subsequently, cyclic AMP (cAMP, cyclic adenosine monophosphate) is increased by activated adenyl cyclase enzyme, which converts ATP to cAMP. Consequently, protein kinase A activated and stimulated calcium exit through the phosphorylation process. This process can lead to bronchodilation increase. The secretory process occurs similar to the bronchodilatory process; the protein kinase A activates and stimulates surfactant secretion, and secretion occurs [5, 6]. The mechanism of action is shown in Fig. 2 [7].

Another α-hederin effect on lung tissues shows that this saponin affects the secretion of IL-7 and IL-2 and alters the expression of miRNA-133a. This mechanism may act as α-hederin anti-inflammatory effect in the lung tissue [3, 7].

4 Effects of *Hedera helix* on Respiratory System

Many articles have examined the effects of *H. helix* components, especially α-hederin. Researchers have chosen trials that had been performed before November 2020. The list of clinical studies is illustrated in Table 2.

A review article in 2003 evaluated ivy extract efficacy in children who suffered from asthma. In this review article, the original data of three ran-

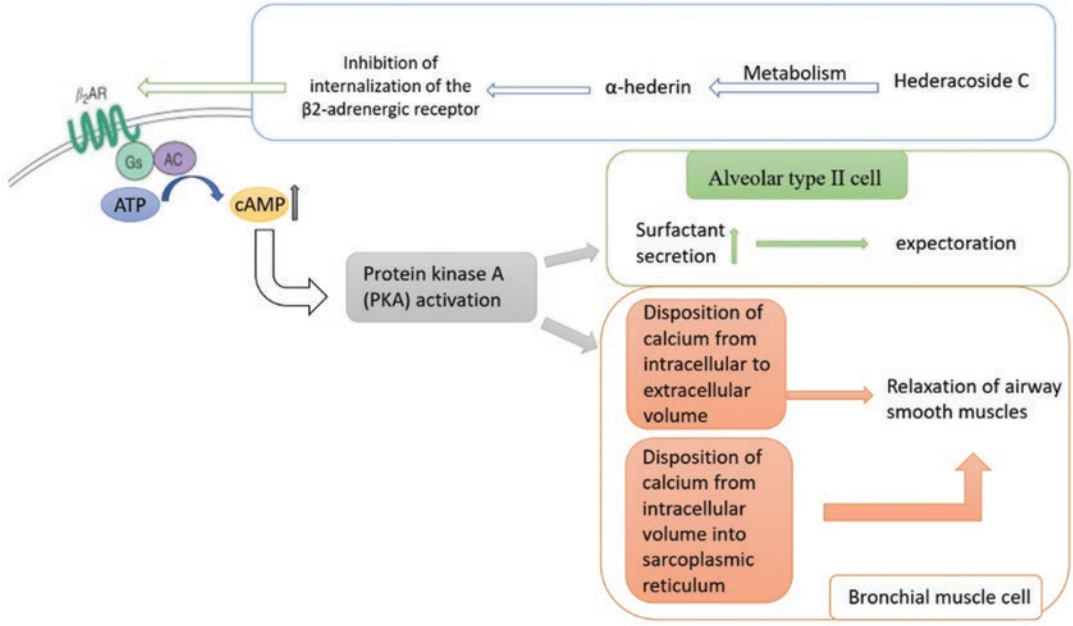

Fig. 2 Mechanism of hederacoside C. *β_2AR* beta2-adrenergic receptor, *Gs* type of G protein receptor, *AC* adenyl cyclase enzyme

Table 2 Clinical studies carried out with preparations based on ivy leaf extract. Ref.: Reference

Author, year	Method	Results		Refs.
		Efficacy	Safety and tolerability	
Meyer-Wegener et al. 1993	Randomized control trial, double-blind *N* = 97 patients, 25–70 years old Prospan® drops 20 drops, 3–5 times daily, + placebo tablet. Ambroxol tablets (30 mg/day) + drops placebo Duration: 4 weeks	There is no significant difference between ivy preparation and synthetic mucolytic	Good in both group Verum group, seven patients experience adverse events. Only two patients were related to treatment In the ambroxol group, six patients experience adverse events	[8]
Gulyas et al. 1997	Randomized, controlled, double-blind *N* = 25, 10–16 years old Prospan® syrup: 5 ml 3 times/day (105 mg extract/day) Prospan® drop: 3 times/day, 20 drop, 42 mg extract/day Duration: 10 days The duration between the two treatments: 2–4 days	Equivalent efficacy for both preparations Significant pulmonary functional improvement	No adverse effects occurred	[19]
Mansfeld et al. 1998	Randomized, controlled, double-blind *N* = 24, 4–12 years old, bronchial asthma Prospan® drop 25 drops, 2 times/daily (25 mg extracted/day) Placebo: drop Duration: 3 days each treatment. The period between the two: 3–5 days	Significant differences between placebo and verum group	Excellent tolerability	[25]

(continued)

Table 2 (continued)

Author, year	Method	Results		Refs.
		Efficacy	Safety and tolerability	
Kraft et al. 2004	Observation, retrospective *N* = 52478, age 0–12 years Duration: not indicated		A total of 115 patients experienced adverse events Total occurrence of adverse events: 0.22% Gastrointestinal side events incidence: 0.17%	[33]
Bolbot et al. 2004	Open, controlled *N* = 50, 2–10 years old Prospan® syrup: 2–6 years 5 ml 3 times daily, 10 ml 3 times daily Acetylcysteine: 2–6 years 100–200 mg, 3 times daily; 7–10 years, 300–400 mg, 3 times daily Duration: 7–10 days	Respiration significantly improved in the Prospan® group than the acetylcysteine group	Tolerability was considered very good to good	[12]
Lässig et al., 1996	Observational, multicenter *N* = 113, 6–15 years 32% patients: 8–10 * 2.5 ml 64% patients: 3*5 ml 4% patients: 3–4*2.5 ml 27% patients: only Prospan® 73% patients: + other drugs Duration: 20–30 days	Coughing and expectoration improved significantly	Very good to good	[26]
Hecker et al. 2002	Observational, multicenter *N* = 1350, 4–25 years Prospan® tablet effervescent: 1.5–2 tablets per day Duration: 1 week	Symptoms improvement: cough (92.2%), dyspnea (83.1%), and pain at breath (86.9%) At least the symptoms of 38% of patients disappeared	Three patients experienced adverse events	[16]
Santoro jr., M. 2005	Multicenter *N* > 5850 Assess productive cough, pulmonary secretion evaluation	Excellent clinical outcome	General tolerability was excellent with minimal effects occurrence	[18]
Schmidt et al. 2012	Two independent non-interventional studies *N* = 257, age 0–12 years old Suffering from acute respiratory catarrh or chronic bronchial disease Group A: 131 patients, drop form Group B: 126 patients, syrup form For measurement: verbal rating scale Duration: 2 weeks	Cough-related symptoms mildly expressed, 94.2%, 97.7% Efficacy was good or very good at 99% in group B and 100% in group A	Five adverse events were reported; none of them was serious Gastrointestinal adverse events occurred in 1 to 10 out of 1000 patients	[9]
Fazio et al. 2009	Observational, multicenter *N* = 9657, all ages Prospan® syrup dosage: 0–5years, 2.5 ml 3 times a day 6–12 years: 5 ml 3 times Over 12 years: 5–7.5 ml 3 times daily Duration: 7 days	Disappear or remission of symptoms in 95.1% of patients	Adverse events occurred in 2.1% of patients, 1.2% of children. Adverse events mainly are gastrointestinal disorders and dermal allergy	[10]

(continued)

Table 2 (continued)

Author, year	Method	Results		Refs.
		Efficacy	Safety and tolerability	
Beden et al. 2011	*N* = 193 children aged 2–4 years old Duration:7 days	Effective in 93.7% of children Clinical signs of children with respiratory disease, including coughing frequency improved	Skin allergy occurred in one child	[11]
Cwientzek et al. 2011	A double-blind, randomized study *N* = 590, with acute bronchitis Treat with Hedelix® and Prospan® Test group *N* = 260 Comparator group *N* = 268	BSS decreased from (test, comparator respectively) 6.2-6.3±1.2 to 4.7-4.9 points in 7 days The test group was non-inferior to the comparator It enhanced acute bronchitis symptoms	2.7% of patients experience adverse events: all of them were non-serious	[14]
Ali et al. 2017	Randomized, placebo-controlled *N* = 110 suffered from a respiratory problem. Cofnovex®: ivy leaf-extracted syrup Patient's outcome: complete, moderate, mild, no improvement	Cofnovex® significantly improves cough symptoms, use as a cough remedy	No adverse events occurred	[15]
Kruttschnitt et al. 2020	Prospective, open,non-interventional cohort study *N* = 139, (group 1, 118 patients received EA 575; group 2, 21 patients received acetylcysteine) Measuring by BSS change Duration: 7 days	After 7 days, BSS was reduced by 1.8 in EA 575 group, 2.1 points in the ACC group No significant differences between the two groups	Both preparations are safe in the treatment of bronchitis	[17]
Olszanecka-Glinianowicz et al. 2020	Multicenter, observational *N* = 5162 suffered from productive cough: 75.5%, occurred at night in 61.6%, very common in 49.8%, affect daily activity: 62.7%	The rate of intensive cough, prevalent cough, night coughing, and cough-affected daily activity significantly decreased		[20]
Schaefer et al. 2016	Randomized, controlled, double-blind, multicenter *N* = 178 Duration: 7 days	The significantly higher efficacy of EA 575 cough liquid in comparison to placebo EA 575 significant relief in 2 days of treatment	Excellent tolerate	[21]
Schaefer et al. 2019	Randomized, placebo-controlled, multicenter, double-blind *N* = 209 Three times/day (5 ml) Two times/day (7.5 ml) Outcome changes in BSS, change in cough severity (measurement by visual analog scale (VAS) and verbal category descriptive (VCD) Duration: 2 weeks	VCD and VAS illustrated the superiority of verum over placebo Significant differences were observed between both treatment groups		[22]

(continued)

Table 2 (continued)

Author, year	Method	Results		Refs.
		Efficacy	Safety and tolerability	
Schönknecht et al. 2017	Non-randomized, non-interventional, multicenter, open-label *N* = 464, 2–12 years with productive cough	93.3% improvement in cough, 84.7% improvement in chest pain on coughing, in dyspnea 88.7%, wheezing 90%, auscultation changes 94.8% The study supported the efficacy of Hedussin prescribed for productive cough	Well tolerated in sick children between 2 and 12 years old	[23]
Zeil et al. 2014	Double-blind, placebo-controlled, randomized cross-over *N* = 30, 6–11 years Suffer from partial or uncontrolled mild persistent allergic asthma Test group: budesonide + ivy leave dry extract Control group: budesonide + placebo Duration: 4 weeks	Significant improvement MEF_{75-25}, MEF_{25}, VC in test group. Uncontrolled mild asthma would benefit from add-on therapy with ivy leaf dry extract		[24]
Büechi et al. 2005	*N* = 62, 16–89 years Mean daily intake: 10 ml (7.5–15) Drug: herbal combination (ivy +thyme+aniseed+marshmallow root) extracts Duration:12 days (3–23 days)	Doctors and patients assessed efficacy as good (86%) and very good (90%)	Only one adverse event occurred	[27]
Kemmerich et al. 2006	Double-blind, placebo-controlled, multicenter *N* = 361 More than ten coughing fit, BSS> = 5 points Duration: 11 days, 5.4 ml 3 times/day Test group (ivy+thyme): *N* = 182, placebo group: *N* = 179	BSS, coughing fit decreased in the test group in comparison with placebo Test groups reduce symptoms faster than placebo	Well tolerated	[29]
Marzian et al. 2007	*N* = 1234, 0–17 years Drug: Thyme+ivy leaf-extracted syrup Duration: 10 days	BSS decreased from 8.8 points on day 1 to 1.3 points on day 10 Coughing fit decreased (81.3%) on day 10	It was rated as good to very good by the physicians in 96.5% of cases	[30]
Ismail et al.	Compare the efficacy of Bronchipret® and ambroxol and acetylcysteine *N* = 7000, three groups	Bronchipret® was superior regarding the clinical effect	Bronchipret® was superior regarding the rate of adverse events	[31]
Stauss-Grabo et al. 2011	Observational, open *N* = 330, 11–85 years old They receive Prospan® tablet at least 2 times per day		Tolerability considered very good (98.5% for doctors, 96.4% for patients) A possible adverse event that related to treatment was nausea	[32]

domized controlled trials have been re-analyzed, which proves that all formulation of ivy-based drugs, including syrups, drops, and suppository, are efficient for the treatment of respiratory tract diseases. They concluded that the ivy extracts enhanced the respiratory function of children with mild to moderately severe chronic respiratory tract disease [1].

A systematic review in 2011 studied the effect of ivy leaf extract on upper respiratory tract infections. In this study, they identified a total of 10 studies on a cumulative 17,463 patients, all of which assessed the ivy extract efficacy alone or combined with other herbal remedies for cough. All studies reported ivy extracts efficacy on respiratory tract diseases and cough, though there was no convincing evidence due to lack of placebo control and methodological flaws [4].

A randomized, controlled, double-blind study, conducted in 1993, recruited 97 patients between 25 and 70 years old who suffered from medium to severe chronic and, in some cases, obstructive bronchitis. The assessment of their effectiveness was based on spirometry. They divided patients into two groups, ambroxol tablet and Prospan® drop. Ambroxol group received 30 mg/day, and Prospan® group received 3–5 times/day of the assigned interventions. The duration of treatment was 4 weeks. Researchers evaluated the symptoms (cough, expectoration, and dyspnea). They could not find any significant difference between the ivy preparation and the synthetic mucolytic [8].

Schmidt et al. assessed the efficacy and safety of two different galenical formulations of the same ivy leaf extract preparation (syrup and drops) on cough and viscous mucus. A total of 257 patients received treatment with the extract of ivy leaf for 2 weeks. They were divided into two groups. Group A (131 patients) received the drug in drop form, and group B (126 patients) received the drug in syrup form. Patients in each group were divided into four subgroups based on their age, and each group received an appropriate dosage of syrup or drop. A verbal rating scale was used to address the drug efficacy regarding cough-related symptoms, which included 0 = not present, 1 = mild, 2 = moderate, 3 = poor, and 4 = very poor. They reported that cough-related symptoms were mildly expressed or absent in 94.2% and 97.7% of patients, respectively. Lastly, the effect was rated as good or very good at 99% in group B and 100% in group A [9].

In a post-marketing trial, 9657 patients were selected with bronchitis (chronic or acute bronchial inflammatory disease) and were treated with a syrup containing dried ivy leaf extract. All the patients suffered from cough (100%), while 74% of them suffered from expectoration, 22% of them suffered from dyspnea, and 21.9% suffered from respiratory-related chest pain. This trial lasted for 7 days. In the end, symptoms were improved or healed in 95.1% of patients. 93% of the patients showed improvement in cough status, and 92.9% of them reported improved expectoration. Lastly, 91.2% and 90.8% of them reported improved dyspnea and pain, respectively [10].

A study was conducted in Slovenia in which the safety and efficacy of ivy leaf extract were investigated in Slovenian children who suffered from respiratory tract infections. The researchers enrolled 193 children aged 2–14 with clinical signs of acute respiratory disease for a 7-day treatment, which turned to be effective in 93.7% of children. At the first visit, 43.6% of children suffered from productive cough, which reached 84.6% at the second visit. Clinical signs of children with respiratory disease, including frequency of cough, improved. The authors stated that ivy leaf syrup was effective and safe in children with coughing and respiratory disease [11].

Bolbot et al. compared the efficacy of ivy syrup (Prospan®) and acetylcysteine in the treatment of children who suffered from acute bronchitis. Fifty children aged 2–10 years with acute bronchitis were recruited. The researchers classified these children into two equal groups. Group 1 received Prospan® syrup, and group 2 received acetylcysteine. Patients were assessed after 1 week of treatment. They assessed the syrup efficacy as "very good" when all clinical parameters were rapidly normalized, "good" when the drug proved to be effective, "moderate" when the drug proved to be effective and managed to normalize many clinical parameters, and "poor" when the drug was insufficient. Seven days after

treatment, both drugs were reported to be effective on the clinical symptoms such as shortness of breath, cough, and pain on respiration. Though Prospan® normalized the respiratory symptoms more rapidly compared to acetylcysteine, note that children better tolerated Prospan® syrup rather than acetylcysteine [12].

An observative clinical assessment scale has been developed to indicate acute bronchitis severity, namely, the bronchitis severity scale (BSS). BSS records five symptoms, including the sputum, cough, chest pain on coughing, pulmonary rales at auscultation, and dyspnea. These symptoms are assessed based on the 5-point Likert scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). The points of these five items are summed up to make the total score, which ranges from 0 to 20. This score may be applied to both children and adults of both sexes [13].

Cwientzek et al. investigated ivy leaf soft (or other) extract tolerability and efficacy. They randomized the enrolled patients into two groups that received Hedelix® and Prospan®, respectively. Two hundred sixty patients received the test products, while 268 patients received comparator products. BSS was used as the comparative scale. Firstly, the BSS score of the test and comparator groups was 6.2-6.3 ± 1.2, respectively. BSS score decreased 4.7–4.9 points until the 7th day. Patients left the study with a mean BSS of 1.4–1.6. Adverse events occurred in only 2.7% of the patients. They concluded that ivy leaf soft extract showed to be non-inferior to the comparator ivy leaf extract in terms of enhancing acute bronchitis symptoms [14].

Cofnovex® is an ivy leaf extract syrup which may be used to treat respiratory problems and cough. Ali et al. investigated Cofnovex® efficacy. They studied patients in two groups, the Cofnovex® group and the placebo group, each including 110 patients who suffered from respiratory problems (including productive and dry cough). Patients did not experience any adverse events in this study. They classified patient outcomes as complete improvement, moderate improvement, mild improvement, and no improvement. The drug effect on coughing is presented in Table 3. Moreover, the drug effect on wheezing is demonstrated in Table 4. The authors concluded that Cofnovex® may significantly improve cough symptoms and may be used as a cough treatment [15].

Hecker et al. designed an experiment to investigate ivy leaf extract tablets. A total of 1350 patients with chronic bronchitis participated in their study (with or without airway obstruction). The observation period was scheduled to be 4 weeks. Comparing with the baseline, the following improvement rates were reported: cough 92.2%; expectoration 94.2%; respiratory pain 86.9%; and dyspnea 83.1%. Considering each of these symptoms, a minimum of 38% of initially affected patients were completely symptom-free. They concluded that ivy leaf tablets can be considered as a therapeutic option to alleviate the symptoms of chronic bronchitis [16].

Table 3 Effect of the drug on cough symptom

Level of improvement	Complete improvement	Moderate improvement	Mild improvement	No improvement
Cofnovex	54 patients (49%)	24 patients (22%)	18 patients (16%)	14 patients (13%)
Placebo	14 patients (13%)	22 patients (20%)	31 patients (28%)	43 patients (39%)

Table 4 Effect of the drug on wheezing symptom

Level of improvement	Complete improvement	Moderate improvement	Mild improvement	No improvement
Cofnovex	45 patients (41%)	32 patients (29%)	20 patients (18%)	13 patients (12%)
Placebo	13 patients (12%)	23 patients (21%)	30 patients (27%)	40 patients (36%)

Kruttschnitt et al. conducted a clinical trial for comparing ivy syrup and acetylcysteine (ACC). ACC decreased the mucus viscosity by hydrolyzing the mucus protein disulfide bonds, thereby facilitating mucus clearance. ACC may be used as an alternative treatment in various conditions in which there are problems with clearance of lung mucosal secretions. They enrolled 139 patients who suffered from acute bronchitis and divided them into two groups: 118 patients received ivy syrup (EA 575), and 21 patients received acetylcysteine. BSS change from baseline was considered as the measurement tool 7 days after treatment. At first, the BSS average point was 6.5 points in EA 575 group and 6.7 points in the ACC group. After 7 days, BSS was reduced by 1.8 points in the EA 575® group and by 2.1 points in ACC groups, which revealed low impairment in both groups. The results also proved comparable continuous improvement of patients in both groups. These results indicated no significant difference between the two groups in terms of efficacy. EA 575® is effective regardless of the indication or the concomitant disease [17].

A multicenter study with over 5850 patients was conducted in Brazil to evaluate *H. helix* as an expectorant in patients with productive cough and evaluated the adverse events and tolerability of *H. helix*. Patients treated with Abrilar® (*H. helix*) showed excellent clinical outcomes with a positive evaluation. The tolerability of the drug was excellent, with minimal effects [18].

A randomized, double-blind, cross-over study evaluated lung function in 25 children between 10 and 15 years old who were suffering from chronic obstructive pulmonary disease. The investigators measured improvement using body plethysmographic and spirometry parameters. The duration of the study was 10 days. Patients received 5 ml 3 times/day of Prospan® syrup (105 mg extract/day), while the other group received Prospan® drops, 20 drops 3 times/day (42 mg extract/day). It was concluded that both preparations had equivalent efficacy. Improvement of pulmonary function was significant. Patients tolerated the intervention very well, and no adverse effects occurred in either of the groups. The investigators also concluded that without the addition of ethanol, a higher dosage of dried ivy leaf extract was required in patients with chronic obstructive pulmonary diseases [19].

Olszanecka-Glinianowicz et al. performed a clinical trial on 5162 patients who suffered from productive cough who received herbal medicine that contained ivy leaf extract. The productive cough was intense in 75.5%, occurred at night in 61.6%, was very common in 49.8% (several times per hour), and affected daily activities in 62.7%. During this study, the rate of intensive cough, prevalent cough, night coughing, and cough-affected daily activity significantly decreased. Using ivy leaf dry extract is suggested as an excellent alternative to the current therapeutic regimens to treat children's productive cough [20].

Schaefer et al. conducted a trial to investigate the safety and efficacy of ivy leave cough liquid (EA 575). Totally, 181 patients with productive cough were randomly allocated two treatment arms, and 178 patients completed the trial. The efficacy outcome in terms of cough severity control in the 7-day treatment period indicated the significantly higher efficacy of ivy leaf cough liquid than placebo. EA 575® demonstrated a swift action onset in comparison with placebo in every clinically relevant variable and provided significant relief within the first 2 days of treatment. Ivy leaf cough liquid, which contains EA 575®, is a useful alternative for treating a patient with an acute cough, which presents excellent tolerability and thus may be considered as an alternative to the adults' chemical cough medicine [21].

Schaefer et al. conducted another trial to investigate the safety and efficacy of a liquid containing ivy leaf dry extract (EA 575). Primary efficacy outcome was measured as BSS change of pooled verum and pooled placebo groups between visit 1 and 5. Additionally, they assessed secondary parameters, including cough severity change based on a visual analog scale (VAS) and verbal category descriptive (VCD) score. They enrolled 209 patients suffering from acute bronchitis and divided them into four groups: 35 patients in the placebo group (receiving twice daily ×7.5 ml), 35 patients in the placebo group (receiving three times daily ×5 ml), 70 patients in the drug group

(receiving twice daily ×7.5 ml), and 68 patients in the drug group (receiving three times daily ×5 ml). The patients were observed for 2 weeks. At first week vest, 79.1% of patients reported to do well or very well in the verum group, while only 50% of patients in the placebo group reported the same result. At the second week's visit, this rate increased to 89.1% in the verum groups. Also, at the first-week visit, 76.9% of the pooled verum groups evaluated their medication as good or very good for cough treatment, while only 44.2% of the patients in the pooled placebo groups reported the same result. In brief, significant differences were observed between both treatment groups considering the assessments of both items at the first-week visit and the second-week visit in favor of ivy leaf cough liquid [22].

Schönknecht et al. performed an open-label study. They enrolled 464 patients aged 2–12 years with productive cough. They reported cough improvement in 93.3% of patients, chest pain improvement in 84.7% of the patients, wheezing improvement in 90% of the patients, dyspnea improvement in 88.7% of the patients, and auscultation improvement in 94.8% of the patients. They concluded the efficacy of liquid ivy extract in the treatment of productive cough [23].

A trial in 2014 investigated 30 children who suffered from uncontrolled or partial mild persistent allergic asthma, despite long-term treatment with 400 μg budesonide equivalent. The trial continued for 4 weeks in which patients received placebo or ivy leaf dry extract, besides their inhaled corticosteroids. The researchers measured their lung function, FeNO, exhaled breath condensate pH, and quality of life and analyzed them after the treatment period. Their study was aimed to assess the possible benefit of ivy leaf dry extracted on therapy in children who suffered from uncontrolled or partial mild persistent asthma. They reported significant MEF_{25}, $MEF75_{-25}$, and VC improvement following treatment with ivy leaf dry extract, unlike placebo treatment. Their study indicated that children with uncontrolled mild asthma despite regular therapy with inhaled corticosteroid would benefit from an add-on therapy with ivy leaf dry extract [24].

A study was conducted in 1998 on 24 patients who had bronchial asthma, and they received placebo or dried ivy leaf extract over 3 days. The study outcome was airway resistance change compared with placebo therapy. Results of dried ivy leaf extract showed a statistically significant and clinically relevant airway resistance reduction, compared with placebo therapy. In conclusion, distinct improvement of respiratory functions was verified in patients who had bronchial asthma, which supported the efficacy of dried ivy leaf extract compared with placebo therapy [25].

Lässig et al. conducted an observational, multicenter study; they recruited 113 patients aged 6–15 years. A total of 32% patients received 8–10 × 2.5 ml (20–25 ml/day), 64% received 3 × 5ml (15 ml/day), and 4% received 3–4 × 2.5ml (7.5–10 ml/day). In total, 27% of children received only Prospan® syrup, while 73% also used aerosols with beta 2-sympathomimetics. They concluded that cough and expectoration improved significantly. The authors considered tolerability in ranges of good (29.5%) to excellent (69.7%) [26].

5 Efficacy of Herbal Cough Syrup Combination with Ivy

Herbal cough syrup combination with ivy was evaluated in a trial. This syrup was prepared using a dry ivy leaf extract (*H. helix folium*), a decoction (boiling water extract) of thyme (*Thymi herba*), and an adjuvant herbal preparation, a decoction of aniseed (*Anisi fructus*), and a mucilage (water macerate) of marshmallow root (*Althaeae radix*). In aniseed, the marshmallow flavor and mucilage are enhanced as a thickener, malt extract, and sugar. In that study, herbal cough syrup was prescribed for 62 patients with appropriate dosage. The patients suffered irritating cough due to common cold ($n = 29$), bronchitis ($n = 20$), and viscous mucus-forming respiratory tract disease ($n = 15$). The primary outcome criteria were the changes of stimulus to cough and expectoration strength (consistency, amount, ease, and color) between the initial and

the final visits. Patients addressed syrup efficacy using four levels of a symptom score for change assessment as follows: (1 for none or little, 2 for medium, 3 for strong, and 4 for very strong). Physicians and patients evaluated the efficacy as very good or good in 86% and 90% of the cases, respectively. Results of this study indicated significant improvements in scores between the initial and the final visits. They attributed these changes to the mucolytic property and mucociliary transportation improvement [27].

A study concluded that thyme and ivy have a synergistic effect. They enrolled 361 patients with acute bronchitis who took a combination of thyme and ivy leaf extract or placebo. Daily coughing fits and BSS were chosen for measuring improvement. After 4 and 10 days of treatment, patients' health and BSS were determined. Before treatment, there were not any significant differences in BSS in both groups. On days 4 and 10, BSS steadily dropped from 8.2 on the first day to 1.6 10 days after the first dose in the thyme-ivy group. However, the BSS of the placebo group was changed from 8.3 on the first day to 3.3 on the 10th day. Almost 83% of the patients' cough improved after 4 days. In conclusion, the ivy-thyme extract had a good impact on cough and respiratory problems [28].

Kemmerich et al. investigated the efficacy of a combination of ivy leaf and thyme herb fluid extract. They enrolled 360 patients suffering from acute bronchitis and randomly assigned them to two 11-day treatment groups: placebo and thyme-ivy combination. The study inclusion criteria included at least ten cough-fits a day, which started 2 days before enrollment and BSS above 5. After the 11-day treatments, coughing and BSS were significantly reduced in patients of both groups. BSS improved rapidly in both groups, though symptom regression occurred faster in the drugs group. On day 4 of the treatment, the drugs group showed a significant reduction in coughing fits. They concluded that acute bronchitis oral treatment with the thyme-ivy combination for 11 days is more efficient compared with placebo [29].

Bronchipret® is a combination of ivy and thyme extract, which is used in adolescents and children who suffer from productive cough and acute bronchitis. Marzian et al. conducted a study to investigate the benefits and tolerability of Bronchipret® in which they enrolled 1234 adolescents and children with BSS above five and a minimum of ten coughing per day. The treatment course was assessed on days 0, 4, and 10 of treatment. The treatment group's average BSS decreased from 8.8 to 4.8 on day 4 of treatment to 1.3 on day 10 of treatment. The number of documented coughing fits decreased to an average of 18.7 on day 10 of treatment. 96.5% of physicians rated its tolerability as good to very good. The researchers concluded that a combination of ivy and thyme extract might be used to treat acute bronchitis with productive cough in adolescents and children. The combination syrup improved the symptoms in 10 days [30].

Ismail et al. conducted a study to compare Bronchipret®, ambroxol, and ACC efficacy as mucoactive substances. They enrolled 7000 patients and divided them into three groups. Each group received one of the Bronchipret® or ACC or ambroxol. The study showed that Bronchipret® was superior regarding clinical effects and the rate of adverse events [31].

6 Tolerance

Fazio et al. enrolled 9657 patients who suffered from bronchitis and treated them with ivy extract syrup for a week. They used the following scale to determine tolerance: (a) very good to good, with no adverse events; (b) moderate, transient adverse events occurred and did not usually lead to medication withdrawal; and (c) poor, adverse events occurred and led to therapy discontinuation. In this study, 96.6% of the patients rated the product tolerance as very good to good. On the other hand, only 0.8% of the patients rated tolerance as poor. No data was available on 1.1% of the patients. They also stated that 2.1% of patients reported adverse events. A total of 112 children were enrolled in this study, most of whom experienced gastrointestinal (GI) disorders. All the reported adverse events were transient and mildly to moderate. Due to adverse events (mostly GI

disorders), 56 patients were withdrawn from the therapy. Conclusively, the authors stated that the ivy extract syrup is well tolerated in patients with bronchitis [10].

Hecker M. et al. enrolled 1350 patients with chronic bronchitis to evaluate ivy extracts (Prospan®) efficacy and safety. They investigated adverse events to assess drug safety. Adverse events were reported by three patients (0.2%). They stated that Prospan® was efficient and safe to be used in chronic bronchitis treatment [16].

As mentioned earlier, Meyer-Wegener et al. enrolled 97 patients with a medium to severe chronic and, in some cases, obstructive bronchitis. They received a Prospan® drop or ambroxol tablet. The study illustrated that tolerability was good to excellent in each treatment [8].

Stauss-Grabo et al. assessed ivy leaf extract tablets safety (Prospan® cough tablets) in their study on 330 patients who suffered from cold and coughing or chronic inflammatory bronchial diseases. The patients underwent 7 days of treatment. The tablets' safety and tolerability were rated by using a questionnaire. Practitioner and patients' legal representative used a 4-point scale ("very good," "good," "moderate" or poor) in a questionnaire for drug tolerability and safety. No case of serious adverse event was reported during the treatment period. Note that one patient experienced nausea without vomiting 10 min after drug intake. Totally, 318 (96.4%) of 330 patients evaluated the tablets as "very good" (N = 234 or 70.9%) or "good" (N = 84 or 24.5%), while 11 patients (3.3%) evaluated its tolerability and safety as "moderate." The practitioner's tolerability rating was reported to be even better and 325 patients (98.5%) evaluated the tablets as "very good" (N = 257 or 77.9%) or "good" (N = 68 or 20.6%). Neither practitioners nor patients reported "poor" tolerability. They suggested Prospan® cough tablets as very well-tolerated and safe drug [32].

The efficacy and tolerability of a fixed fluid extract combination of thyme and ivy leaves were evaluated on 361 patients with acute bronchitis accompanied by 10 or more coughing fits per day. The efficacy was described in the previous section, so safety will be discussed here. Kemmerich et al. assessed the drug's tolerability and safety using a scale ranging from 0 (i.e., very good) to 4 (i.e., very poor). The researchers enrolled 180 patients. the combination syrup was well tolerated. Adverse effects were reported only by seven patients. Lastly, 98.9% of patients in the thyme-ivy combination group rated tolerability as "good" or "very good" compared with 95% of patients in the placebo group. Tolerability was rated as 100% in the thyme-ivy combination group and 97.8% in the placebo group by the researchers. Authors suggested that thyme-ivy combination syrup is safe and tolerable [29].

Marzian et al. assessed the safety and efficacy of thyme-ivy combination syrup. The results regarding efficacy were discussed in the previous section. The tolerability of the 10-day treatment period was rated as good and very good for 96.5% of the cases by physicians. Adverse events were reported in two female patients (mild nausea and stomach ache). In brief, the thyme-ivy combination syrup may be considered as a well-tolerated drug in children [30].

A previously described study compared the safety and efficacy of ivy extract syrup and drop. Schmidt et al. enrolled 268 patients in two groups: syrup (N = 133) and drop (N = 135). Physicians assessed ivy extracts' tolerability. In patients who received syrup, at the interim visit (days 4–7), tolerability was rated as "very good" or "good" in 129 of 131 patients (98.2%) and in 118 of 119 patients (99.2%) in the final visit. In patients who received drop, at the interim visit (days 4–7), tolerability was rated as "very good" or "good" in 133 of 134 patients (99.2%) and in 124 of 124 patients (100%) in the final visit. The reduction of the total number of patients in both groups is due to missing data caused by early termination of the study. Patient compliance was excellent in this study, though two patients in the syrup group and ten patients in the drop group terminated the study because of the unpleasant taste of the drug. Totally, adverse events were reported by five patients, who experienced gastrointestinal disorders (diarrhea, nausea, and vomiting). The authors suggested that ivy extract drop and syrup are tolerable and safe drugs [9].

Kraft et al. conducted a study to assess the tolerability of dry extract from ivy leaves for children. They recruited 52478 patients (aged between 0 and 12 years) and treated with alcohol-free cough mixture from ivy leaf extract. Equivalent Prospan® dose was 227 mg/day for under 1 year old, 364 mg/day for between 1 and 5 years old, 653 mg/day for between 6 and 9 years old, and 710 mg/day for over 10 years old. Duration is not indicated. One hundred fifteen patients experienced adverse events. The occurrence of unwanted side effects was 0.22%. The most important adverse events were gastrointestinal side effects with 0.17% incidence [33].

7 Discussion

In this review, the authors gather articles in which most of them illustrated the efficacy and tolerability of *H. helix* extract products. *Hedera helix* is a product used for respiratory problems such as cough, dyspnea, and expectoration. Some articles evaluate the efficacy of ivy extract liquid (drop and syrup) and tablet for treating cough. They prescribed ivy extract for 1 or 2 weeks and evaluated cough suppression. The frequency of cough, dyspnea, and other symptoms was reduced in the treatment period [9–11, 14–16, 18–23, 25, 26]. In uncontrolled or partial mild allergic asthma, ivy extract could be considered as an add-on treatment, in addition to asthma-related corticosteroid drugs [24]. An article compared ambroxol with Prospan® drop (ivy extract drop) to assess efficacy in cough and respiratory problems, they find out that the efficacy are same and there is no significant difference [8]. Some articles divided patients into two groups to compare ivy extract with acetylcysteine (ACC). ACC is a cough remedy that has been used for a long time for treating cough. Studies that compared ACC with ivy extract concluded that both of them are equal in cough reduction and improving patients' quality of life. For tolerability, patients tolerated ivy extract better than ACC [12, 17]. There are some products with combination of herbal drugs, including ivy extract and thyme. Some studies evaluated the efficacy of these products, and results illustrated that these medicinal herbs could change mucolytic action and lead to improvement in mucociliary transportation and reduction in coughing fit [27–30]. A study compared ACC and ambroxol with Bronchipret® (ivy+thyme combination), and authors concluded that Bronchipret® had a good efficacy in controlling respiratory problems and was better tolerated [31].

For the tolerability part, some patients (2.1% of patients) experience the gastrointestinal disorder, which is mild to moderate and transient, but 46 patients discontinue therapy because of adverse events [10]. In another study, 0.2% of patients experienced adverse reactions. According to another study, just eleven patients rated ivy extract as moderate, while the rest of the patients considered the drug as good and very good, and no one rated it as poor [16]. A study illustrated that just 7 out of 180 patients showed adverse events in a combination of ivy and thyme. In conclusion, the combination of ivy-thyme syrup is safe [29]. In another study, physicians rated the ivy-thyme combination as very good to good [30]. A study reported that 0.01% of patients experience nausea, vomiting, and diarrhea [9]. Another study indicated that 0.22% of patients experienced adverse events, and only 0.17% experienced gastrointestinal side effects [33]. In conclusion, ivy-based products and ivy-thyme combination preparations are well-tolerated and safe for children and adults. Compared to other treatment options, ivy-based preparations seem to be better or equally tolerated and efficient in respiratory problems.

8 Conclusion

In conclusion, *H. helix* is a medicinal herb used to treat cough and respiratory problems. This review illustrated that ivy-based drugs could reduce cough and respiratory problems in patients while being well-tolerated. Ivy extract has been shown to possess an equal effect to ACC in alleviating respiratory problems. In combination with other herbal medicines, ivy has also been demonstrated to be efficacious against respira-

tory problems. With respect to safety, this plant is tolerated very well, and only a few patients have been reported to experience adverse reactions, including gastrointestinal problems [18, 34]. Altogether, the extant evidence suggests the safety and efficacy of ivy extract in children and adults.

Competing Interests None

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Safety and Efficacy of Oral Supplementation of Lentil (*Lens culinaris Medic*) in Dry Eye Patients

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Abstract

Objective: To evaluate the safety and efficacy of dietary lentil capsules in patients suffering from dry eye symptoms.

Methods: A randomized, triple-blind, interventional, placebo-controlled study was done. Sixty patients were randomized in two groups to receive either one capsule containing 500 mg of lentil powder or placebo daily for 3

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months. UCVA, tear film breakup time (TBUT), Schirmer's test, tear film osmolarity, and OSDI score were recorded at baseline and 3 months after intervention. Data analysis was performed using IBM SPSS for Windows version 20 (SPSS, Chicago, IL, USA).

Results: In the lentil group, at baseline, the mean UCVA (LogMAR), OSDI, TBUT (S), tear film osmolarity (mOsm/L), and Schirmer (mm) scores were 0.104 (0.026), 22.66 (19.40), 10.31 (5.32), 301.07 (15.57), and 8.22 (6.87), respectively. These values were 0.101 (0.026), 20.85 (19.44), 13.04 (7.11), 299.81 (11.60), and 9.87 (10.11). In the placebo group, these values were 0.084 (0.027), 25.35 (20.08), 10.56 (4.95), 299.77 (15.09), and 9.35 (8.06) at baseline and 3 months later were 0.077 (0.027), 23.32 (22.90), 13.62 (6.30), 297.54 (12.08), and 8.64 (9.60), respectively. Three patients (one in the lentil group and two in the placebo group) experienced severe gastrointestinal symptoms.

Conclusion: Although consumption of 500 mg of lentil is safe, this amount is not sufficient for reduction of dry eye syndrome in 3 months. For more validation, a clinical study with increased dosage of lentil is proposed.

Keywords

Dry eye disease · Lentil · Randomized controlled trial

1 Introduction

Dry eye is a multifactorial disease of the ocular surface that causes discomfort of patient, visual disturbance, and tear film instability [1]. The disease with potential damage to the ocular surface is associated with increased osmolarity of the tear film and inflammation of the ocular surface [2]. It has remarkable effect on visual function, daily activities, and social performance and life quality of patients. Epidemiology of this disease is different around the world so that it has been reported between 14.6% and 57.5% [3–5].

Dry eye is one of the main reasons for patients' referring to ophthalmologists, and it is estimated that tens of millions are suffering from mild dry eyes [6, 7].

The therapeutic approaches recommended for dry eye disease (DED) are varied based on severity of symptoms and signs of disease such as the discomfort of patient, visual symptoms, corneal and conjunctival stainability, eyelid glands dysfunction, tear film breakup time, and the scores obtained from dry eyes assessment tests. Some of these treatments (but not all) include the use of autologous serum eye drops (ASEDs), artificial tears administration, and lacrimal punctal occlusion [5]. Despite the relative success of these methods, their use has been limited due to their side effects and some disadvantages.

Preservative-free artificial tears are the first step in the treatment of dry eye syndrome. The purpose of using this material is to increase the moisture of the ocular surface and lubrication of the corneal surface [8, 9]. However, artificial tears provide only short-term relief [10]. Natural tears are complex mixture of water, solutes, hydrocarbons, proteins, and fats; thus, artificial tears are unable to be alternative completely. Meanwhile, artificial tears compounds may have complications in the long-term use.

Although autologous serum eye drops have similar biochemical and biomechanical characteristics to tears, they are not identical to natural tears [11]. In addition, there is possibility of transmission of blood-borne infections in this method [12].

Although punctal occlusion increases the quantity and quality of tears and helps to improve the symptoms of patients, this procedure should be performed carefully because of its unwanted adverse effects. It can also cause secondary infections [13, 14].

Studies have shown that nutrition has a direct and strong relation with dry eyes. This has led to use of dietary supplements and changes in the daily diet for the prevention and treatment of dry eye [15–17]. In this regard, the most commonly

considered substance is omega-3 as essential fatty acids [18]. Omega-3 consists of three fatty acids including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic acid (ALA), in which the first and second ones are derived from fish oils and the third one from vegetable fats. Fish oil, seeds, and green vegetables (broccoli and spinach) are respectively top sources of omega-3 fatty acids. Investigations have shown that patients who have higher omega-6 FAs than omega-3 FAs in their daily diets have fewer symptoms of dry eyes. It seems that omega-3 FAs decrease dry eye syndrome by increasing the fatty layer of tears and thus preventing evaporation as well as using anti-inflammatory properties [19, 20].

Lentil, *Lens culinaris Medic*, is one of the cereals with the highest protein after soybean among cereals. Lentil is one of the most widely used foods around the world, including Iran. This edible grain is relatively cheap that is easily available all around Iran and many other countries. Lentils contain several compounds such as vitamins (especially group B), folate, omega-3, omega-6, iron, magnesium, potassium, and copper, in which consumption of some of these substances is recommended for patients with dry eye. The consumption of lentils has also been suggested in Iranian and Islamic traditional medicine to treat dry eye and increase the tears.

No documented study has investigated so far the therapeutic effects of lentil, which is full of nutrients and supplements needed for treatment of dry eye. Given that this plant contains the mentioned compounds and also based on the recommendations of traditional medicine, the present study aimed to investigate the effects of taking this herb in healing the patients with dry eye.

2 Methods

In this triple-blind randomized prospective clinical trial, 60 patients admitted to a hospital in Tehran, Iran, were enrolled in the study according to the following inclusion criteria:

1. Complaints of foreign body sensation, feeling of dry eyes, eye irritation, or photophobia provided that have one of the following conditions:
   (a) Mean TBUT would be less than 10 s for at least one eye in three consecutive measurements.
   (b) Schirmer's test result without local anesthesia would be less than 10 mm in 5 min for at least one eye.
   (c) The corneal fluorescein stainability in at least one eye.
2. If only one eye of the patients had the mentioned conditions, the same eye would be enrolled in the study and if both eyes had the mentioned conditions, the right eye is enrolled.
3. The patients should also have the ability to consume oral capsules.
4. Exclusion criteria were eyes Schirmer's test less than 5 mm, TBUT of eye less than 5 s, severe corneal stainability, corneal ulcer, filamentary keratitis, pregnancy or lactation, any kind of eye surgery in involved eye during the last 6 months, presence of pterygium or pinguecula, any obvious changes in diet over the past month or during treatment, topical eye drugs in the affected eye within 7 days, use of contact lenses, use of ophthalmic corticosteroid within the past month, administration of ocular anti-inflammatory drug over the past month, use of corticosteroids or systemic anti-inflammatory drugs simultaneously with the treatment, history of allergy to consume lentils, and using any kind of oral supplement.
5. The patients who had the mentioned criteria were explained on how to participate in the study and its objectives, and they were asked to sign informed consent form. Written informed consent was obtained from all individual participants included in the study.
6. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

3 Randomization and Blinding

In the current study, 60 patients were equally divided into the two test and placebo groups (n = 30 in each group). Each patient was randomly assigned in one of the two groups based on dedicated code in the table of random numbers. To avoid bias, test group, control group, and examiner were unaware of the contents of groups. The test and placebo groups were encoded to avoid bias of the statistician. Both types of capsules were identical in terms of color, shape, and size.

The patients who had the inclusion criteria were examined by slit lamp examination, and then the following items were recorded for each patient:

1. Scores obtained in OSDI questionnaires: a Persian version of this 12-item questionnaire was prepared [21]. The questions were verbally asked by an interviewer, and the scores of each question were recorded in the range of 0 (never) to 4 (always). Total scores of OSDI were calculated based on previous studies [22, 23].
2. The tear film osmolarity was measured using the TearLab Osmolarity System. Initially, tears were collected by putting an Osmolarity Test Card in the eyelid margin; after inserting the cards in the device, the osmolarity (mOsm/L) was calculated and reported for each patient.
3. The mean TBUT was measured in three consecutive efforts in the affected eye. For this purpose, one drop of saline was poured on the fluorescein paper and putting it carefully on the lower fornix. The patients were asked to close their eyes and open them again. The time between opening the eyes and appearing the first dry spot in the eyes was recorded for each patient. The mean of three repetition of the test was reported.
4. The Schirmer's test was performed without anesthesia in the eye. After placing the filter paper in the eye, the moisture content generated in 5 min was measured. This test was performed twice, and the mean of two measurements was reported.

4 Intervention

The patients in the treatment group received 100 lentil capsules (containing 500 mg of lentil powder), taking one capsule a day. The control group's received capsules are quite similar to the treatment group in terms of appearance, which contained only placebo of lactose. The patients were advised to avoid the consumption of additional dietary supplements during the test period. After 3 months, the patients were visited again and the tests performed in the first examination were repeated. During the study, the patients were asked about gastrointestinal symptoms or any possible complications. The patients also were requested to return the remaining capsules to evaluate the adherence level by counting them. The trial protocol was registered in the Iranian Registry of Clinical Trials (IRCT) under the code IRCT2014072618600N1.

5 Statistical Analysis

Normality of variables was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were expressed as mean (SD) and median ($IQ_{25\text{-}75}$) wherever appropriate. Categorical data were shown as frequency (percentages). Variables which were not distributed normally were changed to their Ln values, in order to be eligible to be computed by one-way repeated measure ANOVA. P-values <0.05 were considered statistically significant. Data analysis was performed using IBM SPSS for Windows version 20 (SPSS, Chicago, IL, USA).

6 Results

6.1 Demographics

In this study, 60 patients with dry eye were studied regarding inclusion criteria. Among these, two patients due to long distance and change of residence (3.33%), three patients due to gastrointestinal complications (5%), and two patients because of unwillingness to continue participa-

Table 1 Patients demographics

Variables	Lentil $N = 27$	Placebo $N = 26$	P value
Age	56.48 (13.74)	55.50 (14.64)	0.802
Sex			
Male	4 (14.8)	5 (19.2)	0.669
Female	23 (85.2)	21 (80.8)	
Number of tab	87.78 (12.32)	84.46 (18.76)	0.449
Target eye			
OD	14 (51.9)	14 (53.8)	0.884
OS	13 (48.1)	12 (46.2)	

tion (3.33%) were excluded from the study, and finally the study was completed with 53 patients (88.33 %). All data related to patients who did not complete the study were deleted from the study. The patients included 44 women and 9 men, and 27 patients in the drug group with an average age of 74.13 ± 48.56 and 26 patients in the placebo group with an average age of 64.14 ± 50.55 years were enrolled in the study. There was no difference in age, sex, number of administrated capsules, and target eye between two groups (Table 1).

Before treatment, the average UCVA was 0.104 (0.026) LogMAR for the patients in the drug group, and it reached to 0.101(0.026) after 3 months. This value was 0.084 (0.027) in the placebo group before the study and 0.077 (0.027) after the study. Comparing the mean visual acuity between the two groups showed no significant difference in these groups after 3 months ($P = 0.554$) (Table 2).

Among the studied factors, TBUT had a significant difference in the test and placebo group before and after treatment ($P = 0.02$). In the case of other factors including Schirmer's test, OSDI, and osmolarity, no significant difference was observed in the drug group before and after treatment ($P > 0.05$) (Table 2).

6.2 Side Effects

The mild side effects including mild stomach fullness and bloating in six patients were resolved by recommending taking the capsule during the meal. Two of them were in the placebo group and four patients in the drug group. Three patients experienced more severe gastrointestinal symptoms (stomachache) that they considered it as consequences of drug taking. One of them was in the drug group (3.4%) and two of them were in the placebo group. These three patients did not complete the treatment course and thus were excluded from the study.

7 Discussion

Dry eye is an important problem in ophthalmology. Treatment of this disease is performed with the long-term use of chemical drugs or surgery, which is associated with side effects. Control of this eye disease with safe, natural, and noncomplicated compounds has always been of interest to researchers and clinicians.

Lentil is one of the most commonly cultivated crops around the world, and it is in the daily diet of many people in the world. This seed is affordable and accessible for most people, and it is rich in tryptophan, manganese, iron, vitamin B1, and potassium. One cup of lentil contains 73.3 mg of omega-3 and 271 mg of omega-6, so it can be considered as a plant omega-3 supplier. Previous studies have shown that consumption of omega-3 in food and supplements may improve dry eye symptoms [24]. In the present triple-blind clinical trial, the efficacy of lentil compared to the placebo was studied in the treatment of mild to moderate dry eye. The main objectives, before and after the study, were to assess visual acuity, osmolarity of tears, questionnaire scores of OSDI, TBUT, and Schirmer-1 test.

The strength of this study is the use of different types of tests to evaluate dry eye, including OSDI questionnaire for subjective symptoms, Schirmer-1 test to evaluate the tear film, TBUT test to assess the integrity and stability of the tear film, and osmolarity measurement to evaluate structure and composition of the tear film.

Several studies have shown that omega-3 can reduce inflammation that has been identified as the main cause of dry eye. In a study, 65 patients in the treatment group received capsules of

Table 2 Results of repeated measures analysis of variance for comparing means of key outcomes in two groups

Parameter		Pre- op	Post- op	P_{Trend}	$P_{across\ time}$	$P_{between\ group}$	$P_{interaction}$
TBUT(S)							
Lentil	Mean ± SD	10.31 (5.32)	13.04 (7.11)	0.020	0.005	0.491	0.582
Placebo	Mean ± SD	10.56 (4.95)	13.62 (6.30)	0.025			
Schirmer(mm)							
Lentil	Mean ± SD	8.22 (6.87)	9.87 (10.11)	0.997	0.294	0.990	0.296
	Ln	1.77 (0.91)	1.77 (1.14)				
Placebo	Mean ± SD	9.35 (8.06)	8.64 (9.60)	0.186			
	Ln	1.94 (0.83)	1.60 (1.18)				
Osmolarity(mOsm/L)							
Lentil	Mean ± SD	301.07 (15.57)	299.81 (11.60)	0.715	0.477	0.536	0.843
Placebo	Mean ± SD	299.77 (15.09)	297.54 (12.08)	0.527			
UCVA(LogMAR)							
Lentil	Mean ± SD	0.104 (0.026)	0.101 (0.026)	0.830	0.476	0.554	0.715
Placebo	Mean ± SD	0.084 (0.027)	0.077 (0.027)	0.358			
OSDI							
Lentil	Mean ± SD	22.66 (19.40)	20.85 (19.44)	0.541	0.266	0.815	0.575
	Ln	2.65 (1.09)	2.55 (1.13)				
Placebo	Mean ± SD	25.35 (20.08)	23.32 (22.90)	0.732			
	Ln	2.81 (1.09)	2.52 (1.39)				

UDVA uncorrected distance visual acuity, *OSDI* Ocular Surface Disease Index, *Pre-op* Pre operation, *Post-op* Post operation

720 mg of EPA + 480 mg DHA/day, and the placebo group received capsules containing olive oil. The results showed that the changes in Schirmer's test and TBUT test after 3 months were significant compared to the placebo group [25].

Kangari et al. also demonstrated that daily consumption of two capsules of omega-3 (each containing 180 mg eicosapentaenoic and 120 mg docosahexaenoic acid) for 1 month caused significant changes in parameters of TBUT, OSDI score, and Schirmer's test compared to the control group [22].

In another study, daily intake of omega-3 was determined 2400 mg per day, and a 45-day follow-up showed that TBUT and Nelson grade have improved in these people [26].

Given that side effects of lentil capsule consumption were not assessed, it was preferred that patients take only one capsule per day with a total of 500 mg of lentil. Mild side effects such as bloating and feeling of stomach fullness were observed in only 13.7% of the patients. All mild side effects were resolved by drug taking during the meal. Severe gastrointestinal side effects leading to discontinuation of the drug consumption were observed in only 3.4% of the patients in the treatment group, which was not a statically significant level. Since in our study, each person received 500 mg of lentils per day, so the daily intake of omega-3 from lentils was 0.366 mg, which was much lower than the amount of omega-3 used in other studies. Therefore, it could be the main reason why patients in the test group were not improved in our study.

Given that in this study, the safety of lentil capsules was proved, so the dosage of this widely known and harmless nutrient can be increased by condensing and embedding more substances in each capsule in future studies. In addition, regarding very low side effects, the number of daily intake of capsule can be raised instead of taking one capsule per day in order to specify the results.

In conclusion, although consumption of 500 mg of lentil is safe, this amount is not sufficient for reduction of dry eye syndrome in 3 months. Condensing and formulation of lentil substrate in capsules can be an appropriate way to evaluate the effectiveness of this herbal substrate.

In addition, since the effectiveness of complementary therapies requires a long time, so it is suggested that further studies be conducted with long-term drug uptake and larger sample size.

Conflict of Interest The authors declare that they have no conflict of interest.

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A Review of *Glycyrrhiza glabra* (Licorice) Effects on Metabolic Syndrome

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Abstract

Metabolic syndrome is a pathological condition characterized by diabetes with insulin resistance, abdominal obesity, dyslipidemia, and hypertension. A wide body of research is emerging on *Glycyrrhiza glabra* L. (licorice) as a traditional herb with various therapeutic effects. Animal and human studies have indicated that licorice affects blood glucose, blood lipid profile, and blood pressure. Licorice seems to be effective in hyperglycemia and dyslipidemia; however, it can increase blood pressure. In this study, we intend to explain its role in regard with metabolic syndrome.

Keywords

Metabolic syndrome · Glycyrrhiza glabra · Fabaceae · Licorice · Hyperglycemia · Hyperlipidemia · Hypertension

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1 Introduction

Metabolic syndrome is a pathological state composed of important risk factors including type 2 diabetes, obesity, insulin resistance, and dyslipidemia. These main metabolic complications expose a person to cardiovascular disease [1]. Insulin resistance, defined as the failure of insulin to stimulate glucose transfer into its target cells, plays a pivotal role in MetS [2].

The incidence rate of metabolic syndrome is increasing worldwide [3]. It is estimated that about a quarter of the population in the world deal with metabolic syndrome. So far, numerous definitions have been proposed for this syndrome. The International Diabetes Federation published the metabolic syndrome diagnostic criteria in 2006 as follows: waist circumference >80 cm in women and more than 94 cm in men with two or more of the following: (1) plasma glucose level higher than 100 mg/dl or diagnosed diabetes, (2) blood pressure >130/85 mmHg or medication use for controlling hypertension, (3) HDL cholesterol <40 mg/dl (men) and <50 mg/dl (women) or medication therapy for HDL-C increase, and (4) triglyceride level >150 mg/dl or medication treatment for decreasing triglyceride [4].

Metabolic syndrome has impacts on community health, thus characteristic new and more practical therapeutic agents is vital. *Glycyrrhiza glabra* L. (licorice) is a popular plant among the Fabaceae family whose members are commonly used as food [5].

Licorice has medicinal values and has been conventionally used to treat several ailments [6], including stomach ulcers, skin diseases, respiratory disorders, fever, hemorrhagic disease, sexual debility, epilepsy, paralysis, rheumatism, and jaundice [7]. The isolated compound from *Glycyrrhiza* species amounts to more than 400 ones. Triterpenes and flavonoids are its main active ingredients that contribute to the pharmacological activities of licorice [8]. The main active component in licorice is triterpenoid saponin, referred to as glycyrrhizin (glycyrrhizic acid or glycyrrhizinate). The amount of glycyrrhizin in licorice is approximately 4–20%, depending on the region and variety [9].

According to Thakur and Raj study, the pharmacological impacts of glycyrrhizin are alike those of glycyrrhetinic acid [10]. The main flavonoids of licorice are liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, and glabridin [11]. In several studies, it has been stated that glycyrrhizin, glycyrrhetinic acid, glabridin, liquiritigenin, isoliquiritigenin, and some other flavonoids in licorice possess an inhibitory effect in diabetes [12] and glycyrrhizin to have a therapeutic potential against metabolic syndrome [13]. In the current paper, we will review the studies that have examined the effect of licorice on metabolic syndrome.

2 Method

In the present study, the literatures with keywords including "hypertension," "blood pressure," "hyperlipidemia," "obesity," "lipid profile," "hyperglycemia," "diabetes," "metabolic syndrome," and "insulin resistance" related to *Glycyrrhiza glabra* L. were reviewed using Google Scholar, PubMed, Scopus, and ScienceDirect databases from 2015 to 2020.

3 Licorice Effect on Diabetes

The chronic high blood glucose level in diabetes causes damage and dysfunction in various parts of the body, particularly the blood vessels, nerves, eyes, and kidneys [4]. The increment in diabetes and its resulting mortality has significant consequences for the social, financial, and health systems [14].

4 The Mechanism of Licorice Action in Diabetes

Peroxisome proliferator-activated receptor α (PPARα) is mostly expressed in the skeletal muscles and liver, where lipid oxidation control occurs. When PPARγ activates, it stimulates glucose uptake and lipid metabolism, resulting in lipid storage. PPARγ increases insulin action on

glucose utilization in the cells and ameliorates glucose tolerance in diabetic models. Some studies reported that licorice extracts and glycyrrhizin can ameliorate diabetes by regulation of PPARα and PPARγ [11]. Three licorice extracts (ethanol, ethyl acetate, and acetone) had PPARγ-ligand-binding activities, and 30 mg/L of these extracts had similar hypoglycemic effect of troglitazone (0.44 mg/L), a synthetic PPARγ agonist. *G. glabra* root (licorice) extracts ensue a powerful response in PPARγ activation assays. Also, glabridin was demonstrated to bind to and activate PPARγ [15].

An in vitro study demonstrated that glabridin considerably increased insulin-stimulated glucose uptake in 3T3-L1 adipocytes and conjointly increased the levels of insulin receptor β-subunit (IRβ), insulin receptor substrate 1 (IRS1), and glucose transporter type 4 (GLUT4) [16]. Licorice flavonoid oil (LFO) promotes GLUT4 translocation in skeletal muscles through activation of AMPK and Akt pathway, potentially contributing to the progress of hyperglycemia in diabetic KK-Ay in mice [17]. Moreover, increase in GLUT4 level accompanied by boosting of PPARγ by means of licorice in metabolic syndrome-induced rats was also recorded.

5 Antidiabetic Effect of Licorice in Animal Studies

Yamashita et al. in 2018 reported that "LFO concentrate solution" (contained 30% licorice ethanolic extract and 70% MCT) administered at doses of 1.0 or 1.5 g/kg in diabetic mice reduced plasma glucose and polydipsia significantly [17].

In medical studies, streptozotocin, a chemical substance, is employed to form diabetes in animals. Streptozotocin is toxic to pancreatic beta cells leading to less insulin production and is used in large doses for type 1 diabetes and in low doses for type 2 diabetes [18]. Based on Thakur AK and Raj P study, oral dose of 100 mg/kg of 18-β-glycirrhetic acid possesses a proper anti-hyperglycemic effect by way of decreasing blood glucose and HbA1C and increasing insulin concentration in diabetic rats by streptozotocin. Streptozotocin effect is compared with glibenclamide in this study [10]. Therefore, it is critically important to consider inquiring a patient with alleged high blood pressure and hypotensive drug treatments resistance to be asked openly about their licorice consumption [19]. Licorice extract (1 g/kg daily) in diabetic rats with STZ decreased blood sugar level and prevented body weight loss [20, 21]. Isoliquiritigenin and liquiritigenin derivatives controlled blood glucose in diabetic mice [21]. Total flavones (200,300 mg/kg) from licorice residues in high-fat diet and STZ-induced diabetic c57 mice cause reduction in fasting blood sugar and had a hypoglycemic effect [22]. In Komolkriengkrai M et al. study, a single dose of streptozotocin (60 mg/kg) was injected to induce a diabetic condition in rats. Diabetic rats were treated with glabridin (40 mg/kg) in one group and with glibenclamide (4 mg/kg) in another experimental group. STZ injection triggered increase in blood glucose after 1 week. The results demonstrated a significant reduction in blood sugar in DM+ glabridin group and in DM+ glibenclamide group at 5–8 weeks in comparison with DM rats [23].

Treatment with glabridin (10, 20, and 40 mg/kg) in diabetic rats using streptozotocin meaningfully elevated body weight and glucose tolerance and reduced blood glucose level. Recently, a 2019 study aimed to estimate the acts of liquiritigenin (LTG) on blood sugar level in hyperglycemic adult zebrafish. After 4 weeks of treatment, LTG could prevent the onset of the hyperglycemia in animals [24].

A bolus intraperitoneal injection of STZ (65 mg/kg) can induce type 1 diabetes in rats. Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor in 5 mg/kg/day, was orally given to rats for 2 weeks before the administration of glycyrrhizic acid. The results indicated that glycyrrhizic acid (dose: 150 mg/kg) failed to modify the plasma insulin levels in these diabetic rats. It can be inferred that endogenous insulin is not involved in the impact of glycyrrhizic acid in this animal model, accompanying the downgradation of hyperglycemia in the diabetic rats by administering sitagliptin at an effective dose for inhibition of DPP-4. The study demonstrated that, by

promotion of circulating levels of GLP-1, GA improved glucose-decreasing effect in STZ-treated rats [25]. El Ghaffar suggested that a single intraperitoneal injection of STZ (60 mg/kg body weight) induced type 1 diabetes in male albino rats. The antidiabetic impact of glabridin (25 and 50 mg/kg) was mediated through boosting the body weight gain and meaningfully lowering serum glucose [26]. Shamim A et al. assessed the effect of *G. glabra* extract against STZ and high-fat diet-induced diabetic rats. The study showed that ethanolic extract of *G. glabra* (500 mg/kg) presented significant antidiabetic potential against streptozotocin and high-fat diet-induced diabetic rats comparable to metformin (0.5 mg/kg) [18].

Alloxan, a chemical harmful substance to pancreatic cells, decreases the secretion of insulin from β cells, leading to extracellular hyperglycemia. Mustafa et al. found out that use of hydroalcoholic extracts of *G. glabra* at doses of 50, 100, and 150 mg/kg in alloxan-induced diabetic mice attenuated the blood glucose after 4 h and exhibited hypoglycemic activity [27].

The most well-known models of diabetes animals are commonly induced by streptozotocin and alloxan. These models are relevant to a small proportion of diabetic patients as type 2 diabetes is associated with a number of metabolic complications. Type 2 diabetic models which caused by a special diet emerge as a more useful protocol. It is demonstrated that intake of fructose-enriched diet in the long term accelerates the risk of insulin resistance, a key factor in metabolic syndrome. The fructose-fed rat model showed several properties of the metabolic syndrome and was employed to investigate the association between insulin resistance and diabetes. A fructose-enriched diet raises energy intake, body weight, and fat in animals. Glycyrrhizic acid (50 and 100 mg/kg) during 45 days is effective to reduce serum glucose, insulin resistance, and HbA1c in fructose-fed rats [28]. Consumption of high-fat, high-sucrose diet caused hyperglycemia, dyslipidemia, and insulin resistance in 24 male rats. Glycyrrhizic acid at dose of 100 mg/kg/day orally had no effect on body weight and daily caloric intake; however, fasting blood glucose, fasting serum insulin, and homeostatic model assessment-insulin resistance (HOMA-IR) were decreased [29].

In the study by Goorani S et al. in Iran, it was indicated that *G. glabra* extract can reduce blood glucose significantly in high-fat diet rats [30]. Treatment with intraperitoneal injection of glycyrrhizin (50 mg/kg body weight) could reduce glucose level and insulin in fructose-fed rats [31]. Also glycyrrhizin at dose of 50 mg/kg/day orally in fat-fed rats decreased blood glucose, serum insulin level, and HOMA-IR and improved insulin sensitivity [32].

6 Antidiabetic Effect of Licorice in Human Studies

In a study performed by Yoko Yamashita and others in 2018, 11 healthy male volunteers participated and received LFO-DS (licorice flavonoid oil diluted by adding a double volume of MCT) containing 1% glabridin. LFO-DS potentially lowered postprandial hyperglycemia (a slight decrease in blood glucose level 90 min after eating rice), which was accompanied by a transient increase in insulin secretion (a significant increase occurred at 30 min after rice ingestion). As the authors point out, it is advisable to perform the experiment in diabetic patients after the LFO approval to perform studies with larger scale in hyperglycemic patients [17].

In the study by Hosoe and his colleagues, 50 patients with metabolic syndrome were assigned to either the interventional or placebo group. The interventional group received 300 mg licorice flavonoid oil (LFO) for 12 weeks. The findings displayed that there was no significant decrease in the level of fasting blood glucose and glycosylated hemoglobin for both groups compared to baseline [33].

Alizadeh and colleagues performed a study over 64 overweight and obese volunteers in a double-blind, placebo-controlled, randomized clinical trial. The experiment design included the participants being randomly allocated to the licorice or the placebo group, and each group received a low-calorie diet with either 1.5 g/day of *G.*

Table 1 Summary of studies published on licorice effects on blood glucose level

Study model	Dose	Result	References
Type 2 diabetic mice	"LFO concentrate solution" (0, 1.0, or 1.5 g/kg body weight)	↓ blood glucose, polydipsia	[17]
STZ-induced diabetic rats with high-fat diet	Alcoholic extract of licorice (500 mg/kg)	↓ blood glucose	[18]
STZ-induced diabetic rat	Licorice extract (1 g/kg)	↓ blood glucose	[20]
STZ-induced diabetic mice	Derivatives of isoliquiritigenin and liquiritigenin	↓ blood glucose	[21]
STZ-induced diabetic mice	Total flavones (200, 300 mg/kg)	↓ FBS	[22]
STZ-induced diabetic rat	Glabridin (40 mg/kg)	↓ blood glucose	[23]
STZ-induced diabetic rat	Glabridin (10, 20, 40 mg/kg)	↑ body weight, glucose tolerance, FBS	[35]
STZ-induced diabetic rat	18-β-Glycirrhetic acid (100mg/kg)	↓ FBS, HbA1c ↑ plasma insulin	[10]
Zebrafish diabetic model	Liquiritigenin (1 nM)	Prevent the onset of hyperglycemia ↓ basal glycaemia	[24]
STZ-diabetic rats type 1	Glycyrrhizic acid (150 mg/kg)	No change in plasma insulin	[25]
Alloxan-induced diabetic mice	Licorice extract (50, 100, 150 mg/kg)	↓ blood glucose	[27]
Diabetic rat with high-fructose diet	Glycyrrhizic acid (50 and 100 mg/kg	↓ serum glucose, insulin resistance, HbA1c	[28]
Diabetic rat with high-fat, high-sucrose diet	Glycyrrhizic acid (100 mg/kg)	↓ FBS, fasting serum insulin, HOMA-IR	[29]
STZ-induced diabetic rat	Glabridin (25, 50 mg/kg)	↓ serum glucose	[26]
High-fat diet rat	Licorice extract (20, 60, 180 mg/kg)	↓ serum glucose	[30]
High-fructose diet rat	Glycyrrhizin (50 mg/kg)	↓ serum glucose, insulin	[31]
High-fat diet rats	Glycyrrhizin (50 mg/kg)	↓ blood glucose, serum insulin levels, and HOMA-IR ↑insulin sensitivity	[32]
Eleven healthy male volunteers	LFO-DS (1200 mg/day)	↓ postprandial hyperglycemia, ↑insulin secretion	[17]
Fifty patients with metabolic syndrome	LFO (300 mg/day)	No significant decrease in FBS and HbA1c	[33]
Sixty-four volunteers	Licorice extract (1.5 g/day)	No decrease in FBS, insulin concentrations, and HOMA-IR	[34]

glabra extract or placebo for 8 weeks. By drawing a comparison between the two groups, it was indicated that insulin concentrations and HOMA-IR were decreased in both groups, and meaningful reduction in insulin and insulin resistance was only observed in the licorice group compared to the baseline. Therefore, the authors concluded that supplementation with dried licorice extract plus a low-calorie diet didn't bring about change in insulin resistance and body composition. It is better to conduct a study with higher dosages, and different forms (oil, pure components of licorice, e.g., glabridin and other flavonoids) of licorice extract assess their effect on the obesity control [34]. In Table 1, we summarized the studies performed on licorice effects on blood glucose level and diabetes.

6.1 Licorice Effect on Hyperlipidemia

Hyperlipidemia can induce atherosclerosis, which may lead to stroke, coronary heart disease, and myocardial infarction. Statistics show that in the USA, many adult populations have elevated

cholesterol levels, and millions of people worldwide are affected. The incidence of hyperlipidemia is increasing by degrees, and may worsen with an aging population, making the prevention and supervision of hyperlipidemia of high importance [36]. It is assumed that obesity and insulin resistance are the main factors in the association with dyslipidemia and obesity [37].

6.2 The Mechanism of Licorice Action in Hyperlipidemia

There are many possible mechanisms about licorice effect on body composition: First, the active form of glycyrrhizin, glycyrrhetic acid, adjusts energy metabolism and fat distribution with the aid of 11β-HSD1 enzyme's inhibition at the adipocyte level. Second, it affects gene expression of enzymes related to the lipid oxidation pathway in the liver. Third, licorice is well-known to suppress food cravings. A recent meta-analysis including 26 clinical trials in human subjects has demonstrated that licorice consumption reduced body weight [38]. El Magd and his colleagues suggested that the body weight loss effect of glycyrrhizin might be due to these mechanisms: antioxidant and anti-inflammatory effects of glycyrrhizin and lowering of some factors such as insulin resistance, absorption and digestion of exogenous lipid, adipose tissue expansion, and improvement in lipid metabolism [32].

Licorice flavonoids are beneficial in preventing and reducing oxidative inflammatory status related to overweight by regulating various molecular pathways, such as PPARα and SREBP-1c [39]. Licorice flavonoid oil decreased abdominal adipose tissue weight by mediating the transcriptional regulation of PPARα in the liver of high-fat diet-induced obese rats. Cheng and his colleagues established that glycyrrhizic acid upregulated both PPARα and PPARγ in different tissues (the kidney and skeletal muscles) of rats exposed to stress and on a high-calorie diet. In their study, the treatment of glycyrrhizic acid increased the expression of PPARγ to the normal levels in the quadriceps muscle of high-fat diet rats [11]. Tyagi et al. concluded that the presence of flavonoids and polyphenols found in *G. glabra* extracts could be considered responsible for increasing HDL and decreasing LDL and VLDL in rats treated with *G. glabra* [40]. Another mechanism for the anti-dyslipidemic effect of licorice is suppression of HMG-CoA synthase activity by glycyrrhizin, the active component of *G. glabra* [41].

The amount of glycyrrhizic acid (GA) is important for regulating the activity of 11β-HSD types 1 and 2. 11β-HSD type 2 activity is inhibited and leads to pseudohyperaldosteronism, instead of 11β-HSD type 1, at higher dose [29]. Inhibition of 11β-HSD1 activity also improves lipid profiles and prevents lipid storage, making glycyrrhizic acid a potential compound for use in metabolic syndrome [28].

7 Antihyperlipidemic Effect of Licorice in Animal Studies

Qureshi et al. found that *G. glabra* extract (50 mg/kg) meaningfully lowered total cholesterol (TC), LDL-C, and TG levels and boosted HDL-C [8]. Yoko Yamashita et al. demonstrated that a solution of licorice flavonoid oil (containing 3% glabridin) not only decreased triacylglycerol (TAG) and free fatty acid (FFA) levels in KK-Ay mice but also caused reduction in body weight and lipid accumulation, yet it failed to affect total cholesterol levels [17]. In many experiments, various high-fat diets with different compositions were used to induce hyperlipidemia in experimental rats. In one study in 2016, *G. glabra* extract (500 mg/kg) was administered to streptozotocin-induced diabetic rats fed with high-fat diet, and the treated groups showed a significant decrease in lipid profile, i.e., total cholesterol, triglycerides, LDL-C, and body weight. Moreover, in groups treated with licorice extract, HDL levels were elevated as a favorable lipoprotein with an inhibitory effect in the pathogenesis of atherosclerosis. The atherogenic index (AI) is a key parameter to assess the plaque formation which is the leading cause of atherosclerosis and ischemic heart disease (IHD). This extract presents significant control in the atherogenic index.

The substance was compared to clinically used drugs, i.e., atorvastatin (10 mg/kg) and metformin (0.5 mg/kg) [18].

Hosseinzadeh and Nassiri-Asl stated that the ethanolic extract of licorice and its fractions in hamsters fed with high-fructose diet could cause increment in HDL and decrease in serum total cholesterol, LDL, and triglyceride, through decreasing the sensitivity of LDLs to oxidation and preventing the biosynthesis of cholesterol and free radicals [21]. Singh S et al. mentioned that glycyrrhizic acid (50 and 100 mg/kg) was highly effective in lowering weight gain, total cholesterol, TG, LDL, and atherogenic index and enhancing HDL level in high-fructose diet rats [28].

Cheng and colleagues studied some rats fed with high-fat, high-sucrose diet and were treated with GA 100 mg/kg. According to the results, GA caused an increase in LPL expression and decrease in FFA level. There was not significant changes in the levels of TAG, TC, LDL, and HDL [29].

In the study of El-Ghaffar, the administration of glabridin (25 or 50 mg/kg) in diabetic rats with STZ presented significant decline in total cholesterol, TG, LDL, and atherogenic index, although the level of HDL increased. All the parameters are compared to STZ group only [26].

Goorani et al. experimented on 50 rats (10 rats as the negative control and 40 rats fed with high-fat diet) for 4 months. Then the groups received the licorice aqueous extract at 20, 60, and 180 mg/kg concentrations. After 2 months, a decline in concentrations of triglyceride, cholesterol, and LDL and a rise in HDL were demonstrated considerably [30]. For investigation of the effect of glycyrrhizin on metabolic syndrome, in Sil study, rats with metabolic syndrome underwent a high-fructose (60%) diet and received glycyrrhizin (50 mg/kg, i.p.). The study found increase in HDL level and weight of rats despite decrease in triglycerides [31].

El-Magd and his colleagues study was performed on 70 male Wistar rats, randomized in two groups. The treatment group received glycyrrhizin 50 mg/kg/day orally along with high-fat diet for 10 weeks. Glycyrrhizin decreased the levels of TG, TC, and LDL-C and normalized them. In addition, glycyrrhizin noticeably boosted HDL-C ranges in prophylactic model while amplifying HDL-C in the treatment model. The oral intake of glycyrrhizin did not bring about any change in food intake [32].

Methionine and choline-deficient (MCD) diet in mice could increase the amount of triglyceride, free fatty acid, and total cholesterol and showed significant triglyceride accumulation. After glycyrrhizic acid (GA) treatment at dose of 50 mg/kg, the aforementioned lipid profile was markedly decreased [42]. Compared to the normal-fat diet group, all serum lipids in low-fat diet mice group were lower than those of high-fat diet-induced mice with obesity. Even though oral intake of *G. glabra* extract (200 mg/kg/day) did not considerably change LDL-C, HDL-C, or total cholesterol levels, it reduced the TG level compared to high-fat diet [9].

Doğan et al. deduced from their research that the addition of *G. glabra* root powder at the amounts of 0.5%, 1.0%, and 1.5% to the feed of laying quails had no adverse effects on performance and can be used to reduce cholesterol and triglyceride concentrations and increased HDL-C in quails [43].

In Awad study, the effect of *G. glabra* root extract on the plasma lipid profile of rats was assessed. Thirty male albino rats were used for this investigation. Rats were fed with different doses of licorice extract (150, 250, and 400 mg/kg) for a period of 60 days, and this extract exhibited significantly decreased levels of total cholesterol, total LDL, and total triglycerides and increased levels of HDL as compared with positive control merely fed with high-cholesterol diets [36].

The antihyperlipidemic effect of ethanolic root extracts of *G. glabra* at dose of 400 mg/kg was studied in Wistar rats using high-fat diet. The efficacy of the extract was compared with simvastatin (10 mg/kg). When the diet was co-administered with *G. glabra* extracts, the elevated levels of TC, TG, and LDL-C condition at dose 400 mg/kg of licorice extract showed a considerable decline. There was a significant elevation in plasma HDL-C in *G. glabra*-treated rats as com-

pared to high-fat diet rats. Due to the effect of ethanolic extract of licorice in reducing triglycerides, cholesterol, and LDL, it seems to be useful as an antihyperlipidemic agent or adjuvant drug for the treatment of hyperlipidemia [40].

Ismaiel studied albino Wistar rats fed with aqueous extract of licorice tea (10, 30, and 50 mg/kg.BW/ml). The teas were found to decrease the serum cholesterol, LDL, VLDL, triglycerides, and atherogenic index particularly at their higher concentrations but were found to slightly increase the HDL levels as compared to the control group (normal healthy rats fed with clean water). The study demonstrated that aqueous extract of *G. glabra* tea possesses hypolipidemic effect at higher concentration [44].

Bagheri and his colleagues revealed in their recent research that treatment with licorice extract and licorice banana mixture on 40 female albino rats had significant decrease in LDL, total cholesterol, and total triglyceride. In contrast, HDL level was increased in groups of licorice extract and licorice banana mixture, respectively [45].

Thirty-two male Wistar rats were applied and fed high-cholesterol diet (HCD) for 12 weeks. The treatment with glycyrrhizin at dose of 100 mg/kg could attenuate the increase observed in weight as compared to the normal diet supplemented with 2% additional cholesterol group. On the other hand, GL treatment caused a decrease in TC, TG, and LDL-C levels. HDL-C level was increased significantly in the treated groups with glycyrrhizin [46].

In a review study by Sabreen et al., they reported ethanolic extract from licorice that was fare linked with reducing fat absorption in obese rats revealed anti-obesity effect. High-fat diet induced obesity in rats and rise in total cholesterol, total triglyceride, and body weight compared to normal rats. Licorice extract (100–400 mg/kg) administration with high-fat diet for 8 weeks led to significantly decreased body weight and triglycerides in rat models [47].

Aqueous extract of licorice tea treatment at doses of 10, 30, and 50 mg/kg BW/ml did not improve the serum content of HDL significantly [48]. The results in Fogelman et al. research also suggested that supplementation with licorice can reduce the serum concentrations of cholesterol, triglycerides, LDL, and VLDL and atherogenic index in Wistar rats [49].

8 Antihyperlipidemic Effect of Licorice in Human Studies

Alizadeh and others performed a study on 50 patients with metabolic syndrome in which patients received licorice flavonoid oil (LFO) at dose of 300 mg for 12 weeks. The results showed a significant reduction from the baseline levels in total body fat mass and visceral fat area, total cholesterol, and LDL-C during week 8. Also, body weight and BMI at weeks 4 and 8 were decreased. In addition, a significant difference in changes from the baseline was observed in body weight and BMI in the LFO-treated group compared to the placebo group. Their study verified that LFO is a promising dietary nutrient for improving metabolic syndrome, specially through its effect on normalizing body weight, BMI, and possibly the amount of fat tissue and HDL-C [33].

Licorice flavonoid oil (LFO) has been described to have valuable effects on insulin resistance and obesity. In some overweight postmenopausal women who consumed 900 mg/day of LFO, decrease in BMI, visceral fat, and levels of LDL-C was observed. The beneficial effects of licorice on changes in body composition are also presented in people with normal body weight. A dose of 3.5 g/day of licorice resulted in reduced body fat mass in healthy individuals with normal weight without significant changes in body mass index. In another clinical trial, LFO administration had favorable effects on body composition with a reduction of body fat mass and increase of muscle mass in 54–90-year-old adults who underwent rehabilitation for osteoarthritis [38].

Hautaniemi and colleagues reported that patients with high level of cholesterol and without significant stenosis showed a decrease in total cholesterol and low-density lipoprotein after consumption of licorice ethanolic extract (0.2 g/day) for 12 months [49]. Mirtaheri et al. stated that

during the intervention, the dosage of licorice was 120–300 g/day of a product in 22 healthy volunteers, showing after 2 weeks that the levels of triglycerides and cholesterol in licorice group were decreased with HDL levels being elevated [50]. In a clinical trial on 64 overweight and obese subjects, both groups received 1.5 g/day of dried licorice extract or placebo, respectively, concurrent with weight loss diet for 8 weeks. Only 58 participants completed this clinical trial. At baseline, there were no significant differences for lipid profile except for LDL-C level between the groups. After the administration of licorice extract, TC, LDL-C levels, TC/high-density lipoprotein (HDL-C), LDL-C/HDL-C ratios, and the log of TG/HDL-C were significantly decreased, but no changes were observed in TG and HDL-C levels. The authors concluded that the licorice extract supplementation used concurrently with a low-calorie diet can improve the serum lipid profile [51]. We mentioned the studies in Table 2 related to licorice effects on hyperlipidemia.

9 Licorice Effect on Hypertension

High blood pressure is considered as the main risk factor of cardiovascular disease [52]. Hypertension is a chronic condition that triggers numerous cardiovascular disorders, such as heart failure, ischemic stroke, and peripheral artery disease [53]. There is a difference in studies about the amount of licorice that causes serious cardiovascular symptoms. Factors such as diagnosed previous hypertension, female sex, older age, and elongated gastrointestinal transit time increase the risk of elevated blood pressure with licorice [54].

10 The Mechanism of Licorice Action in Hypertension

Licorice pseudoaldosteronism was first reported in 1968 and is considered as an undesirable complication. Most cases of licorice-induced pseudoaldosteronism have been reported in patients consuming great amounts of glycyrrhizin (>500 mg daily). In contrast, a recent study in Japan showed that a small amount of licorice causes pseudoaldosteronism [55]. The active ingredients of licorice root (glycyrrhizic acid and glycyrrhetic acid) lowered potassium serum level [54]. This corticosteroid-like action causes the antiallergenic and anti-inflammatory effects of licorice [56, 57]. Daily consumption of licorice ranges from 3 to 15 g of dried root to 500 to 1500 mg of extract. The European Union, in 1991, suggested a tentative dose of 100 mg/day as the upper limit for glycyrrhizin (roughly the amount found in 60–70 g licorice) because an overdose of licorice that contains glycyrrhizin and hydrolyzed metabolite glycyrrhetic acid can result in mineralocorticoid excess syndrome [58]. Licorice is listed in many articles to cause resistant hypertension [59, 60].

11 Licorice Effect on Hypertension in Animal Studies

In an animal study performed by Singh et al., glycyrrhizic acid was used for evaluating its effect on blood pressure and heart rate of mice. Followed by the administration of 10 mg/kg i.p. of GA, both blood pressure and heart rate fell considerably. Following 3–4 h, blood pressure together with heart rate stabilized back to normal [61].

12 Licorice Effect on Hypertension in Human Studies

A review study on food products which might increase blood pressure explained about a crossover study completed among 64 healthy volunteers. The participants received licorice 50, 100, and 200 g daily for 2–4 weeks. The results showed an increase in mean blood pressure. In another study, 25 healthy subjects and 11 patients with hypertension received licorice at dose of 100 g/day (i.e., 150 mg glycyrrhizic acid) for about 4 weeks. During a day of blood pressure

Table 2 Summary of studies carried out on licorice effects on lipid profile

Study model	Dose	Result	References
Rabbits with high-cholesterol diet	Licorice extract 50 mg/kg	↓ TC, TG, and LDL ↑ HDL	[8]
Mice with high-fat diet	Extract of licorice (200 mg/kg/day)	↓ serum TG No change in TC, HDL, and LDL	[9]
Type 2 diabetic mice	"LFO concentrate solution" (0, 1.0, or 1.5 g/kg body weight)	↓TAG, FFA, visceral fat accumulation No change in cholesterol	[17]
STZ-induced diabetic rats with high-fat diet	Alcoholic extract of licorice (500 mg/kg)	↓TC, TG, LDL, AI, body weight ↑ HDL	[18]
Hamsters with high-fructose diet	Ethanolic extract of licorice (100 mg/kg)	↓ serum TC, LDL, TG ↑ HDL	[21]
STZ-induced diabetic rats	Glabridin (25, 50 mg/kg)	↓ TC, TG, LDL, AI ↑ HDL	[26]
Diabetic rats with high-fructose diet	Glycyrrhizic acid (50 and 100 mg/kg	↓ TC, TG, and LDL-C, AI ↑ HDL	[28]
Rats with high-fat, high-sucrose diet	Glycyrrhizic acid (100 mg/kg)	↓ FFA No change in TAG, TC, LDL, and HDL	[29]
Rats with high-fat diet	Licorice extract at 20, 60, and 180 mg/kg	↓ cholesterol, LDL, triglyceride ↑ HDL	[30]
Rats with high-fructose diet	Glycyrrhizin (50 mg/kg)	↓ weight gain and TG levels ↑ HDL	[31]
Rats with high-fat diet	Glycyrrhizin (50 mg/kg/day)	↓ TG, TC, LDL levels ↑ HDL	[32]
Rats with high-cholesterol diet	Licorice extract (150, 250, and 400 mg/kg)	↓ TC, TG and LDL ↑ HDL	[36]
Wistar rats with high-fat diet	Licorice extract (400 mg/kg)	↓ TC, TG, and LDL ↑ HDL	[40]
Methionine- and choline-deficient (MCD) diet in mice	Glycyrrhizic acid (12.5, 25, 50 mg/kg)	↓ serum TG, FFA, TC	[42]
Quails	0.5, 1, and 1.5% of feed with licorice root powder supplementation	↓ serum TC, LDL, TG ↑ HDL	[43]
Albino Wistar rats	Licorice tea (10, 30, and 50 mg/kg.BW/ml)	↓ TC, TG and LDL-C, VLDL, AI ↑ HDL	[44]
Normal rats	Licorice extract and licorice banana mixture	↓ total lipids, LDL, TC, TG ↑ HDL	[45]
High-cholesterol diet rat	Glycyrrhizin (100 mg/kg)	↓ TC, TG, and LDL ↑ HDL	[46]
Rats with high-fat diet	Licorice extract (100–400 mg/kg)	↓ body weight and TG	[47]
Wistar rats	Licorice tea (10, 30, 50 mg/kg BW/ml)	↓ TC, TG, LDL, AI, VLDL No change in HDL	[48]
Overweight women	LFO (900 mg/day)	↓ visceral fat, body mass index, and LDL	[38]
Healthy, normal weight people	3.5 g/day of licorice	↓ body fat mass without significant changes in body mass index	[38]

(continued)

Table 2 (continued)

Study model	Dose	Result	References
Adults 54–90 years old	LFO	↓ body fat mass	[38]
Hypercholesterolemia patients	Ethanolic extract of (licorice 0.2 g/day)	↓ TC, LDL	[49]
Twenty-two healthy volunteers	Glycyrrhizin (290–370 mg/day)	↓ TG, TC ↑ HDL	[50]
Sixty-four overweight and obese subjects	1.5 g/day of dried licorice extract	↓ TC, LDL, TC/HDL, LDL / HDL ratios and log TG/HDL No change in TG and HDL-C	[51]
Fifty subjects with metabolic syndrome	LFO (300 mg/day)	↓ body weight, BMI, visceral fat, TC, LDL ↑ HDL	[33]

monitoring for healthy people, systolic and diastolic blood pressures were intensified by 6 mmHg and 4 mmHg, respectively. However, patients with chronic hypertension showed increment in systolic and diastolic blood pressures by 12 mmHg and 9 mmHg [54]. During the intervention in 22 healthy volunteers, with 120–300 g/day of licorice depending on the amount of glycyrrhizin in the product, elevated systolic blood pressure was observed after 2 weeks [50].

In a systematic review in 2018, in which a total of 26 clinical trials were included, results showed a rise in the diastolic blood pressure (DBP) with licorice, which is related to licorice-related hypernatremia [62]. The study comprised of meta-analyses of 18 researches, resulting in statistically meaningful rises in mean systolic blood pressure and diastolic blood pressure following constant ingestion of a product containing at least 100 mg glycyrrhizic acid. Potassium, aldosterone concentration, and plasma renin activity were all significantly decreased. Therefore, regular licorice intake is related to an increase in blood pressure and a reduction in plasma potassium, even at moderate doses [63]. We summarize the human studies about the effects of licorice on blood pressure in Table 3.

12.1 Case Reports About Increase in Blood Pressure by Licorice

The following are some case reports in regard to the use of licorice increasing blood pressure in some patients.

Smedegaard and Svart mentioned a 43-year-old woman admitted to a clinic with a high blood pressure (177/98 mmHg) with her blood test revealing low plasma potassium levels (1.9 mmol/L). The ECG test revealed flattened T-waves and long QT interval. It is determined that the patient had increased licorice consumption daily (about 70 g). After she stopped licorice intake and potassium was administrated, plasma potassium and ECG got normalized [64]. A 65-year-old woman presented to the emergency room with acute chest pain, headache, nausea/vomiting, and high blood pressure in the previous days. Relatively small amounts of licorice, at least 50 g daily for 2 weeks, can cause high blood pressure, and this patient has been taking a total of 256–512 g licorice daily for 6 months [65].

Varma and Ross reported on a 70-year-old woman with recent diagnosis of hypertension who was admitted to the acute medical unit. Her systolic blood pressure was 200 mmHg and serum potassium of 2.4 mmol/L. In the patient's history, she noted she had consumed averagely six tea bags of "Twining's Comforting" licorice tea daily [57].

A 66-year-old man diagnosed with new high blood pressure has had hypokalemia for the past 4 months on routine blood tests. The patient had high blood pressure (152/80 mm Hg), and his recent laboratory tests showed a lowered serum potassium level (2.5 mmol/L) and a metabolic alkalosis. Additional patient history revealed that he had been taking an abnormally great amount of licorice-containing lozenges for his neuropathic pain for about the past 3–4 months [66].

Table 3 Summary of human studies mentioned licorice effects on blood pressure

Study model	Dose	Result	References
Sixty-four healthy volunteers Thirty-six subjects	Licorice 50 g,100 g, 200 g/day Licorice 100 g/day	↑ mean blood pressure ↑ blood pressure	[54]
Systematic review	Licorice consumption	↑ diastolic blood pressure	[62]
Twenty-two healthy volunteers	Licorice 120–300 g/day	↑ systolic blood pressure	[50]
Systematic review	Glycyrrhizic acid 100 mg	↑ mean systolic blood pressure and diastolic blood pressure	[63]
Patients with hypercholesterolemia	0.2 g/day of ethanolic extract of licorice root	↓ blood pressure	[49]

Another case is a 57-year-old man with acute visual impairment. Initial examination revealed a blood pressure of 250/110 mmHg and hypertensive retinopathy. Further assessments of the patient's habits revealed a marked weekly intake of minimally three packs of licorice (each 300 g) from a German candy producer for the previous 3–4 months. Since this dietary behavior was considered to be supposedly in association with the patient's high blood pressure, the licorice consumption was stopped [67].

Another article states that a 57-year-old male patient had irregular palpitation recently. Upon physical examination, his heart rate was 160 bpm/min and his blood pressure was 90/60 mmHg with reduced potassium level, which was 2.0 mmol/L. It was found that the patient was drinking licorice root syrup daily during the month of Ramadan in order to reduce the thirst [68].

A 65-year-old man case with a blood pressure of 159/71 mmHg who presented to the emergency department was reported. Blood test indicated hypokalemia (potassium level = 1.8 mEq/liter). A detailed history and physical examination disclosed that the patient consumed a large amount of black licorice for several weeks [69].

Lee concludes that it is of critical importance to consider inquiring a patient with alleged high blood pressure and resistance to the normal hypotensive pharmacological treatments to be asked openly about their consumption of uncommon food, specifically licorice [19].

An 18-year-old healthy woman with early-onset preeclampsia, possibly intensified by licorice consumption, was presented with high blood pressure of 200/145 mmHg and pulse rate of 100 bpm at 18 weeks' gestation. She had a strong family history of preeclampsia and was consuming great amounts of licorice [70].

A 51-year-old man presented with elevated blood pressure of 174/62 mmHg and lowered potassium of 2.6 mmol/L. It was determined that he had lately started eating large amounts of black licorice flavored jelly beans (one bag of approximately 50 jelly beans daily) [71].

Also hypokalemic effect of licorice was recorded in a case presentation conducted on a 55-year-old male consuming 25 g/day licorice for 1 year after quitting smoking [72].

A 60-year-old Korean woman was presented with ventricular tachycardia secondary to hypokalemia. The patient was not taking any other medication and denied any vomiting or diarrhea, but on repeated inquiry, she admitted of taking herbal medicine containing licorice, though [73].

A 47-year old woman with a diagnosis of primary biliary cholangitis was found with a history of consuming 225 mg of glycyrrhizin daily for 3 years. She had a dramatically elevated blood pressure of about 230/110 mmHg without a history of hypertension and was referred to the medical service [74].

The induction of licorice hypertension because of pseudo-hyperaldosteronism has been extensively reported. By presenting these cases, we are reminded of glycyrrhizin tempted hypertension, a condition which could lead to medical emergencies [74]. Therefore, caution should be taken when consuming licorice products.

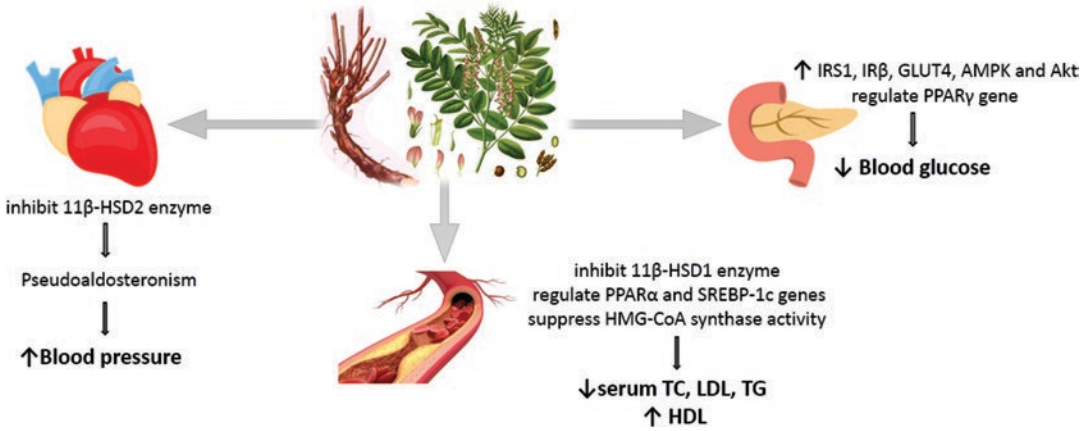

Fig. 1 Effects of licorice on hypertension, hyperlipidemia, and hyperglycemia

13 Conclusions

According to the studies on the effect of licorice, this plant has been referred to as one of the effective plants in the treatment of type 2 diabetes and hyperlipidemia (Fig. 1). Regarding the effects of licorice on diabetes, animal studies have demonstrated that licorice extract can have hypoglycemic effects. It appears that research on the antidiabetic effect of licorice in animals had been done, and no comprehensive human studies have been performed on the subject.

In the current research, no distinction has been made between type 1 and type 2 diabetes. There are also a limited number of studies on the antidiabetic outcomes of licorice in patients with metabolic syndrome. Given that diabetes and metabolic syndrome are common ailments in society, it seems that more studies are to be performed on the topic so that more valid inferences can be made. Studies of the effects of licorice on hyperlipidemia have shown positive consequences on reducing triglycerides and increasing HDL.

Due to the fact that metabolic syndrome is associated with an increase in triglycerides and a decrease in HDL, despite numerous studies on animals proving the certain effect of licorice in improving hyperlipidemia, human studies in this area are limited, and it appears that more comprehensive clinical trials in this regards are needed to be conducted in patients undergoing metabolic syndrome. Since licorice can cause hypertension and hyperkalemia and can cause cardiovascular problems, high blood pressure with licorice seems to be a negative factor in the treatment of metabolic syndrome.

Most studies on the effects of licorice on blood pressure in humans have been performed as case studies, and it is of high importance to obtain accurate measurements in human groups with high blood pressure. In a range of studies, the antidiabetic and antihyperlipidemic effects of licorice have been shown to point out that it can be one of the most necessary compounds in the treatment of metabolic syndrome. It is advisable to conduct extensive studies in people with metabolic syndrome to obtain a comprehensive view of the effects of licorice in people suffering from high blood pressure, dyslipidemia, and hyperglycemia at the same time.

Conflict of Interests None

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Natural Insulin Sensitizers for the Management of Diabetes Mellitus: A Review of Possible Molecular Mechanisms

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Abstract

Diabetes mellitus is a growing health challenge globally which is increasing in epidemic proportion. Naturally occurring pharmacological agents are more likely to provide beneficial therapeutic effects without undesirable side effects compared to the synthetic agents. There is a growing evidence that some naturally occurring pharmacological agents derived from plants have potential antihyperglycemic effects. In this study, we have reviewed the molecular mechanism behind potential hypoglycemic properties of four well-known herbal-based agents, namely, ginger, curcumin, garlic, and cinnamon. Also, we present the related clinical data confirming experimental results aiming to develop novel therapeutic strategies based on these herbal agents potentially for the management of patients with diabetes.

Keywords

Diabetes mellitus · Ginger · Curcumin · Garlic · Cinnamon · Oxidative stress · GLUT-4 · Pharmaceutical · Herbal medicine

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1 Introduction

The incidence of diabetes mellitus (DM) is rising exponentially [1]. This chronic disorder has a negative effect on most metabolic pathways [2, 3]. DM is a potent upstream event for the development of various complications such as diabetic nephropathy, retinopathy, neuropathy, and cardiovascular diseases [2]. Uncontrolled DM can trigger other pathophysiologic pathways such as

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oxidative stress, inflammation, fibrotic process, and apoptotic events and thereby impose deleterious impacts on most tissues contributing to tissue dysfunctions [2, 3]. Many antidiabetic drugs with different therapeutic potentials have been developed to normalize glycemia and to reduce the risk of diabetic complications [4–6]. Since these agents are associated with some unfavorable side effects [7, 8], the use of naturally derived compounds in the management of patients with diabetes is growing [9–11]. These natural-based agents can potentially increase insulin sensitivity and improve insulin resistance, thereby could be potentially used as therapeutic agents for the management of diabetes [9–11]. In the current study, we review the possible antidiabetic effects of some well-known natural-based agents.

The two common subtypes of DM are type 1 diabetes (T1DM) and type 2 diabetes (T2DM) [12]. About 90–95% of patients with DM have T2DM and is mainly contributed by insulin resistance in peripheral tissues [12–14].

2 Insulin Signal Transduction and Insulin Sensitivity

Insulin signal transduction (IST) is a complex molecular pathway with sequential steps involving different enzymes and mediators resulting in glucose entering into the cells facilitated by GLUT-4 (glucose transporter-4) transporters [15, 16]. GLUT-4 is a protein mainly localized in adipocytes, muscles, and myocardial cells and is responsible for glucose uptake into these cells in response to circulating insulin [17]. The IST is initiated by binding of insulin to its specific receptors known as insulin receptors (IRs) [17]. This binding process induces downstream events such as recruitment of different adaptor proteins including insulin receptor substrates (IRSs), Shc (SHC-transforming) protein, and APS protein (an adapter protein) [18, 19]. These events provide a binding site for the IRS-1 (insulin receptor substrate type 1) [19]. IRS-1 is also sensitive to other types of kinases such as ERK1/2 (extracellular signal-regulated kinase 1/2), atypical PKC (protein kinase C), S6K1 (ribosomal protein S6 kinase beta-1), SIK2 (serine/threonine-protein kinase 2), Akt (protein kinase B), mTOR (mammalian target of rapamycin), ROCK1 (Rho-associated protein kinase 1), AMPK (AMP-activated protein kinase), and GSK3 (glycogen synthase kinase 3) which are activated after phosphorylation [19, 20]. Activated IRS-1 binds to PI3K (phosphoinositide 3-kinase) and activates it which in turn catalyzes the conversion of PIP_2 (phosphatidylinositol 4,5-bisphosphate) to PIP_3 (phosphatidylinositol 3,4,5-trisphosphate) [21]. PIP_3 is itself a potent activator for Akt, which induces GLUT-4 localization and thereby facilitates glucose entering into the insulin-dependent cells [21, 22]. Any disturbance in these sequential delicate steps can potentially impair normal IST and thereby induce varying degrees of insulin resistance and DM [16]. Hence, any factors which could potentially promote these sequential steps can induce insulin sensitivity and thereby improve insulin resistance [15, 23–25].

3 Natural Insulin Sensitizers

In addition to synthetic medications, some plants and/or their extract can be considered as natural pharmaceuticals which have hypoglycemic effects through different molecular pathways [9–11, 26]. Emerging in vitro and in vivo evidence suggest that the five main naturally occurring agents that have potential antihyperglycemic effects are saffron, ginger, curcumin, cinnamon, and garlic [9, 27–30]. We have previously reviewed the antihyperglycemic potentials of saffron and its active ingredients [9]. In the following sections, we have discussed the four main naturally derived plants with antihyperglycemic properties, viz., ginger, curcumin, garlic, and cinnamon, and their potential molecular mechanisms.

1. Curcumin

Curcumin is an active diarylheptanoid compound from the curcuminoid family which is mainly found in turmeric species and is responsible for the yellow color of this plant [31, 32].

Besides as a dietary supplement, this phytochemical has various pharmacological actions [33–39] as well as insulin-sensitizing and hypoglycemic effects [36, 40–45]. It can exert its antidiabetic effects in T2DM through various molecular pathways [27]. Curcumin has strong anti-inflammatory potentials which enable it to lower inflammation-induced insulin resistance in DM [46]. It can attenuate the inflammatory events in the beta cells by suppressing the activity of T lymphocytes and reducing the expression of inflammatory cytokines in the diabetic milieu [46]. Evidence suggests that curcumin is a potent antioxidant which neutralizes the oxidative stress involved in promoting insulin resistance [47]. It can induce Nrf2 activity and upregulate elements of the antioxidant defense system [48, 49]. It has also been shown that curcumin might improve mitochondrial function and reduces the free radical generation leading to lower oxidative damages in the beta cells [50, 51]. Moreover, curcumin may provide an insulin sensitizer effect by stimulating the GLUT-4 expression in the diabetic milieu [52]. Curcumin could also promote beta cell function and thereby improve insulin sensitivity [40, 52]. Improvement in lipid metabolism can be considered as another possible molecular pathway by which curcumin increases insulin sensitivity [53–55].

There is also clinical evidence suggesting the potential role of curcumin as an antihyperglycemic agent [27, 56, 57] (Table 1). Na et al. in 2013 demonstrated that it can reduce HbA_{1c} (hemoglobin A_{1c}) and improve insulin resistance via lowering FFAs (free fatty acids) in patients with diabetes [27]. Chuengsamarn et al. after a randomized controlled trial of 6 months reported that curcumin reduced the fasting blood glucose and HbA1c via improvement in insulin sensitivity and glucose homeostasis in patients with T2DM [56]. Jiménez-Osorio et al. have shown that curcumin markedly reduced fasting plasma glucose in patients with T2DM [58]. Moreover, Hodaie and coworkers have shown that curcumin markedly reduced fasting hyperglycemia and HbA1c in patients with T2DM [57]. These clinical trials have confirmed the experimental data suggesting that curcumin has antihyperglycemic effects by improving insulin sensitivity in a diabetic milieu [27, 56, 57].

2. **Ginger**

Ginger is a flavoring plant belonging to Zingiberaceae family which has pharmacological effects beyond its use as a food additive [59]. Evidence demonstrated that the rhizomes of the ginger roots widely used in ancient medicine have significant hypoglycemic effects [59–61]. Ginger can induce insulin sensitivity via different molecular pathways such as antioxidative, anti-inflammatory, lipid modulatory pathways and by preventing lipid peroxidation [62–64]. It can also modulate the molecular mechanisms of IST as PI3K activity, Akt activation, IRS-1 phosphorylation, and GLUT-4 localization in 3T3-L1 adipocytes [29].

Clinical evidence has confirmed these findings [62]. Khandouzi and coworkers in 2015 surveyed the antidiabetic effects of ginger and found that it reduces hyperglycemia, fasting blood glucose, HbA1c, and MDA (malondialdehyde) in patients with T2DM potentially mediated by its antioxidative properties [62]. Mozaffari and colleagues in 2014 conducted a clinical trial demonstrating that ginger powder reduces fasting blood glucose and HbA1c and induces insulin sensitivity in patients with T2DM [65]. Moreover, Bahramian et al. in 2018 demonstrated that daily administration of ginger in women with gestational diabetes has no significant effects on fasting hyperglycemia and HbA1c, but increased the glucose tolerance in these patients [66]. Similarly, Haas and coworkers in 2015 reported that daily usage of ginger supplements reduced fasting hyperglycemia and HbA1c as well as increased insulin sensitivity in patients with T2DM [67]. This evidence suggests that ginger species has potential insulin sensitizer effects that could be of potential benefit in patients with T2DM.

3. **Garlic**

Garlic (*Allium sativum*) plant is an ancient species possessing a wide range of pharmacological effects including antimicrobial, anticancer, anti-

Table 1 Clinical evidences about insulin-sensitizing effects of ginger, curcumin, garlic, and cinnamon

Natural agent	Population of study (without placebo groups)	Dosage/duration	Clinical effects	Refs.
Curcumin	50 patients with T2DM	300 mg/day/6 months	Decreased FBS, HbA1c, HOMA-IR, and insulin sensitivity	[27]
	113 patients with T2DM	250 mg/day/6 months	Reduced FBS, HbA1c, and LDL	[56]
	105 patients with diabetic or nondiabetic CKD	320 mg/day/8 weeks	Declined FBS	[58]
	53 patients with T2DM	1500 mg/day/10 weeks	Reduced FBS and body weight	[57]
Ginger	22 patients with T2DM	2 g/day/12 weeks	Reduced FBS, HbA1c, and Apo lipoproteins	[62]
	88 patients with T2DM	3 g/day/8 weeks	Decreased FBS, HbA1c, and insulin resistance	[65]
	76 women with gestational diabetes	500 mg/day/8 weeks	No significant effects on FBS and HbA1c, but improved the glucose tolerance	[66]
	33 patients with T2DM	1600 mg/day/12 weeks	Markedly reduced FBS, HbA1c, and insulin sensitivity	[67]
Garlic	210 patients with T2DM	300, 600, 900, 1200, and 1500 mg/day/24 weeks	Reduced FBS and HbA1c	[78]
	60 patients with T2DM	250 mg/day/12 weeks	Induced insulin sensitivity via attenuating inflammatory events and improving lipid profile	[79]
	26 women with gestational diabetes	400 mg/day/8 weeks	Reduced FBS and HbA1c	[80]
Cinnamon	137 patients with T2DM	500 mg/day/2 months	Declined FBS, HbA1c, and HOMA-IR	[87]
	79 patients with T2DM	3 g/day/4 months	Reduced FBS, LDL, HDL, and HbA1c	[90]
	40 diabetic women with PCOS	1 g/day/8 weeks	Increased insulin sensitivity and declined HOMA-IR	[91]
	137 patients with hyperglycemia	500 mg/day/2 months	Reduced LDL, HDL, FBS, and increased insulin sensitivity	[94]
	66 women with PCOS	1.5 g/day/12 weeks	Declined FBS and HbA1c	[95]

CKD chronic kidney disease, *FBS* fasting blood glucose, *HOMA-IR* homeostatic index of insulin resistance, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *HbA1c* glycosylated hemoglobin, *PCOS* polycystic ovary syndrome

inflammatory, immunomodulatory, neuroprotective, antioxidative, as well as antidiabetic properties [68–71]. Evidence demonstrated that garlic extract can modulate some molecular mechanisms involved in IST [72–76]. It can induce AMP-activated protein kinase and increase insulin sensitivity in adipocytes [30]. Also, garlic extract can reduce the oxidative stress leading to an improvement in insulin sensitivity [76], which was confirmed by other studies [77].

There are also clinical data demonstrating the antihyperglycemic properties of garlic [78]. Ashraf et al. in 2011 has shown that aged garlic extract can exert obvious hypoglycemic effects by lowering the fasting blood glucose (FBG) and HbA1c in patients with T2DM [78]. Kumar and coworkers in 2013 reported that garlic extract induces insulin sensitivity by reducing the inflammatory response and deaminase levels as well as resulted in an improvement in lipid profile in

patients with T2DM [79]. Faroughi et al. in 2017 provided data in gestational diabetes demonstrating garlic pill significantly increased insulin sensitivity in women with gestational diabetes [80]. Although more clinical trials are needed, the available evidence suggests potential antihyperglycemic effects of garlic and its extracts.

4. Cinnamon

Cinnamon is a spice of the genus *Cinnamomum*. It has been primarily recognized as a food additive, but has potent medicinal effects and thereby used for thousands of years in ancient medicine [81]. There is evidence suggesting that cinnamon and/or its active flavoring ingredient, cinnamaldehyde, can improve glucose homeostasis and induce insulin sensitivity in adipocytes and muscle tissues via several molecular pathways (Fig. 2) [82, 83]. It can increase glucose transport across the cell membrane by promoting GLUT-4 expression/localization [84]. Also, cinnamon can promote different steps of IST such as IRS-1 phosphorylation and PI3K activity, thereby inducing insulin sensitivity [84]. Modulatory effects on the pathophysiologic pathways involved in insulin resistance such as AGE-RAGE interaction, oxidative damages, and inflammatory responses are the other possible ways by which cinnamon induces insulin sensitivity in adipose and muscle tissues [84]. Treatment with cinnamon extract decreases the mRNA expression of the inflammatory mediators such as IL (interleukin)-1β, IL-6, and TNF-α (tumor necrosis factor-alpha) and modulates the mRNA expression of IR, IRS-1 and IRS-2, PI3K, and Akt [84, 85]. It can also improve insulin sensitivity via PPAR (peroxisome proliferator-activated receptor) activation in 3T3-L1 adipocyte [28]. These effects are accompanied by improved insulin signaling in brain tissues confirming the effect of cinnamon on the IST [86].

There are also clinical studies demonstrating the effect of cinnamon on insulin sensitivity [87]. Stoecker et al. in 2010 showed that cinnamon therapy in T2DM patients reduced FBG, HbA1c, and HOMA-IR [87]. It also modified glucose homeostasis by promoting postprandial GLP-1 (glucagon-like peptide-1) secretion [88, 89]. Mang et al. in 2006 demonstrated that cinnamon increases insulin sensitivity by improving the lipid metabolism in patients with T2DM [90]. Wang et al. in 2007 provided further evidence in patients with diabetes and polycystic ovary syndrome demonstrating the insulin-sensitizing effects of cinnamon [91]. More clinical evidence

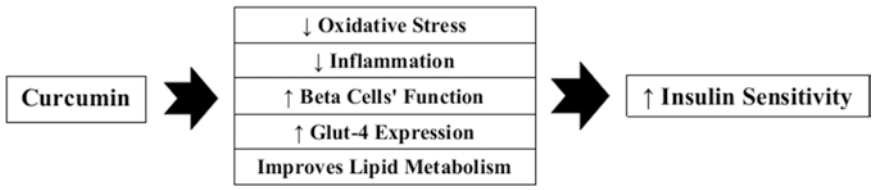

Fig. 1 Curcumin induces insulin sensitivity via at least five molecular mechanisms

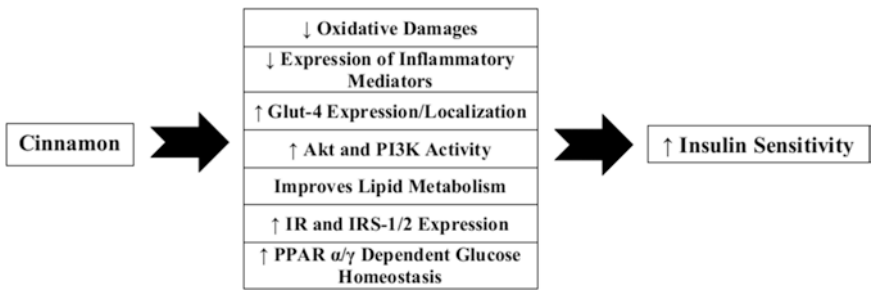

Fig. 2 Main molecular pathways by which cinnamon induces insulin signal transduction

is presented in Table 1. We also have some reports indicating no significant effects on cinnamon extract on insulin sensitivity [92, 93].

4 Conclusion

Herbal-based therapeutic approaches for patients with diabetes have been tried for thousands of years and have received more attention recently. There is a growing evidence that ginger, curcumin, garlic, and cinnamon have potent antihyperglycemic effects and thereby their extracts can be potentially useful in the management of patients with T2DM. Although some clinical trials have confirmed the experimental evidence, there is a need for more clinical trial evidence, especially for garlic and cinnamon. This suggests that herbal-based agents could be the next generation of therapeutic intervention for the management of diabetes. However, more clinical trials are needed for identifying the ideal dosage, duration of therapy, and formulation.

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Conflict of Interests The authors clearly declare that they have no conflict of interest in this study.

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Evaluation of the Anti-constipation Effects of Abdominal Application of Olive Oil Ointment in Children 1–4 Years Old: A Pilot Placebo-Controlled, Double-Blind, Randomized Clinical Trial

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Abstract

Objective: With a prevalence of 0.7 to 29.6%, functional constipation (FC) is a common pediatric complaint worldwide. Current therapeutic strategies for FC mainly include prevention and treatment of fecal impaction, by administration of oral laxatives or rectal medications. However, these agents have been reported to have limited efficacy and a number of serious side effects. In traditional Persian medicine, local application of olive oil was used to relieve childhood constipation. In this pilot placebo-controlled, double-blind, randomized clinical trial, the laxative effects of the external use of olive oil ointment in 1- to 4-year-old children with functional constipation were investigated.

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Methods and Materials: Forty patients with FC were randomly assigned in olive oil ointment or placebo groups, receiving either an ointment containing 85% olive oil or a comparable placebo or an ointment containing 85% liquid paraffin adjusted to have color and odor similar to those of intervention ointment, twice a day for 4 days. Rome III criteria for functional gastrointestinal disorders (FGIDs) were used to identify eligible patients from three hospitals. The primary outcome measure was treatment success, defined as ≥1 spontaneous stools per day, without episodes of fecal impaction at endpoint (day 4). The secondary outcome measure was the frequency of fecal incontinence, abdominal discomfort or flatulence, painful defecation, and dermal irritations (adverse effect).

Results: Improvements in stool frequency started from day 1 and continued up to day 4 (end of the study) and were significantly greater in patients receiving olive oil ointment in comparison with placebo group ($p < 0.05$). No adverse effect (including fecal incontinence, painful defecation, gripe and skin reaction, etc.) was reported in intervention and placebo groups during the study.

Conclusion: Olive oil ointment used in this study can be presented as a safe, well-tolerated, and effective herbal preparation in children with functional constipation.

Keywords

Olive oil · Constipation · Herbal medicine · Clinical trial

1 Introduction

Functional constipation (FC) is a common pediatric complaint worldwide, with an approximate prevalence of 0.7 to 29.6% [1]. The constipation symptoms are often consisting of infrequent, painful, and difficult defecation. The pathophysiology of FC is multifactorial and not well understood [2]. Constipation is mainly defined based on the number and frequency of bowel movements (BMs). However, patients commonly define it as a complex disorder accompanied with bloating, hard stools, abdominal straining and pain, and a sense of incomplete evacuation of rectum on defecation [3]. The Rome III criteria is a recognized, standardized, symptom-based set of criteria which provide a structured framework for the diagnosis and clinical study of functional gastrointestinal disorders, including chronic constipation [4]. The Rome III criteria for pediatric FC are different in children <4 years of age and children aged 4 or more years [1].

Current therapeutic strategies for FC mainly include prevention and treatment of fecal impaction, by administration of oral laxatives or rectal medications [5]. New approaches to manage FC include chloride channel activators, neurotrophins, and serotonergic agents with enterokinetic properties. However, these agents have been reported to have limited efficacy and a number of serious adverse effects [3]. Moreover, diet therapy and lifestyle changes, toilet training, psychological assessment, and behavior modification have been considered helpful [6].

Olive is the fruit of the olive tree, *Olea europaea* L., from Oleaceae family. Olive tree is a species native to the Mediterranean Basin and some parts of Asia and is a source of valuable nutrients and phytochemicals of medicinal interest [7]. Olive oil is an important constituent of Mediterranean diet and mainly contains fatty acids (mainly oleic, palmitic, and linoleic acids), steroids, simple phenols, secoiridoids, lignans, and squalene [8]. The results obtained from a number of clinical trials revealed that extra-virgin olive oil can alleviate constipation through improving BMs and possessing lubricant effect [4, 9, 10]. In Persian medicine (PM), olive oil has been widely used for the treatment of constipation. Topical use of olive oil on abdominal regions of infants and young children has been recommended by PM scholars such as Avicenna, Jorjani, and Aqili Khorasani to alleviate constipation [11–13]. However, to the best of our knowledge, there is no clinical trial studying the effects of topical application of olive oil in children with FC. In the present placebo-controlled, double-

blind, randomized pilot clinical trial, we aim to evaluate the anti-constipation effects and safety profile of an ointment containing 85% w/w olive oil in children 1–4 years old and to compare it to a placebo ointment.

2 Material and Methods

2.1 Intervention Medications

The study products (olive oil ointment and placebo) were prepared in the industrial pharmacy lab at the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Organic extra-virgin olive oil was purchased from the Fadak integrated Agro-Industrial company, Fadak, Qom, Iran. The product had been produced by cold pressing and complied with the EU Regulation (Council Regulation (EC) No 834/2007). An ointment containing 85% w/w olive oil in solid paraffin was prepared. An ointment containing 70% w/w solid paraffin in liquid paraffin was also prepared to be used as placebo. Olive oil and placebo ointments were similar in terms of appearance, thickness, color, and odor. Each ointment was instructed to be gently massaged on the abdominal area for 5 min twice daily for a period of 4 days.

2.2 Study Design and Population

This study was designed as a pilot, multicenter randomized double-blind placebo-controlled parallel-group trial and was conducted between September 2017 and August 2018. The study was conducted in Emam Reza, Qaem, and Akbar Pediatric Hospitals of Mashhad. The study was approved by the Regional Committee for Research Ethics at the Mashhad University of Medical Sciences under the approval code of IR.MUMS.fm.REC.1396.43. The protocol for this study was registered at the Iranian Registry of Clinical Trials under the accession code of IRCT2017072535304N1.

Fifty-seven children with FC who met the inclusion criteria entered this study. The participants were children 1 to 4 years of age with FC who were admitted to the hospital according to the Rome III criteria for functional gastrointestinal disorders (FGIDs) recommended by the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)* [14]. The inclusion criteria for functional gastrointestinal disorders (FGIDs) included the presence of two or more of the following criteria for at least 1 month: two or fewer defecations in the toilet per week, at least one episode of fecal incontinence per week, history of retentive posturing or excessive volitional stool retention, history of painful or hard bowel movements, presence of a large fecal mass in the rectum, and history of large diameter stools which may obstruct the toilet.

Exclusion criteria included organic constipation, hypothyroidism, intestinal pseudo-obstruction, cystic fibrosis, neural anomalies, intestinal obstruction, patients receiving constipation-inducing medications including atropine, hyoscine, homatropine, ipratropium bromide, cyclopentolate, tropicamide, propantheline, clidinium, dicyclomine, oxybutynin, trihexyphenidyl, and biperiden, calcium- and iron-containing supplements, antihistamines, tricyclic antidepressants, phenothiazines, and antacids.

The children's caregivers were provided with oral explanation and written information concerning the trial before signing the participation consent form. They were all required to sign the form before being recruited in the study. Seventeen out of 57 patients were excluded from study due to suffering from concomitant diseases (2 patients), the presence of abdominal lesions (1 patient), an upcoming medical procedure preventing the patient from remaining in the trial (1 patient), unusual family conditions, and cultural or religious restrictions (13 patients). Accordingly, a total of 40 patients completed the study.

2.3 Study Procedure

The study period was 4 days during which four visits with the investigators were performed.

Parents or caregivers of the children who met the inclusion criteria were asked to assess the bowel patterns of their children and record them in a stool diary for 4 days. Changes in stool frequency, stool consistency, fecal incontinence, abdominal colicky pain, and painful defecation and side effects were assessed during daily visits by investigators.

Patients were assigned randomly to receive an ointment containing 85% w/w olive oil or a comparable placebo, twice daily, for a period of 4 days. All patients were required to discontinue previously administered oral laxatives. In case of no defecation for three consecutive days, polyethylene glycol (PEG) 3350 was allowed at a single dose of 1.5 mg/kg/d until the occurrence of bowel movement. All subjects' caregivers were required to report any side effects during the study period. Randomization was carried out by a member of the study team not involved in patient recruitment using computer-generated random allocation of practices to one of two groups: group A (treatment group) and group B (placebo group). Neither the caregivers of patients nor the investigators were aware of the allocated intervention. Identical bottles and labels were used for both groups.

2.4 Outcome Measures

The primary outcome measure was treatment success, defined as ≥1 spontaneous stools per day, without episodes of fecal impaction at endpoint (day 4). The secondary outcome measure was the frequency of abdominal discomfort or flatulence, painful defecation, and dermal irritations.

2.4.1 Baseline Assessments

Chronic constipation in subjects was diagnosed based on "The Rome III criteria" by a pediatrician. Moreover, the patient's history was carefully examined to rule out diseases such as hypothyroidism, intestinal pseudo-obstruction, cystic fibrosis, neural anomalies, intestinal obstruction, etc. Also, patients receiving constipation-inducing medications, calcium- and iron-containing supplements, antihistamines, tricyclic antidepressants, phenothiazines, and antacids were excluded at the baseline. Demographic information (e.g., gender, age, and weight) was assessed by structured short questionnaires (Table 1).

2.4.2 Follow-Up Assessments

Follow-up research assessments were performed for four consecutive days. Research assessments included physical examination by a pediatrician. Another structured (short) questionnaire is used to collect information on frequency of defecation, from patient's caregivers.

2.4.3 Safety Assessments

During each visit, all patient's caregivers and researchers were requested to report any observed adverse effects related to the treatment. The pediatrician made a decision whether or not to continue the study based on the information disclosed by the patients. For subjects

Table 1 Demographic characteristics of the patients

Variables	Placebo group	Intervention group	P-Value
Age (years)*	2.09 ± 0.7839	2.19 ± 0.8129	0.747**
Weight (Kg)	12.75 ± 2.0995	12.3 ± 1.75019	0.379***
History of laxative consumption (% yes)	80%	80%	1***
Cow milk consumption	10%	15%	0.643***
Formula consumption	60%	65%	0.752***
Breast milk consumption	30%	20%	0.478***
Duration of constipation (months)	16.8 ± 7.1789	21.6 ± 10.0021	0.089**

*Mean ± SD; **Two independent t-test; ***Fisher exact test

who completed the study, the undesirable adverse events including fecal incontinence, abdominal discomfort or flatulence, painful defecation, and dermal irritations were checked.

2.5 Statistical Analyses

Descriptive statistics were calculated for baseline characteristics. All data were statistically analyzed with SPSS software version 24 (SPSS, Chicago, Illinois). The Kolmogorov-Smirnov test was performed to check the normality assumption. Two independent *t*-tests were applied to determine the statistical differences between the means of two groups for continuous variables with a normal distribution. For variables not normally distributed, Mann-Whitney U test was performed to indicate the statistical differences between the medians of two groups. A two-sided *p*-value of <0.05 was considered as statistically significant.

3 Results

3.1 Demographic Characteristics

Forty children completed this randomized, double-blind, and placebo-controlled pilot trial. Patients were randomly assigned to receive intervention medication or placebo. Demographic properties are presented in Table 1.

3.2 Comparing Daily BMs Frequency Between Intervention and Placebo Groups

Comparisons between the medians of daily BMs frequency of intervention and placebo groups at baseline and days 1, 2, 3, and 4 were performed. The results revealed that the medians of daily BMs frequency in intervention and placebo groups were not significantly different at baseline ($p = 0.07$). However, the results were significantly higher in intervention group at days 1, 2, and 3 and at the endpoint compared to those of the placebo group. The results have been shown in Table 2.

3.3 Comparing the Medians of the "Differences Between Daily BMs Frequency in Different Days and Baseline" in Intervention and Placebo Groups

The medians of the differences between "daily BMs frequency at days 1 to 4 compared with baseline" in intervention and placebo groups were measured. The results revealed that the medians of the differences were significantly higher in intervention group compared to the placebo group. They remained significantly unchanged from day 1 to day 4. The results are shown in Table 3.

Table 2 Comparisons between the medians of daily BMs frequency of intervention and placebo groups at baseline and days 1 to 4

	Daily BMs frequency* placebo	Daily BMs frequency intervention	P-value***
Baseline	0.2 (0.0–1.33)**	0.2 (0.0–1.33)	0.656
Day 1	0 (1–0)	1 (0.3–5)	0.0001>
Day 2	0 (1–0)	1 (0.3–5)	0.0001>
Day 3	0 (1–0)	1 (0.3–5)	0.0001>
Day 4	0 (1–0)	1 (0.3–5)	0.0001>

*Median; **Range; ***Mann-Whitney U test P-value

Table 3 The medians of the differences between "daily BMs frequency at days 1 to 4 compared with baseline" in intervention and placebo groups

Differences between days and baseline	Placebo*	Intervention*	P-value***
Day 1 and baseline	−0.171 (−0.0–33.8)**	0.858 (0.2–3.86)**	0.0001>
Day 2 and baseline	−0.2 (−0.0–33.8)	0.858 (0.2–3.86)	0.0001>
Day 3 and baseline	−0.2 (−0.0–33.75)	0.879 (0.2–3.86)	0.0001>
Day 4 and baseline	−0.171 (−0.0–33.8)	0.879 (0.2–3.86)	0.0001>

*Median; **Range; ***Mann-Whitney U test P-value

Table 4 The comparisons between the medians of "differences between daily BMs frequency at baseline and days 1, 2, 3, and 4 in intervention and placebo groups

Medians of differences between days	P-value (placebo)	P-value (intervention)
Days 2 and 1	1	1
Days 3 and 1	0.414	0.276
Days 4 and 1	1	0.276
Days 3 and 2	0.414	0.276
Days 4 and 2	1	0.276
Days 4 and 3	0.414	1

3.4 Comparing the Medians of "Differences Between Daily BMs Frequency in Different Days" in Intervention and Placebo Groups

The comparisons between the medians of "differences between daily BMs frequency at baseline and days 1, 2, 3, and 4 in intervention and placebo groups revealed that the differences were only significant in days 1, 2, 3, and 4 compared to the baseline. The results are shown in Table 4.

3.5 Safety

The patients in both intervention and placebo groups were required to report the presence of any side effects including fecal incontinence, abdominal colicky pain, and painful defecation and dermal irritations following the treatment. However, no adverse effect was reported by patients during the study.

4 Discussion

The results of the present clinical trial demonstrated that abdominal application of an olive oil ointment (85% w/w) can be an effective treatment for 1–4-year-old children with FC. The frequency of BMs significantly increased from 0.2 per day at day 0 to once a day at day 4 ($P < 0.0001$). Children treated with olive oil ointment had a significantly higher defecation frequency than children receiving a placebo at days 1, 2, 3, and 4 of treatment ($P < 0.0001$). There were no clinical adverse events related to intervention or placebo treatment.

The pathophysiology of FC is known to be multifactorial. Withholding behavior, due to experiencing hard, painful, or frightening BMs; changing from breast milk to formula or introducing infants diet; psychosocial factors, such as major life events; behavioral disorders, such as autism and attention deficit/hyperactivity disorder; and parental socioeconomic and educational status can play important roles in FC etiology [15, 16]. It is clinically evident that only 69% of the children with severe FC had recovered within 6 months after initial evaluation, and in 15% of the children, a recurrence was observed within 3 years [16]. Accordingly, looking for easily applicable and safe therapeutic strategies with

long-lasting anti-constipation effects would be of great importance.

Olive oil is considered to be one of the most commonly recommended alternative medicine for the treatment of chronic constipation in Persia, Mediterranean region, and Europe [9, 11].

Hitherto, a few studies have been performed on the efficacy of oral olive oil for the treatment of constipation in adults. A 4-week, double-blind, randomized, controlled trial was conducted to investigate the effects of daily intake of olive oil and flaxseed oil compared with mineral oil for the treatment of constipation in patients undergoing hemodialysis. The results revealed that olive oil or flaxseed oil was as effective as mineral oil in these patients [4]. The results of another randomized, controlled study showed that administration of 60 mL olive oil followed by 2 L of PEG-electrolyte lavage solution increased both patient satisfaction and the quality of right-side colonic cleansing compared to administration of a high volume (4 L) of PEG-electrolyte lavage solution for colonoscopy preparation [9].

It is generally thought that intake of olive oil on an empty stomach enhances the absorption capacity of the small intestine. This leads to the passage of the oil to the colon where it acts as a lubricant. Moreover, olive oil has the ability to coat the intestinal wall and stool mass with an oily film which provides easier passage through the colon and rectum [9]. Oleic acid, a monounsaturated fatty acid which comprises nearly 70% of olive oil, has been shown in an experimental model to possess motor effect on the human colon, which leads to reduction of fluid tolerance, stimulation of high-pressure contractions, and acceleration of colonic transit [17]. Chronic constipation has been shown to be associated to the intestinal dysbiosis, which refers to an imbalance in the composition of the intestinal microbiota [18, 19]. Dysbiosis is related to the proliferation of *Bilophila wadsworthia*, which leads to an inflammatory reaction mediated by Th1 cells. By maintaining a normal intestinal microbiota, olive oil and some other components of Mediterranean diet could prevent dysbiosis and thus alleviate chronic constipation [19].

In Persian medicine, application of olive oil on the abdominal region accompanied by a gentle massage has been recommended for the treatment of pediatric constipation [11–13]. The results of our study supported the abdominal use of olive oil to relieve FC. However, potential mechanisms for the effects of topical use of olive oil are to be studied. Herbal oils phenolics have been reported to enhance distal colonic contractility and motility, ex vivo [20]. It has been evident that oleic acid acts as a selective penetration enhancer for small molecules through mechanisms including lipid fluidization and lipid phase separation [8]. Therefore, oleic acid can possibly increase the transdermal penetration of olive oil phenolics through abdominal layers, which results in the feasibility of delivering significant amounts of phenolics through the skin. Oleuropein glycoside, the main phenolic secoiridoid present in the extra-virgin olive oil, has been shown to inhibit the production of interleukin-1β (an important inflammatory mediator) by human whole blood cultures, by 80% at a concentration of 10^{-4} M [21, 22]. Oleuropein also can inhibit tumor necrosis factor alpha (TNFα)-induced matrix metalloproteinase 9 (MMP-9) in a monocyte cell line. Other phenolic compounds present in olive oil including oleocanthal and hydroxytyrosol have also been reported to possess anti-inflammatory activities with various mechanisms [23]. This elucidates the possible effect of olive oil on inflammatory related chronic constipations.

A limitation of this study is the short follow-up period provided for participants. The short study period was due to the fact that parents tend to discontinue the study when symptoms of constipation had improved. Moreover, as a pilot study, the sample size was small and so replication on a larger scale is warranted. Therefore, more prospective clinical trials with a longer follow-up period and greater study population are

needed to confirm and explain the results of this study.

In conclusion, the present randomized, placebo-controlled, double-blind pilot trial demonstrated the efficiency and safety of abdominal application of olive oil in 1–4-year-old children with FC to improve stool frequency. On the basis of our results, topical use of olive oil can be a natural, easily accessible, safe, and well-tolerated treatment for pediatric functional chronic constipation. The mechanisms for the observed effect need to be studied in the future studies.

Conflict of Interests None.

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Therapeutic Potential of Pomegranate in Metabolic Disorders

Maryam Akaberi, Zahra Boghrati, Amirhossein Sahebkar, and Seyed Ahmad Emami

Abstract

Metabolic syndrome and associated disorders have become one of the major challenging health problems over the last decades. Considerable attention has been paid to natural products and herbal medicines for the management of metabolic disorders in recent years. Many studies have investigated the therapeutic effects of different parts (arils, peels, seeds, and flowers) of pomegranate (*Punica granatum* L.) for the prevention and treatment of this syndrome. This study aims to provide an updated review on the in vitro and in vivo studies as well as clinical trials investigating the effects of pomegranate and its active compounds on different components of metabolic problems such as hyperglycemia, hyperlipidemia, hypertension, as well as obesity over the last two decades. Besides, the key mechanisms by which pomegranate affects these pathogenic conditions are also discussed. The studies show that although pomegranate has promising beneficial effects on diabetes, hypertension, hyperlipidemia, and obesity in various cellular, animal, and clinical models of studies, there are some conflicting results, particularly for hyperglycemic conditions. The main mechanisms include influencing oxidative stress and anti-inflammatory responses. Overall, pomegranate seems to have positive effects on the pathogenic conditions of metabolic syndrome according to the reviewed studies. Although pomegranate is not suggested as the first line of therapy or monotherapy, it could be only used as an adjunctive therapy. Nevertheless, further large and long-term clinical studies are still required.

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Keywords

Punica granatum · Pomegranate · Lythraceae · Punicic acid · Punicalagin · Ellagic acid · Metabolic syndrome

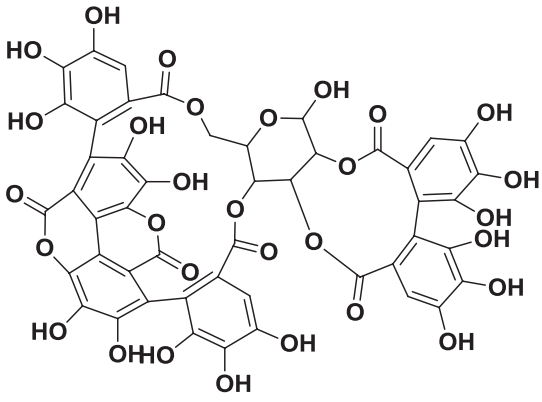
Abbreviations

AGEs	Advanced glycation end products
ALT	Alanine transaminase
AST	Aspartate transaminase
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
CPT-1a	Carnitinepalmitoyltransferase-1a
DBP	Diastolic blood pressure
EA	Ellagic acid
FA	Fatty acid
FBG	Fasting blood glucose
FBI	Fasting blood insulin
GLUT-4	Glucose transporter type
HADMSC	Human adipose-derived mesenchymal stem cells
HbA1c	Glycated hemoglobin
HDL	High-density lipoproteins
HOMA-IR	Homeostatic model assessment of insulin resistance
HT	Hydroxytyrosol
IAUC	Incremental AUC
IFG	Impaired fasting glucose
IL-6	Inteleukine-6
IR	Insulin resistance
ITM	Islamic traditional medicine
LBP	Lipopolysaccharide-binding protein
LDL	Low-density lipoproteins
MDA	Malondialdehyde
MetS	Metabolic syndrome
NF-κB	Nuclear factor κB
OGTT	Oral glucose tolerance test
PAE	Pomegranate aqueous extract
PCG	Punicalagin
PE	Pomegranate extract
PJ	Pomegranate juice
PFE	Pomegranate fruit extract
PLE	Pomegranate leaves extract
PON1	Paraoxonase 1
PPE	Pomegranate peel extract
PSO	Pomegranate seed oil
RCT	Randomized controlled clinical trial
SBP	Systolic blood pressure
SIRT1	Sirtuin1 protein
SREBP-1c	Sterol regulatory element binding protein-1c
STZ-NA	Streptozotocin-nicotinamide
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TNF-α	Tumor necrosis factor alpha
VLDL-C	Very low-density lipoproteins cholesterol
WC	Waist circumference
WHO	World Health Organization

1 Introduction

Metabolic syndrome (MetS) is a constellation of several cardiometabolic risk factors including central obesity, low high-density lipoproteins (HDL), high triglyceride (TG) content, high blood pressure (BP), and high fasting blood glucose (FBG) levels. Most of the time, MetS is associated with obesity. An individual having a BMI of over 25 is considered overweight and over 30 obese according to WHO definition. Obesity and the resulting disorders like type 2 diabetes mellitus (T2DM), atherosclerosis, and cardiovascular diseases are growing problems all over the world. Over the last four decades, rates of overweight and obese people have raised more than fourfold globally; not only this increase is in adults but also in children. Developing countries constitute the majority of overweight or obese children [1]. Compared to obesity, there is no similar global data on MetS which is harder to measure. However, the global prevalence can be estimated to be over a billion people in the world [2].

Growing a sedentary lifestyle is supposed to be a major cause for increased prevalence rate of MetS. Exercise and a healthy diet are two key preventive factors against metabolic disorders. Physical activity helps to expend energy and make it balanced. Moreover, in recent years, studies have revealed that the Mediterranean diet which is mostly based on using different vegetables, fruits, herbs, and spices has benefi-



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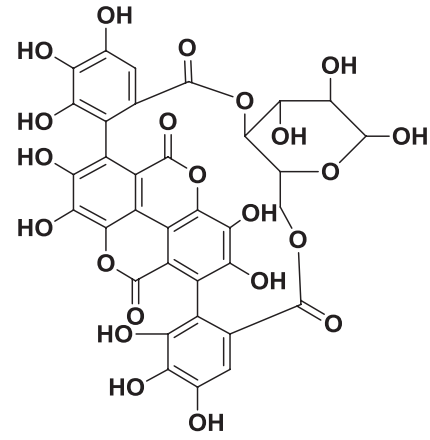

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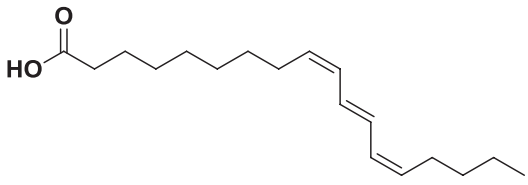

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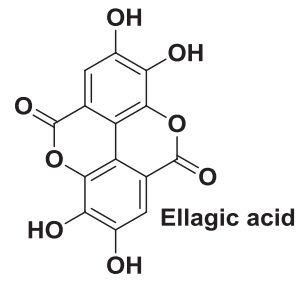

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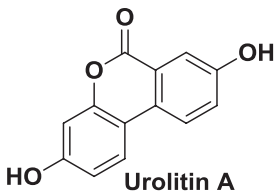
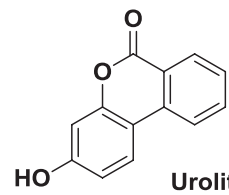


Urolitin A



Urolitin B

Fig. 1 Some of the main bioactive components of pomegranate against metabolic disorders. Urolithin A and B are the metabolites of ellagic acid produced in the colon

cial effects in preventing obesity, diabetes, and MetS [3]. Several herbs and natural products such as olive oil, *Capsicum* spp., turmeric, cinnamon, rosemary, and plants containing polyphenols are useful for the management of MetS [4–6]. For instance, soy isoflavones, citrus secondary metabolites like hesperidin, and quercetin can improve lipid metabolism, and administration of cocoa is reported to regulate blood glucose and high BP. Green tea is also a type of tea rich in polyphenols that can significantly reduce BMI and WC and improve lipid metabolism [4].

Pomegranate (*Punica granatum* L., Lythraceae) is originally from the regions including Iran to northern India. In these countries, pomegranate and its products, like pomegranate juice (PJ), paste, and seeds (known as Nardoon in Persian), are a part of people's diet. With one million metric tons of output, Iran is the largest producer of this valuable fruit in the world. Pomegranate is cultivated almost in all cities of the country. Out of 31 important pomegranate cultivars/varieties in the world, 26 are from Iran [7]. Pomegranate has a long reputation in the traditional medical system of ancient Persia. In Iranian traditional medicine,

Table 1 Antidiabetic activities of pomegranate

	Plant part	Extract/component	Study design	Effects	References
Clinical	Aril	PJ	A randomized, controlled, triple-blinded, parallel trial; patients with polycystic ovary syndrome; aged 30.04 ± 6.39 years old; 2 L; weekly; 8 weeks	↑ Insulin sensitivity	[35]
			RCT; 1.5 ml/kg of the body weight; 28 patients (10 males, 18 females) with impaired fasting glucose (IFG); aged 28–59 years old	↑ Insulin level, ↓ IR, ↓ Serum glucose ($P < 0.0001$)	[36]
			A clinical trial; 1.5 mL kg/b.w. orally; 59 patients with T2DM (25 males and 34 females); aged 37–60 years old	↓ The level of fasting serum erythropoietin ($P = 0.0087$)	[37]
			RCT; 50 patients with T2DM; 250 mL/day; aged 55 ± 6.7 years old; 12 weeks; n = 25 in each group	Anti-inflammatory effect through increasing Sirtuin1 protein (SIRT1) in peripheral blood mononuclear cell ($P = 0.001$)	[38]
			A crossover RCT; healthy individuals; aged 18–75 years old; n = 16	↓ Glucose incremental AUC (IAUC) ($P = 0.000005$) ↓ Glucose concentration ($P = 0.0004$)	[39]
			A single-blind RCT; 60 patients with T2DM; 40–65 years old; 200 ml of PJ daily; 6 weeks	↓ Oxidative stress via reducing serum oxidized LDL ($P < 0.05$) and anti-oxidized LDL ($P < 0.05$) ↓ Fasting plasma glucose ($P < 0.05$)	[40]
			A double-blind RCT; 50 patients with T2DM; 40–65 years old; 250 mL/day PJ; 12 weeks	↓ Plasma C-reactive protein ↓ Interlukin-6 ($P < 0.05$) No effect on FPG or HOMA-IR	[41]
			RCT; 85 patients with T2DM; 1.5 mL of PJ/kg b.w.; aged 37–60 years old; short-term effects on diabetic variables: 3 h after administration	↓ IR ↑ β-cell function ↓ FBS ($P < 0.05$)	[42]
			A quasi-experiment trial; 40 patients with T2DM; 50 g of PJ daily; 4 weeks	↓ Serum IL-6 ($P < 0.05$) ↑ Serum TAC ↑ Plasma antioxidant levels No significant effect on FBS	[43]
			A quasi-experimental interventional study; 50 patients with T2DM; mean age 45 ± 8 years old; 200 ml of PJ daily; 6 weeks	↓ FBS ↓ Total cholesterol ↓ LDL-C ↓ MDA ($P < 0.001$) ↑ PON1 and its arylesterase activity ($P < 0.001$)	[44]

	Peel	70% ethanol extract (PoPEx) containing PCG, punicalin, EA, and gallic acid	A double-blind RCT; 37 patients with T2DM; aged 40–65 years old; BMI ≥ 25 kg/m^2; HbA1C ≥ 6.5%; capsules twice a day; complemented with metformin; 8 weeks	↓ HbA1c ($P < 0.05$)	[45]
	Seed	PSO	RCT; 52 obese T2DM patients; 3 capsules/day containing 1 g PSO	↑ GLUT-4 gene expression ↓ FBS No side effects	[12]
			RCT; 52 obese T2DM patients; 3 g PSO daily in soft gel capsules; 8 weeks	↓ FBS ($P = 0.008$) ↓ IL-6 ($P = 0.049$) ↓ TNF-α	[13]
			A double-blind RCT; 80 patients with T2DM; 1000 mg PSO twice daily (2000 mg PSO); 8 weeks	No effect on FBS and IR	[46]
In vivo	Aril	PJ and PCG	Streptozotocin-nicotinamide (STZ-NA) T2DM rats; PJ: 100 or 300 mg/kg/b.w., PCG: 2.6 or 7.8 mg/kg/b.w.; orally/daily; 6 weeks	PJ: ↓ IR ↓ Serum TNF-α concentration ↓ Hepatic c-Jun N-terminal kinase expression ↑ Hepatic insulin receptor substrate-1 expression ($P < 0.001$) ↓ Oxidative liver injury No side effects PCG: No significant antidiabetic activity Many side effects	[47]
		Extract	STZ-induced diabetic rabbits; 100 mg/kg; orally; 21 days	↓ Serum glucose thrombospondin-1 ↓ Nitric oxide ↓ Alanine aminotransferase ↓ Aspartate aminotransferase ↓ Lactate dehydrogenase alkaline phosphatase ↓ C-reactive protein	[48]
		PAE	Alloxan-diabetic male Wistar rats; 100, 200, and 350 mg/kg b.w.; oral glucose tolerance test (OGTT), short-term and long-term PAE consumption periods models	↓ FBS, ↑ mRNAs expression levels of insulin receptor substrate 1 (IRS-1), Akt, GLUT-2, and GLUT-4	[49]

(continued)

Table 1 (continued)

	Plant part	Extract/component	Study design	Effects	References
		PAE	STZ-induced diabetic mice; 150 and 300 mg/kg b.w./day; positive control: libitum; 21 days	↓ Blood glucose level ($P < 0.05$) ↓ AST and ALT enzyme	[50]
		PJ	STZ-NAD-induced T2DM Sprague-Dawley rats; 1 ml; orally; daily; 21 days	↓ Plasma glucose concentration No impact on plasma insulin	[51]
		Extract	Mice; antiglycation assays	↓ Glycation products such as glycoalbumin, hemoglobin A1c, and serum AGEs (advanced glycation end products) ($P < 0.05$)	[52]
		EA	Wistar rats; 0.8 g/kg; 8 weeks	↑ Glucose tolerance	[53]
		PAE	Alloxan-diabetic male Wistar rats; 100, 200, or 350 mg/kg b.w.; 21 days	↓ FBG ($P < 0.001$) ↓ ROS generation ($P < 0.001$) ↑ Insulin level ($P < 0.001$)	[54]
	Flower	Powder	Sprague-Dawley rats; 5–5000 mg/kg; positive control: metformin	LD_{50}: 100 and 200 mg/kg	[55]
		Aqueous-ethanolic extract (50%, v/v)	Alloxan-induced diabetic rats; 400 mg/kg, b.w.; orally	↓ Blood glucose	[56]
	Leaves	Ethyl acetate fraction	Rats; STZ-induced diabetic rats; 50, 100, and 200 mg/kg; oral gavage; positive control: glibenclamide (5 mg/kg); 28 days	↓ FBS ($P < 0.01$) ↑ Liver antioxidant status No significant effect on serum insulin	[57]
	Peel	PPE	T2DM male albino Wistar rats; 50, 100, and 200 mg/kg b.w./day PPE; 14 days	↓ FBS	[58]
		PCG	T2DM mice; 5 weeks	↓ IR, ↑ Insulin sensitivity ↓ Serum-free FAs levels and hepatic steatosis	[59]
		Phenolic-rich hydro-methanol PJ	Wistar albino rats; positive controls: glibenclamide and atorvastatin; 200 mg/kg PJ daily; orally; 56 days	↓ FBS ($P \leq 0.001$) ↓ HbA1c levels	[60]
		Calcitriol and/or PPE	Sprague-Dawley female rats	↓ Troponin I, endothelin-1, creatine kinase-MB, lactate dehydrogenase levels ↑ Interleukin-1, interleukin-1β, transforming growth factor β, and oxidative redox ↑ Activation of Raf/MEK/ERK signaling and mitigation of apoptotic pathways	[61]
		Extract	STZ-induced diabetic male guinea pigs	↓ Fasting serum glucose	[62]
		PCG	Mice; 20 mg/b.w./day via gavage; 4 weeks	↑ Autophagy via the Akt/FoxO3a signaling pathway ↓ FBS and HOMA-IR	[63]
		Ethanol fraction	STZ-induced diabetic rats; 100 and 200 mg/kg b.w./day	↓ Blood glucose levels and glycated Hb ↑ Increase TAC and GSH ↓ MDA, TNF-α, and IL-6 levels	[64]

	Plant part	Extract/component	Study design	Effects	References
	Seed	Punicic acid	_	↓ Inflammatory cytokines ↑ Glucose homeostasis ↑ Antioxidant properties	[11]
In vitro	Aril	PJ, ellagitannin-rich fraction, PCG (A), punicalin (B), and EA (C)	α-Glucosidase inhibitory assay	IC_{50} of A, B, and C: 140.2, 191.4, and 380.9 μmol/L, respectively	[65]
		PJ, main polyphenols EA and PCG, and the main gastrointestinal metabolite urolithin A (URO-A)	α-Glucosidase inhibitory bioassay; positive control: acarbose; PJ: 0, 50, and 100 μg/mL, EA: 0, 10, 50 μM/mL, PCG: 0, 10, and 20 μM/mL	IC_{50}: 0.0055, 0.015, 0.025, 0.38, and 1.01 mg/mL for PCG, URO-A, EA, acarbose, and PJ, respectively	[21]
	Flower	Ethanol extract	α-Glucosidase activity assay; positive control: galanthamine (3.74 ± 0.28 μg/mL); 250 μg/ml	α-Glucosidase activity with IC_{50} 29.77 ± 1.50 μg/mL	[66]
		Phenolic-rich fractions including fractions A, B, and C + D, tricetin, tricetin 4′-O-β-glucopyranoside	α-Glucosidase and α-amylase inhibition assays; positive control: acarbose	IC_{50}s in α-glucosidase inhibitory assay: tricetin (2.37 mg/ml), fraction A (0.80 mg/ml), fraction B (0.44 mg/ml), fraction C + D (1.46 mg/ml), acarbose (3.96 mg/ml) IC_{50}s in α-amylase inhibitory assay: tricetin (0.43 mg/ml), tricetin 4′-O-β-glucopyranoside (1.17 mg/ml), acarbose (0.038 mg/ml)	[67]
	Fruit	Extract, phenolic constituents PCG, EA, and gallic acid, and their colonic metabolites urolithin A and urolithin B	Anti-glycation assays; positive control: aminoguanidine	↓ The formation of advanced glycation end products (AGEs)	[68]
	Peel	Polyphenol-rich extract	α-Glucosidase inhibition and glucose uptake assays; L6 rat skeletal muscle cells	Inhibit α-glucosidase activity; IC_{50} 0.0453 μg/ml ↑ Glucose uptake	[69]
	Seed	Punicic acid	3 T3-L1 adipocytes; 5, 10, and 30 mM; positive control: rosiglitazone	↑ GLUT4 expression ↑ Adiponectin secretion	[70]
			3 T3-L1 adipocytes	Protects 3 T3-L1 adipocytes from TNF-α-induced IR Maintains mitochondrial transmembrane potential Ameliorates imbalance in mitochondrial dynamics in IR ↑ ATP production in IR	[71]

almost all parts of the plant have been applied to cure respiratory, digestive, cardiovascular, and metabolic problems, among others. Polyphenolic compounds including anthocyanins, flavonoids, catechins, organic acids, tannins, and their colonic metabolites such as urolithin A with antioxidant and anti-inflammatory activities constitute the bioactive components of pomegranate (Fig. 1) [8].

This study summarizes the potential therapeutic effects of pomegranate and its active constituents on the individual components of the MetS including high blood glucose content, high BP, insulin resistance (IR), hyperlipidemia, and high body mass and discusses the potential underlying mechanisms.

2 Pomegranate and MetS Components

Pomegranate juice (PJ) has been vastly studied against MetS. However, many studies have reported the effects of various preparations from other pomegranate parts such as leaves, peel, flowers, and seeds as well as some pure compounds punicalagin (PCG), ellagic acid (EA), and punicic acid [9, 10].

2.1 Diabetes

According to Table 1, different in vitro and animal models of study have revealed the potential antihyperglycemic activities for different pomegranate parts. PJ has been found to be able to regulate glucose and insulin levels, influence the hepatic enzymes, and stimulate anti-oxidative and inflammatory responses. Several randomized control trials (RCTs) have been designed to evaluate the effects of PJ consumption in patients with T2DM. The trials have revealed that PJ could increase insulin sensitivity, total antioxidant capacity (TAC), and plasma antioxidant levels and decrease IR, serum glucose, interleukin-6, plasma C-reactive protein, and malondialdehyde (MDA) that all imply anti-inflammatory and antioxidant activities of pomegranate. However, a few reports have studied the effects of other parts of the plant like seeds and peels on the pathogenic conditions of T2DM. Interestingly, very promising results have been observed by pomegranate peel administration. According to the data from some clinical trials (Table 1), seed and peel of pomegranate may have also beneficial effects on the FBG levels. Punicic acid, as one the major components of pomegranate seed extract, has shown promising therapeutic potential against hyperglycemia. Modulating glucose homeostasis, reducing inflammatory cytokines, and increasing antioxidant properties are among the most important mechanisms of this acid against diabetes [11]. In a RCT, administration of the oil obtained from pomegranate seeds (PSO) could increase GLUT-4 (glucose transporter type 4) gene expression and improve hyperglycemia in patients suffering from T2DM [12]. The data from another RCT have shown that the PSO administration in obese people with T2DM could reduce the FBS levels and improve inflammatory conditions by decreasing tumor necrosis factor-α (TNF-α) and interleukin-6 [13]. As abovementioned, inhibiting oxidative stress and reducing lipid peroxidation are important mechanisms for antidiabetic activity of pomegranate. Increasing the activities of antioxidant enzymes, like metal chelation activity, decreasing reactive oxygen species, inhibiting or activating PPAR-γ and nuclear factor κB (NF-κB) transcriptional factors, and reducing resistin formation are among the main targets of pomegranate [14].

Although most of the studies have reported the beneficial effects of pomegranate in controlling T2DM, the results of a few trials are conflicting. For instance, in a meta-analysis, 16 eligible trials investigating the efficacy of pomegranate supplementation for the management of glucose were reviewed systematically [15]. The results revealed that although pomegranate did not affect FBG, FBI, or HbA1c significantly, it caused significant heterogeneity in FBI and HOMA-IR [15]. A meta-analysis of 12 RCTs, studying the possible effects of PJ on insulin sensitivity and glucose control in adults, has shown that PJ did not affect significantly on FBG and insulin concentrations [16]. Further,

Table 2 Anti-obesity activities of pomegranate

	Plant part	Extract/component	Study design	Mechanism	Effects	References
Clinical	Aril	PJ	A double-blind RCT; 20 obese subjects; 120 ml of PJ; 1 month; n = 10	BMI, weight, and fat mass did not increase in the treated group compared to the placebo	Anti-obesity effects	[72]
	Fruit	PE	A crossover RCT; 49 overweight-obese subjects (BMI > 27 kg m^{-2}) with mild hyperlipidemia; daily; 3 weeks; 3-week washout periods	↓ Plasma lipopolysaccharide-binding protein (LBP) ($P < 0.05$) ↓ High-sensitivity C-reactive protein ($P = 0.054$)	Decreases endotoxemia by reshaping the gut microbiota, mainly through the modulation of *Faecalibacterium*, *Odoribacter*, and *Parvimonas*	[73]
In vivo	Aril	PJ	HFD-induced obese rats; 0.15% PJ (wt/vol); 4 weeks	↑ Hepatic mRNA expression of hormone-sensitive lipase, pyruvate kinase, and adiponectin ↓ Ghrelin mRNA expression ↓ Number and size of lipid droplets in hepatocytes	Regulates obesity markers	[74]
	Fruit	PFE	Male Zucker diabetic fatty (ZDF) rats; 500 mg/kg, p.o.; 6 weeks	↓ Ratio of liver weight ↓ Hepatic triglyceride contents ↓ Lipid droplets ↑ Hepatic gene expression of PPAR-α ↑ Carnitine palmitoyltransferase-1 and acyl-CoA oxidase (ACO) ↓ Stearoyl-CoA desaturase-1	Ameliorates obesity-associated fatty liver by activating hepatic expression of genes responsible for FA oxidation	[75]
		PFE	Ovariectomized mice; 30 mg/kg b.w./day; 12 weeks	↓ Serum resistin levels ↑ Resistin mRNA expression in white adipose tissue ↓ Secretion and intracellular protein levels of resistin in differentiated murine 3 T3-L1 adipocytes	Suppresses resistin secretion by a novel mechanism involving the degradation of intracellular resistin protein in adipocytes	[76]

(continued)

Table 2 (continued)

	Plant part	Extract/component	Study design	Mechanism	Effects	References
		Pomegranate vinegar	High-fat diet (HF)-induced obese rats; 0, 6.5, or 13% w/w; 16 weeks	Adipose tissue: ↑ Phosphorylation of AMP-activated protein kinase (AMPK) ↑ Hormone-sensitive lipase and mitochondrial uncoupling protein 2 ↓ Sterol regulatory element binding protein-1c (SREBP-1c) ↓ PPARγ in adipose tissue Liver tissue: ↑ PPAR ↑ Carnitinepalmitoyltransferase-1a (CPT-1a) ↓ SREBP-1c	Attenuates adiposity through coordinated control of AMPK signaling in the liver and adipose tissue	[77]
	Leaves	Pomegranate leaf extract (PLE)	High-fat diet mice; 400 or 800 mg/kg/day of PLE; 5 weeks	↓ Body weight ↓ Energy intake ↓ Various adipose pad weight percent ↓ TC, TG, glucose levels, and TC/HDL-C ratio ↓ Intestinal fat absorption	Inhibits obesity development	[78]
		Ethanol extract	High-fat diet-induced mice; 50 mg/kg and 100 mg/kg b.w.	↓ Body weight ↓ Faces index ↓ Total fat index ↓ Food index ↓ Lee's index	Anti-obesity effects	[79]
	Seed	PSO, Punicic acid	High-fat diet mice; 1% PSO; 12 weeks	↓ Body weight ($P = 0.02$) ↓ Body fat mass ($P = 0.02$)	Ameliorates high-fat diet-induced obesity	[80]
	_	PCG	High-fat diet (HFD)-fed mice; PCG in 2% ethanol; oral gavage five times a week	↓ Lipid accumulation in adipocytes ↓ Adipocyte-induced inflammatory responses via Nrf2/Keap1 pathway ↓ Body and white adipose tissue weights Regulates pro- and anti-inflammatory cytokines ↓ Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)	Ameliorates obesity and obesity-induced inflammatory responses via activation of Nrf2/Keap1 signaling	[19]

	Plant part	Extract/component	Study design	Mechanism	Effects	References
In vitro	Seed	PSO, SHAMstat3pg (a fatty acid composite extracted from PSO)	HADMSC; 10 μg/ml of SHAMstat3pg (24 h)	Regulate the mRNA expression of the obesity-associated gene transcripts Regulate the expression of the obesity-linked proteins and genes in HADMSC	Inhibits the differentiation of preadipocytes to adipocytes	[18]
	–	PPE, PCG, EA	3 T3-L1 mouse adipocytes	↓ FA synthase (FAS): IC_{50}, 4.1 μg/ml (PPE), 4.2 μg/ml (4.50 μM, PCG), and 1.31 μg/ml (4.34 μM, EA) ↓ Lipid accumulation inside FAS overexpressed 3 T3-L1 adipocytes	Anti-obesity effects	[81]

RCTs in large scales with longer duration are necessary to corroborate the efficacy of pomegranate supplementation in the management of diabetes.

2.2 Obesity

The prevalence of cardiovascular diseases and MetS would be increased in overweight and obese individuals. Diet intervention is one of the main strategies for weight maintenance and weight loss. Studies have shown that PSO can help in controlling diet-induced obesity [17]. SHAMstat3pg, a FA composite extracted from PSO containing three dietary FAs punicic acid, linoleic acid, and oleic acid, could inhibit HADMSC (human adipose-derived mesenchymal stem cells) adipogenesis, reduce glucose uptake, attenuate ATP production, and ameliorate inflammation. It could also favorably regulate the mRNA expression of gene transcripts associated with obesity [18]. Another bioactive component from pomegranate is PCG that is able to suppress obesity and the resulting inflammatory responses through different signaling pathways including the Nrf2/Keap1 [19]. Consumption of ellagitannin-containing foods such as pomegranate produces some microbial metabolites known as urolithins having a great impact on adipogenesis and lipid accumulation [20–22]. Urolithin A is one of the most important urolithins produced in the gut after pomegranate consumption.

Similarly, the main mechanisms for anti-obesity activity of pomegranate are decreasing oxidative and inflammatory responses. In a double-blind RCT studying the effects of pomegranate extract (PE) on 48 obese and overweight participants, administration of 100 mg PE daily for 30 days significantly decreased mean serum levels of plasma MDA, IL, and hs-CRP [23]. Downregulating the genes and proteins associated with obesity, decreasing adipogenesis, and lipid accumulation are among other anti-obesity mechanisms of pomegranate products (Table 2).

2.3 Cardiovascular Protective Activities

Evidence to date reveals the beneficial effects of pomegranate products in a heart-healthy diet [24]. In fact, obesity, high blood glucose levels, hypertension, and dyslipidemia are all risk factors for cardiovascular problems, but we have discussed these pathogenic conditions in separate paragraphs to have a better overview of the conditions. In the above paragraphs, the effects of pomegranate products on obesity and diabetes were reviewed. In the following paragraphs, the benefits of this valuable fruit for the management of high BP and dyslipidemia are presented. Studies to date emphasize that pomegranate and its active constituents can be applied as dietary supplements for the treatment of cardiovascular diseases, such as hypertension, dyslipidemia, and peripheral and coronary artery disease. In fact, pomegranate is reported to have cardiovascular protective activity via decreasing platelet aggregation and oxidative stress, diminishing lipid uptake, regulation of BP, and positively influencing endothelial cell function [25]. The therapeutic potential of pomegranate for cardiovascular disorders has been revealed by several in vitro, in vivo, and clinical studies (Tables 3 and 4). For instance, the results from a meta-analysis studying 16 RCTs (572 subjects) reported that PJ supplementation could significantly reduce IL-6, TNF-α, and hs-CRP levels [26]. In an in vivo model, PCG could ameliorate cardiac mitochondrial impairment via AMPK activation in obese rats [27]. In addition, the administration of 500 mg/kg pomegranate fruit extract (PFE) in Zucker diabetic fatty rats could decrease cardiac fibrosis via modulation of NF-κB and cardiac endothelin-1 pathways that interactively regulate fibroblast growth [28].

2.3.1 Hypertension

Daily pomegranate use can help in lowering BP evidenced by different lines of experimental and clinical studies (Table 3) [29]. Pomegranate is reported to improve antioxidant and anti-atherosclerotic activities resulting in an improved cardiovascular health [30]. A meta-analysis of

Table 3 Antihypertensive and cardiovascular protective activities of pomegranate

	Plant part	Extract/component	Study design	Effects	References
Clinical	Aril	PJ	Single-blind RCT; 30 patients with T2DM; aged 54.6 ± 8.4 years old; 200 ml/day PJ; 6 weeks	↓ SBP ($P < 0.001$) ↓ DBP ($P < 0.05$)	[82]
		PJ	A crossover RCT; 41 hemodialysis patients; 100 ml PJ immediately after their dialysis session; three times a week; 8 weeks	↓ SBP and DBP ($P < 0.001$)	[83]
		PJ	Single-blind clinical trial; 21 hypertensive patients; aged 30–67 years old; 150 ml/day in a single occasion between lunch and dinner; n = 11; 2 weeks	↓ SBP ($P = 0.002$) ↓ DBP ($P = 0.038$) ↓ Serum levels of VCAM-1 ($P = 0.008$)	[84]
		PJ	Single-arm study; 13 hypertensive men; aged 39–68 years old; 150 ml/day	↓ SBP ($P = 0.013$) ↓ DBP ($P < 0.010$)	[85]
		PJ	A triple-blinded, parallel RCT; patients with polycystic ovary syndrome; aged 30.04 ± 6.39; 2 L; weekly; 8 weeks	↓ BP	[35]
		PJ	RCT; 220 mL PJ, daily; 100 patients with unstable angina or myocardial infarction; (n = 50, each); 5 days during the hospitalization period	↓ Serum troponin and MDA levels ↓ Intensity, occurrence, and duration of angina pectoris	[86]
		PJ	A double-blinded, crossover RCT; 30 patients with MetS; 500 mL daily; 1 week	↓ SBP ($P = 0.00$) ↓ DBP ($P = 0.00$) ↓ hs-CRP ($P = 0.018$)	[87]
		PJ	A crossover RCT; 28 volunteers at high CVD risk; mean age: 50·4 years old; 500 ml of PJ; 4 weeks	↓ SBP ($P = 0{\cdot}034$) ↓ DBP ($P = 0{\cdot}031$)	[88]
		PJ	RCT; 101 hemodialysis patients; 100 cc PJ (0.7 mM polyphenols); three times a week for 1 year	↓ SBP ↓ DBP	[89]
		Pomegranate ellagitannin-enriched extract	Pilot study; 22 overweight subjects; two capsules daily (1000 mg of extract)	↓ Thiobarbituric acid reactive substances (TBARS) in plasma No toxicity	[90]
	Fruit	Capsules: extract of the whole pomegranate (Pomanox®) containing 210 mg of PCGs, 328 mg of other pomegranate polyphenols (such as flavonoids and EA), and 0·37 mg of anthocyanins	A double-blind RCT; aged 18–65 years old; 8 weeks	↓ DBP ($P < 0{\cdot}05$)	[91]
	Peel	70% ethanol extract	A double-blind RCT; 37 patients with T2DM; 40–65 years old; (BMI) ≥ 25 kg/m^2, HbA1C ≥ 6.5%; capsules twice a day; supplemented with metformin; 8 weeks	↓ SBP ($P < 0.01$) ↓ DBP ($P < 0.05$)	[45]

(continued)

Table 3 (continued)

	Plant part	Extract/component	Study design	Effects	References
	_	PPE	A double-blind, randomized, placebo-controlled pilot study; 38 obese women (30 < BMI >35 kg/m2) with dyslipidemia; 500 mg PPE daily; 8 weeks; n = 19	↓ SBP ↓ hs-CRP	[92]
		PCG and hydroxytyrosol (HT)	A double-blinded crossover RCT; middle-aged healthy adults; 9.9 mg of HT and 195 mg of PCG; 20 weeks	↓ SBP ($P < 0.001$) ↓ DBP ($P < 0.001$) ↑ Endothelial function	[93]
In vivo	Aril	PJ	Wistar rats; 100 and 300 mg/kg; p.o.; 4 weeks	↓ BP	[94]
	Flower	PFE	Zucker diabetic fatty rats; 500 mg/kg, p.o.; 6 weeks	↓ Overexpressed cardiac fibronectin and collagen I and III mRNAs ↓ Upregulated cardiac mRNA expression of ET-1, ETA, inhibitor-κBβ, and c-Jun Normalizes downregulated mRNA expression of inhibitor-κBα Modulation of cardiac endothelin-1 and NF-κB pathways	[28]
	Peel	PPE	Spontaneously hypertensive rats; orally; 30 days by gavage	↓ SBP ↓ Angiotensin-converting enzyme (ACE) coronary activity ↓ Vascular wall areas ↓ Superoxide anion levels	[95]
		PPE containing 40% PCG	Spontaneously hypertensive rats; 150 mg/kg/day; 8 weeks	↓ BP ↓ Cardiac hypertrophy ↑ Mitochondrial function by activating AMPK-Nrf2 in the paraventricular nucleus	[96]
		PPE	Spontaneously hypertensive female rats; 250 mg/kg PPE by gavage; 30 days	↓ SBP	[97]

Table 4 Lipid-lowering activities of pomegranate

	Plant part	Extract/component	Study design	Effects	References
Clinical	Peel	70% ethanol extract (PoPEx)	A double-blind RCT; 37 patients with T2D; 40–65 years old; BMI ≥ 25 kg/m^2, HbA1C ≥ 6.5%; capsules twice a day; complemented with metformin; 8 weeks	Reduces triglyceride plasma levels ($p < 0.01$), LDL-C/HDL-C ratio ($p < 0.001$) Increases the level of HDL-C ($p < 0.001$) Improves the plasma FAs content ($p < 0.05$ and $p < 0.01$)	[45]
	Aril	PJ	A randomized crossover trial; 41 hemodialysis patients; 100 ml PJ immediately after their dialysis session; three times a week; 8 weeks	↓ Triglycerides ($P < 0.001$) ↑ HDL-cholesterol ↑ TAC ($P < 0.001$)	[83]
	–	PCG and hydroxytyrosol (HT)	A randomized, double-blinded, placebo-controlled, crossover trial; middle-aged healthy adults; 9.9 mg of HT and 195 mg of PCG; 20 weeks	↓ oxLDL ($P < 0.05$)	[93]
	Peel	PPE	A double-blind, randomized, placebo-controlled pilot study; 38 obese women (30 < BMI >35 kg/m2) with dyslipidemia; 500 mg PPE; daily; 8 weeks; n = 19	↓ Serum TC ($P = 0.014$) ↓ LDL-C ($P = 0.021$) ↓ TG ($P = 0.036$) ↑ HDL-C ($P = 0.020$)	[92]
	Aril	PJ	RCT; 101 hemodialysis patients; 100 cc PJ (0.7 mM polyphenols); three times a week; 1 year	↓ Triglycerides ↓ HDL level	[89]
In vivo	Fruit	Aqueous extract	In vivo, STZ-induced diabetic mice; 150 and 300 mg/kg body weight (b.w.)/day; positive control: libitum; 21 days	↓ Total cholesterol levels ($p < 0.05$) ↓ Triglycerides ($P < 0.05$) ↓ LDL-cholesterol ($P < 0.05$) ↑ HDL-cholesterol ($P < 0.05$)	[50]
	Aril	PJ	In vivo; STZ-nicotinamide (NAD)-induced T2DM Sprague-Dawley (SD) rats; 1 ml; orally; daily; 21 days	↓ Plasma TC ↓ Triglyceride	[51]
			A double-blinded, crossover RCT; 30 patients with MetS; 500 mL daily; 1 week	↑ Very low-density lipoprotein cholesterol (VLDL-C) ($P = 0.014$)	[87]
			Mice fed a high-fat diet; 300 µL of PJ (0.35 mmol total polyphenols); oral gavage; daily; 5 months	↑ PON1 expression and activity	[34]
	Peel	PPE	Spontaneously hypertensive female rats; 250 mg/kg PE by gavage; 30 days	↓ TC ↓ LDL	[97]
In vitro	Aril	PJ and main polyphenols (EA and PCG and the main gastrointestinal metabolite (urolithin-A (0, 10, and 50 µM/mL)	In vitro, lipase inhibition bioassay; positive control, orlistat; PJ, 0, 50, and 100 µg/ml; EA, 0, 10, and 50 µM/mL; PUN, 0, 10, and 20 µM/ml	IC_{50} values were 0.00074, 0.032, 0.092, 0.16, and 2.50 mg/mL for orlistat, urolithin-A, EA, PCG, and PJ, respectively	[21]

eight RCTs has supported the regulatory effects of PJ in BP [31]. This analysis concluded that PJ supplementation could significantly reduce both systolic BP (SBP) and diastolic BP (DBP) regardless of the duration and dose consumed [31].

2.3.2 Dyslipidemia

In 2020, Aziz et al. have investigated the effects of PJ on lipid profiles in a systematic review meta-analyzing 17 clinical trials [32]. Overall, according to the reviewed studies, they concluded that pomegranate did not decrease serum lipid levels significantly. However, they emphasized on the low and inconsistent quality of evidence in the included trials and suggested more comprehensive and precise clinical trials [32]. Nevertheless, it was found in a previous systematic review studying 12 RCTs (545 participants) that while the administration of pomegranate did not affect on HDL-C and total cholesterol significantly, it could decrease triglyceride levels significantly [33].

There are many studies investigating the effects of pomegranate on the lipid profile regulation, and several mechanisms are proposed (Table 4). For example, studies have found that the administration of PJ can increase the binding of HDL to PON1 (paraoxonase 1), an antioxidant arylesterase in association with HDL, and thus enhance PON1 catalytic activity [34].

3 Conclusion

Pomegranate is a plant with a long reputation of use from ancient times to the present. Pomegranate is considered a medicinal plant in different traditional systems of medicine like Iranian traditional medicine (ITM), traditional Chinese medicine, and Ayurveda. The therapeutic potential of this valuable plant for the prevention and treatment of MetS is mentioned in many textbooks of ITM. On the other hand, MetS has been a growing health challenge in the last recent decades in both developing and developed countries. The present review paper shows that pomegranate is effective in the management of MetS and can be used as a supplementary or adjuvant therapy for controlling different components of this disorder. However, because of a few contrasting data in some studies, further research and clinical trials with more sample sizes are necessary to have a better insight.

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Conflict of Interests None.

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Resveratrol as a Probable Multiheaded Treatment Approach for COVID-19

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Abstract

The COVID-19 pandemic has plagued the world for more than 1 year now and has resulted in over 77 million cases and 1.7 million related deaths. While we await the rollout of the vaccines, new treatments are urgently needed to reduce the effects of this devastating virus. Here, we describe a number of preclinical studies which show promising effects of the polyphenol resveratrol.

Keywords

Resveratrol · COVID-19 · Herbal medicine

1 Introduction

SARS-CoV-2, the causative agent of COVID-19 disease, represents one of the leading causes of morbidity and mortality globally and is still spreading rapidly, even 1 year after its first identification in Wuhan, China. As there is no well-established drug therapy, testing of both existing and new compounds to combat this terrible disease has received considerable attention across the world [1]. One such approach is the use of natural products. Medicinal plants and their bioactive ingredients have been used comprehensively to treat human disorders and infections for thousands of years. Some phytochemicals and medicinal plants have been suggested to possess promising effects against COVID-19 disease [2]. Resveratrol (RSV), a senolytic phytoalexin clas-

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sified as a stilbenoid, can be produced by some species of plants after fungal or bacterial infections. Grape and peanut are major sources of RSV [3]. RSV has been shown to have diverse biological activities, which may provide a new means in our struggle with COVID-19 [4]. Herein, we provide an overview on the potential therapeutic effects of RSV against COVID-19 as well as the underlying mechanisms that could explain such protective effects.

2 Therapeutic Mechanisms Underlying the Efficacy of RSV in COVID-19

Evidence suggests that RSV could be beneficial in four contributory aspects of COVID-19 viral challenge: (1) protection of lung tissue as the most vulnerable part of the body in SARS-CoV-2 infection, (2) the host immune system response, (3) the coronavirus infectivity cycle, and (4) the ensuing effects of infection on the host.

Treatment with RSV was found to attenuate airflow limitations in several experimental models of respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD), and it has been investigated extensively in various lung injury conditions [5]. In an animal model of asthma, RSV was shown to reduce mucosal hypersecretion and eosinophilia along with anti-inflammatory effects [6]. Numerous mechanistic reports stated that after SARS-CoV-2 infection and creation of high levels of reactive oxygen species (ROS), extreme inflammation can cause considerable injuries to bronchial epithelial and endothelial cells [7]. In this manner, beneficial effects of RSV were found to be mediated by its antiapoptotic, anti-inflammatory, and antioxidant properties in alveolar spaces [5, 8].

One of the procedures for COVID-19 patients is to provide respiratory support by mechanical ventilation, especially in severe cases. High tidal volume mechanical ventilation by itself is a trigger for inflammation and cytokine release and can cause serious damages to both patients with and without lung disorders [9]. In vivo and in vitro studies carried out by Dong et al. demonstrated that RSV mitigated mitochondrial oxidative damage induced by an increase in the architectural chromatin-binding factor high-mobility group box 1 (HMGB1) induced by high tidal volume mechanical ventilation [10]. In another study on mechanically ventilated mice, despite no change observed in interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α levels by RSV, nuclear factor (NF)-κB DNA-binding activity was inhibited by pretreatment with this polyphenol [11]. Hypoxia-reoxygenation is another pulmonary-related disorder with the major contributing factors of oxidative stress and inflammation. Pretreatment of type II alveolar epithelial cell line with RSV led to reduction in IL-1β and IL-6 levels. In addition, amelioration of the anti-inflammatory factor IL-10 and surfactant proteins was also observed [12].

Viral infection at early stages may spontaneously be halted by the host immune response. Conversely, inappropriate response of the immune system can cause some deleterious and potentially long-term sequelae on different tissues. Due to this, evaluation of the patient's condition is vitally important to ensure the right decisions are made for amplification or attenuation of the immune system. An extreme immune response and its subsequent hyper-inflammation are called the "cytokine storm," phenomena which is an important factor in COVID-19 pathogenesis and severity [7]. Previous studies have shown elevated concentrations of pro-inflammatory cytokines like IL-6, TNF-α, IL-1β, monocyte chemoattractant protein 1 (MCP-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients infected by SARS-CoV-2 [13]. This oversecretion of pro-inflammatory elements from type II pneumocytes, activated alveolar macrophages, infiltrated neutrophils, and T cells in a destructive cycle can cause acute respiratory distress syndrome (ARDS) and multi-organ failure [7]. In this light, the protective effects of RSV in disorders caused by autoimmune or inflammatory procedures are likely to be due to its anti-inflammatory properties [3]. Numerous in vivo and in vitro investigations have shown anti-inflammatory properties of

RSV to decrease several pro-inflammatory cytokines and modulate related intracellular signaling pathways [3, 14].

Activated neutrophils by neutrophil extracellular trap (NET) formation in this inflamed environment are one of the main factors in alveolar capillary permeation [15]. In an isolated neutrophil model, RSV attenuated neutrophil activities in a dose-dependent manner by inhibiting phosphorylation of Src family kinases [16]. Furthermore, Vargas et al. hypothesized that treatment via RSV could cleave DNA in NETs and improve cell survival [15].

A considerable decrease of the increased IL-1β and IL-18 was confirmed by RSV treatment due to its NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome-attenuating activities [17]. In a cellular context, NLRP3 inflammasome inactivation causes an inhibitory effect on neutrophil infiltration and ARDS progression [7, 17].

The progressive cycle of cytokine and ROS production to activate cells which can generate more ROS and release further chemokines finally ends in uncontrollable systemic inflammation [7]. Cytokine gene expression is predominantly regulated by the NF-κB pathway [5]. Thus, suppressing SARS-CoV-2-induced NF-κB signaling would be a potential strategy to counteract the associated destructive effects. Convergent evidence from cellular and animal experiments suggest that RSV could act as an agent for NF-κB inhibition and modulation [3, 18].

Two of the main pathways for cytokine release after lipopolysaccharide (LPS)-induced lung injury are NF-κB and mitogen-activated protein (MAPK) signaling, although corticosteroid treatment effects are restricted to the NF-κB pathway. On the other hand, evidence has shown that RSV modulates both of these signal transduction pathways [19]. Furthermore, Bhakti et al. [20] showed that RSV can reinforce dexamethasone anti-inflammatory properties in acute lung inflammation by a significant decrease in TNF-α and IL-8 concentrations in comparison to control group. Dexamethasone (2.5 mg/kg) combined with RSV (50 mg/kg) caused a significant reduction in neutrophil counts and lung edema. This combination impeded matrix metallopeptidase 9 (MMP-9) and myeloperoxidase activity as important factors involved in pulmonary neutrophil infiltration [20]. Despite widespread studies on anti-inflammatory activities of RSV, a systematic review and meta-analysis of clinical trials showed a significant decrease in C-reactive protein (CRP) but no significant effects on IL-6 or TNF-α reduction [21].

Studies have reported interruptive properties of RSV in the life cycle of various respiratory viruses [22]. Significant antiviral properties have been observed in influenza, respiratory syncytial, varicella zoster, Epstein-Barr, and herpes simplex virus models by RSV [23]. A recent in vitro study showed that RSV exhibited anti-MERS-CoV activity [24]. Although MERS-CoV is only about 50% similar at the phylogenetic level to SARS-CoV-2 and MERS-CoV entry into host cells occurs via binding to dipeptidyl peptidase (DPP)-4 receptors versus angiotensin-converting enzyme (ACE)-2 receptors in the case of SARS-CoV-2, Lin et al. found that RSV inhibited MERS-CoV at the level of viral replication [22, 24]. Using in silico approach, two studies demonstrated the binding affinity of RSV itself and its derivatives to an RNA-dependent RNA polymerase and papain-like protease which are essential in the virus life cycle [1, 25]. Computational analysis revealed that the RSV dimer, δ-viniferin, inhibited the 3C-like protease and RNA-dependent RNA polymerase enzymes [1]. In addition, a recent computational docking study found that RSV can theoretically bind to the ACE-2 and spike complex, which is essential for viral entry, with superior affinity to chloroquine as positive control [4]. A schematic showing the potential protective mechanisms of RSV in COVID-19 patients is shown in Fig. 1.

Some studies have reported complications related to COVID-19 are thromboembolism and lung tissue fibrosis. A recent retrospective study [26] analyzed chest computed tomography (CT) images of COVID-19 patients and showed that a high prevalence of pulmonary embolism was associated with poor prognosis. Major etiological factors related to pulmonary thromboembolism are ROS production and the cytokine storm

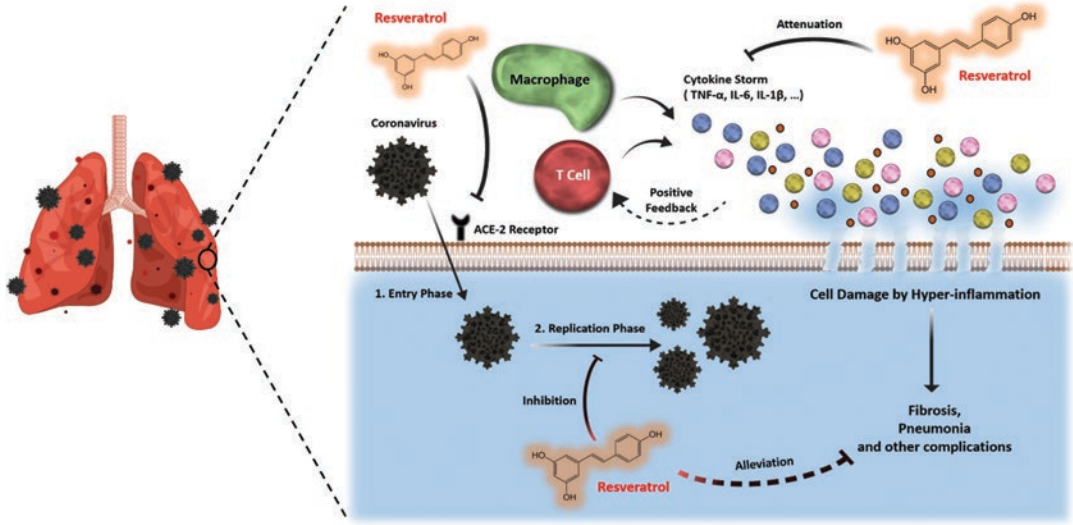

Fig. 1 Holistic scheme of resveratrol's potential effects against COVID-19

which can activate platelets. The interaction between these activated platelets and neutrophils results in the maintenance of the hyper-inflammatory and pro-coagulant status in the lung tissue [7]. In an animal study, it was shown that intraperitoneal pretreatment with RSV (10 mg/kg) decreased monocyte chemoattractant protein-1 (MCP-1) expression and MAPK phosphorylation in an acute pulmonary thromboembolism-induced pulmonary artery hypertension model [27]. Another study showed that oral administration of a RSV hybrid with furoxan had antithrombic effects in a mouse pulmonary thromboembolism model [28].

Another pathological consequence of infection via SARS-CoV-2 and its subsequent cytokine release syndrome is pulmonary fibrosis. Transforming growth factor-β 1 (TGF-β1) plays a pivotal role in this pathologic status which finally causes gross collagen deposition in lung tissue [29]. In in vivo models of LPS- and bleomycin-induced pulmonary fibrosis, RSV was found to exert its anti-fibrotic properties by TGF-β suppression along with attenuation in the Smad signaling pathway [30, 31].

In ARDS patients, excessive heme may contribute to a pro-inflammatory and pro-fibrotic condition, as found in COVID-19 cases [32]. RSV, via its capacity to increase intracellular heme oxygenase enzymes [33], could be beneficial in attenuating complications related to NLRP3 inflammasome activation, platelet hyperactivity, and hyper-inflammatory syndrome [32].

3 Delivery of RSV to the Lung

All of these beneficial effects on pulmonary system and antiviral properties of RSV are dependent on its concentration. Due to RSV poor bioavailability, several nanoparticulate drug delivery systems have been applied to enhance its solubility and potency. In a recent study, beneficial effects of RSV encapsulated in lipid nanoparticles were demonstrated. LPS-induced elevation of inflammatory markers like IL-6, macrophage inflammatory protein-1 alpha (MIP-1α), and MCP-1 was suppressed efficiently. Furthermore, in contrast to RSV alone or empty lipid nanoparticles, RSV loaded in lipid core nanoparticles reduced histological changes and leukocyte gathering in the bronchoalveolar fluid in a mouse model [34].

To provide suitable localized concentrations of antiviral agents for respiratory infections, inhalation-based formulations have been suggested [35]. Therefore, another instrumental strategy for RSV administration to COVID-19 patients is via

a pulmonary drug delivery approach. Co-delivery of RSV and budesonide microparticles via inhalation led to a significant decrease in TNF-α and IL-6 levels in rat alveolar macrophage cells [19]. Using a vibrational atomization spray-drying method, polymeric microparticles of RSV were prepared to achieve deep lung displacement for pulmonary arterial hypertension by Dimer et al. [36]. In an in vitro study, an inhalable dry powder of RSV significantly inhibited IL-8 expression in the Calu-3 lung epithelial cell line [37]. Generally, nanoformulations and respirable forms of RSV could provide better stability and bioavailability properties for patients with active respiratory infection and inflammation.

4 Conclusion

In conclusion, what we know about RSV in this field is largely based upon cellular, animal, and computational studies that investigated how it could be beneficial. However, the findings taken together suggest that RSV may provide a novel treatment strategy to reduce the effects of the devastating SARS-CoV-2 virus at the level of individual patients and on societies worldwide. These findings warrant the testing of RSV in further preclinical and clinical studies of COVID-19 and other pathogens.

Conflict of interests None.

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A Review on the Phytochemistry, Pharmacology, and Therapeutic Effects of *Rheum ribes*

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Abstract

Herbal medications are typically used for the treatment of diverse diseases without significant adverse effects. The *Rheum ribes* (*R. ribes*), commonly called rhubarb, is a hardy perennial herb and is consumed all over the world. There is growing evidence of the therapeutic benefits of *R. ribes*. Extensive in vitro and in vivo investigations have shown that *R. ribes* reveals beneficial properties via different mechanisms. In the current article, various pharmacological and therapeutic effects of *R. ribes* have been reviewed. For this purpose, different online databases using keywords such as *R. ribes*, therapeutic effects, and pharmacological effects were searched until the end of December 2020. *R. ribes* has been suggested to be effective in the treatment of a wide range of disorders including stomachache, nausea and vomiting, hemorrhoids, and measles. Additionally, different studies have demonstrated that *R. ribes* possesses numerous pharmacological properties including anti-inflammatory, anticancer, antibacterial, and antiviral, and may also function as an expectorant. The present narrative review provides a detailed survey of scientific investigations regarding the pharmacological properties and therapeutic effects of *R. ribes*.

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Keywords

Rheum ribes · Antibacterial · Anticancer · Antioxidant · Anti-inflammatory · Antidiabetic · Neuroprotective · Pharmacological properties

1 Introduction

Polygonaceae is a major plant family with nearly 1200 species around the world [1]. The genus *Rheum* of this plant family contains about 103 species, which are located in temperate and subtropical areas mostly found in Western Asian countries such as Iran, Iraq, Lebanon, Azerbaijan, Turkey, and Pakistan [2, 3]. The *Rheum ribes* (*R. ribes*), commonly called rhubarb, is a hardy perennial herb, growing about 40–150 cm tall with large, brown-green leaves, edible flower stalks, and small flowers (Fig. 1), which often grows in mountainous regions. This plant is usually consumed either raw or cooked and is traditionally used in "folk medicine" for the treatment of various diseases. Indeed, *R. ribes* has a wide range of bioactive compounds that have several therapeutic properties.

In traditional Iranian medicine, some medicinal properties have been attributed to this plant, such as hepatoprotective, appetite-stimulating, blood-purifying, and bile-reducing [4]. Rhubarb root has been used for treating ulcers, obesity, diarrhea, diabetes, hypercholesterolemia, hypertension, constipation, and some skin infections [5]. The extract of some parts of this plant are used as a treatment for stomachache, nausea and vomiting, hemorrhoids [6], and even measles. Other reported pharmacological actions of *R. ribes* include anti-inflammatory, anticancer, antibacterial [7, 8], and antiviral [9] properties, as well as its use as an expectorant [10]. It has been reported that its root extract has been shown to have hypoglycemic effects in animal models of diabetes, while no glucose-lowering effect occurs in normal animals [11]. Various methanol and chloroform extracts of different parts of the plant have been reported to possess antioxidant effects [12–14]. In this narrative review, we summarize the pharmacological and therapeutic effects of *R. ribes* (Fig. 2).

2 Methods

We conducted a literature search available in ISI Web of Knowledge, MEDLINE, PubMed, Scopus, and Google Scholar databases for articles in English that had been published through December 2020. For this purpose, we used appropriate keywords including *Rheum ribes*, antibacterial, anticancer, antioxidant, anti-inflammatory, antidiabetic, neuroprotective, and anti-trichomoniasis. Fifty-nine studies were considered for inclusion in this narrative review.

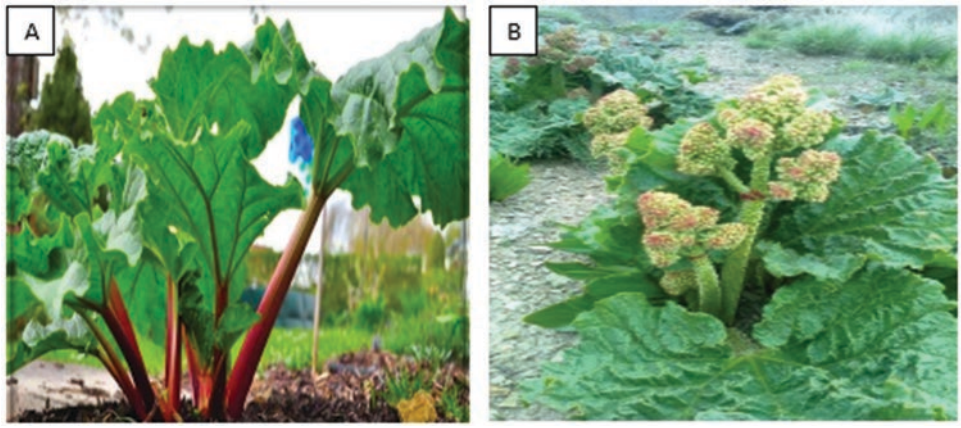

Fig. 1 *R. ribes* L. (stems and leaves), (**a**), *R. ribes* L. (flowers), (**b**)

Fig. 2 Various effects of *R. ribes*

3 Phytochemistry

R. ribes has been reported to be comprised of a variety of biologically active phytochemicals, such as carbohydrates, flavonoids, alkaloids, glycosides, anthocyanins, stilbene, and anthraquinones, all having different health benefits [15, 16]. Two different methods were used by Al-Shammari et al. to analyze the chemical components of *R. ribes* rhizomes, both qualitatively and quantitatively. One of the methods employed was gas chromatography/mass spectrometry (GC-MS). The two methods demonstrated a total of 15 different constituents in rhubarb rhizomes. These components possess a wide range of pharmacological effects. For instance, a certain concentration of oxalic acid in the blood is necessary for appropriate immune defense against infection from various pathogens. Otherwise, the immune system is challenged in terms of protecting humans from various diseases [17]. Ascorbic acid is a well-known antioxidant with antinociceptive and anti-inflammatory properties [18]. Bis-(diiodarsino) methane is a compound that is needed to promote the natural metabolism of hormones such as estrogen in the body. Studies have also shown that this compound can induce apoptosis of human carcinoma cells in various neoplasms, such as cervical cancer and breast cancer [19]. Stearic acid is a pharmaceutical product that is used in drug delivery systems due to its excellent biocompatibility and high capacity for biodegradation [20].

Using a hydroxylation method and GC/MS analysis, the composition of essential oil extracted from the roots and stems of rhubarb was determined in another study. The main hydrocarbons present in the oil were long-chain n-alkanes. Of the 30 constituents that were identified, tricosane and heneicosane were the predominant, followed by pentacosane, heptacosane, and palmitic acid [21].

Nutritional value (total ash, nitrogen, crude protein and fiber, and pH) and mineral compositions (14 mineral elements) of *R. ribes* were determined in a study by Tuncturk et al. [1]. The results indicated a high percent of nitrogen, crude protein, and fiber content in some edible parts of this plant, as well as high concentrations of Mg, Ca, Mn, Cu, and Zn among the elements that were analyzed.

Furthermore, 21 compounds with anticancer activity have been identified in the whole plant *R. ribes* (WBFRR) when extracted using butanol [15]. The major polyunsaturated fatty acids that have been identified in the extract of *R. ribes* flowers are oleic acid, γ-linolenic acid, linoleic acid, palmitic acid, and stearic acid [22]. The percentage of unsaturated fatty acids (66.0%) is greater than the saturated fatty acids (10.5%); the former of which have been proven to reduce serum low-density lipoprotein cholesterol. In addition, analysis of distilled oil of *R. ribes* flowers has revealed 23 compounds, with germacrened (22.3%) and α-pinene as the major constituents present.

4 Pharmacological and Therapeutic Effects

4.1 Antibacterial Effects

The antibacterial and antifungal activity of several parts of the *R. ribes* plant has been evaluated in several studies. For example, an ethanol extract of the *R. ribes* root, EERR, has shown antibacterial activity against *Escherichia coli* (*E. coli*) and *Enterobacter aerogenes* (*E. aerogenes*) at concentrations of 0.6, 1.2, and 2.5 mg/ml and against *Staphylococcus aureus* (*S. aureus*) at higher concentrations (10 and 20 mg/ml). An antifungal effect of *R. ribes* against *Saccharomyces cerevisiae* has also been reported at high doses [23]. However, a different study could not confirm any antifungal activity of compounds contained in various extracts of this plant [24].

The antibacterial effects of various rhubarb root extracts [aqueous, ethanol, and an organic solvent (chloroform)] at low (31 μg/ml) and high (4000 μg/ml) concentrations were evaluated for activity against *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis*, and the fungus *Candida albicans* in a study by Alaadin et al. Additionally, four biologically active compounds (aloe emodin, chrysophanol, physcion, and emodin) were also isolated from the extracts and evaluated against the aforementioned strains of bacteria at low and high concentrations (2 and 250 μg/ml, respectively). Neither of the two extracts (aqueous and ethanol) demonstrated any activity against the bacterial strains mentioned above, although the chloroform extract showed marked activity against *S. aureus* and *E. coli* and very weak activity against *P. aeruginosa*. Despite the chloroform extract demonstrating marked activity against *E. coli*, none of the three extracts could "completely" inhibit *P. aeruginosa* and *E. coli*, even at the highest concentrations evaluated, nor did chrysophanol and physcion "completely" inhibit *S. aureus* at a concentration of 250 mg/ml. However, the other two compounds mentioned above (i.e., aloe emodin and emodin) were active against *S. aureus* at the minimum concentrations used in that study.

A different study that examined the antibacterial effect of rhubarb stalk extract on various gram-positive and gram-negative bacteria demonstrated greater antibacterial activity against *Streptococcus agalactiae* and *Listeria monocytogenes* than other bacteria (*E. coli*, *S. aureus*, *K. pneumonia*, *Ps. aeruginosa*, and *S. epidermidis*) [25]. In yet another study by Aygün et al., it was reported that silver nanoparticles (NPs) formulated to contain *R. emodi* root extract were bactericidal against gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*Salmonella typhimurium* and *E. coli*) bacteria. The zones of inhibition for *B. subtilis* was found to be

19.0 ± 0.5 mm, whereas it was 29 ± 1.3, 24 ± 1.2, 28 ± 0.9, and 31 ± 1.1 mm against *S. aureus*, *S. pyogenes*, *E. coli*, and *S. typhimurium bacteria*, respectively [26].

In a very interesting antimicrobial activity study, the antibacterial effects of different parts of plant extract (root, stalk, and leaves) were evaluated against various hospital strains of bacteria including *E. coli*, *Proteus* spp., *Neisseria gonorrhoeae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* This study showed that the root and leave extracts had more antibacterial activity than that of the stalk when compared to the positive control, and the activity was more effective against *P. aeruginosa* and *Proteus* spp. than the other pathogens studied [27]. It has been suggested that the plant *R. ribes* may be effective against pathogens like *P. aeruginosa* that can develop "antibiotic resistance."

Finally, in a study that evaluated the antimicrobial effect of rhubarb flowers, it was observed that essential oil and the hexane extract of rhubarb markedly inhibited the growth of *Staphylococcus epidermidis*, with zones of inhibition of 15.0 and 16.2 mm, respectively [22]. These authors suggested that the hexane extract and essential oil of *R. ribes* may induce an antimicrobial effect due to unsaturated fatty acids and terpenoid components, respectively. Moreover, Bagheri et al. studied the antimicrobial activities of hydroalcoholic and aqueous extracts of *R. ribes* L. on *Acinetobacter baumannii* and reported that the aqueous extract had a greater minimum inhibitory concentration (MIC) against *A. baumannii* than the hydroalcoholic extract [28]. (Table 1)

4.2 Anticancer Effects

For decades, many natural products isolated from plants have been screened in vitro and in vivo for their anticancer activity. In this regard, the cytotoxic effects of the ethanol extract of *R. ribes* root (EERR) in prostate cancer cells (PC3) was studied by Tartik et al. [23]. Their results revealed that EERR has marked cytotoxic activity on PC3 and suggested that EERR may induce apoptosis of PC3 via DNA fragmentation. Further studies by Azadpour et al. [25] concerning the cytotoxic effect of different concentrations of rhubarb extract on KB and A549 cancer cell lines showed that although rhubarb caused cytotoxicity in the cell lines tested, it did not cause mutations or damage to the DNA of normal cells. They suggested rhubarb may represent a promising herbal plant for cancer treatment, because it has no genotoxic effects on normal cells. However, it should also be noted that Abudayyak et al. [29] demonstrated a DNA mutagenic effect of *R. ribes* extract at a dose of 25 μg/ml.

Another group who investigated the cytotoxic potential of rhubarb extracts for anticancer activity was Keser et al. This group evaluated the cytotoxic activity of an extract of *R. ribes* stalk against different cell lines (A2780, PC-3, MCF-7, and HCT-116) [30]. However, it was Uyar et al. who attempted to elucidate a mechanism of action for *R. ribes* extract. These authors proposed that the extract of *R. ribes* can induce apoptosis in the HL-60 cell line through an increase in the expression of pre-apoptotic genes, such as caspase 3, and by mediating a reduction in antiapoptotic genes such as BCl-2 [31]. In an attempt to further elucidate the mechanism of action for the anticancer activity observed with various extracts of rhubarb, Achakzai et al., using different solvents (butanol, methanol, n-hexane, and aqueous) to extract the active components/compounds from the whole rhubarb plant, investigated their anticancer potential against the MCF-7 cell line. GC-MS analysis showed that just the butanol fraction of this plant (composed of 21 compounds) had marked anticancer activity with minimum toxicity [15].

As mentioned above, when discussing the antibacterial action of various Rhubarb extracts, silver nanoparticles (Ag NPs) have also been utilized when testing the anticancer activity of compounds isolated from various portions of the Rhubarb plant. For example, *R. ribes* has been utilized for the "green synthesis" of Ag NPs and assessed for anticancer activity against the MDA-MB-231 breast cancer cell line [26]. The MTT cell viability test showed a cell survival rate of 60% and 40% after 24 and 48 h of exposure,

Table 1 Summary of results reporting antibacterial and anticancer effects of *R. ribes*

Dose	Experimental model	Effect	References
31 to 4000 µg/ml	Bioautographic assay	No inhibitory effect on *P. aeruginosa* and *E. coli* at the highest concentration tested	[7]
250 and 500 µl of plant extract per cup or disc	Cup plate and paper disc methods	More effective against *P. aeruginosa* and *Proteus* spp. compared with the positive control	[8]
Hexane fraction 0.1 gm, whole plant aqueous fraction 23.6 gm, in vitro	Brine shrimp lethality assay	Marked anticancer activity of whole plant butanol fraction	[15]
30 µL of the hexanic extract and essential oil, in vitro	Disc diffusion method (DDM)	Moderate effects of both samples on some gram-positive and gram-negative bacteria	[22]
Various doses, in vitro	Agarose gel electrophoresis WST-1 assay	Antimicrobial activity against several microorganisms DNA fragmentation of cancer cell lines	[23]
250 and 500 µg per cup or disc	Cup plate and disc diffusion method	Significant antibacterial activities	[24]
Different concentrations, in vitro	MTT assay MBC and MIC Ames test	Cytotoxic effect against cancer cell lines at different concentrations Antibacterial activity against some optional bacteria Lack of mutagenic effect	[25]
Ag NPs (green synthesis using *R. ribes*), different concentrations, in vitro	MTT cell viability test drug concentration dilution method	Cytotoxicity effects against breast cancer cell line at low concentrations tested High antimicrobial activity	[26]
100 µl of plant extract, in vitro	Agar well diffusion method	Beneficial effect on controlling some microbial infections	[27]
Different concentrations of aqueous and hydroalcoholic of plant extract, in vitro	Disk diffusion, MIC and MBC	Effectiveness of hydroalcoholic *R. ribes* extract in control of *A. baumannii*	[28]
1333.33 µg/ml to2.60 µg/ml, in vitro	DPPH method	Potential cytotoxic effects via induction of apoptosis	[31]

respectively, and the Ag NPs IC_{50} values were 165.6 g/ml and 98.96 g/ml at these same time points. Thus, it would seem to suggest that metallic nanoparticles of rhubarb extract at low concentrations may induce cell apoptosis. Furthermore, another study reported that ethyl acetate extracts of rhubarb root showed cytotoxic activity against a promyelocytic leukemia cell line with an IC_{50} value of 149 g/ml after treatment for 24 h and an IC_{50} value of 74 g/ml after 48 h treatment [31]. This study revealed that the rhubarb root extracts presumably exert their toxicity through the induction of apoptosis. That is, the cytotoxic effect of the rhubarb extracts is most likely attributed to active components/compounds within the extracts that exert apoptotic effects. For instance, emodin, an anthraquinone derivative of rhubarb, has been reported to induce apoptosis in various cancer cell lines by activating caspases 3 and 9, as well as upregulating Bax [32] (Table 1).

4.3 Anti-ulcer Effects

The gastroprotective effects of methanolic and aqueous extracts of *R. ribes* (200 mg/kg, p.o.) have been investigated using an ethanol plus pylorus ligation (EPPL) method. Previous results have shown that *R. ribes* significantly reduced gastric volume, free acidity, and total acidity. Extracts from *R. ribes* have also been found to

increase the level of mucoproteins, such as total protein, hexoses, hexosamine, fucose, and sialic acid. It is also noteworthy that extracts obtained from *R. ribes* have been shown to decrease gastric lipid peroxidation. In fact, histopathological data has shown that treatment with extracts obtained from *R. ribes* have resulted in a hyperplastic gastric mucosa, indicating repair/regeneration of epithelial tissue after ulcerative damage [33].

Lastly, the effect of an extract obtained from *R. ribes* (2.5 mL for children less than 15 kg, every 6 h for 5 days) has previously been studied in 150 children (age between 12 and 72 months) with suspected *Shigella dysenteriae*. Body temperature, abdominal pain, need for antipyretics, defecation frequency, stool volume, and consistency and microscopic stool examination were used as measures of treatment outcome. The results showed that the *R. ribes* extract was very effective in reducing the duration of dysentery, fever, and abdominal pain and that it can be regarded as a complementary treatment for children with *Shigella dysenteriae* [34].

4.4 Anti-inflammatory Effects

The anti-inflammatory properties of 25 ul of an *R. ribes* extract have been reported by using an "oxidative burst" assay, which employs a chemiluminescence technique. Various fractions of extracts derived from *R. ribes* (25 ul) were incubated for 15 min at 37 °C with whole blood in Hank's balanced salt solution (HBSS). The whole plant aqueous fraction of *Rheum ribes* (WAFRR), the whole plant butanol fraction of *Rheum ribes* (WBFRR), and whole plant methanol extract of *Rheum ribes* (WMERR) demonstrated anti-inflammatory activity on reactive oxygen species (ROS) having IC_{50} values of 24.2 ± 2.7, 12.0 ± 0.6, and 23.2 ± 1.9 [15].

Consistent with the anti-inflammatory effects for *R. ribes* determined in vitro and described above using whole blood, Ag NPs of *R. ribes* have been investigated for their effects on carrageenan-induced paw edema in male albino mice. The extract of *R. ribes* incorporated into the Ag NPs provided a dose of either 50, 100, or 150 mg/kg, and each dose was administered by intraperitoneal injection 30 min prior to a carrageenan paw injection. All of doses tested, especially the 150 mg/kg dose, exhibited an anti-inflammatory effect [35].

4.5 Antioxidant Effects

As it pertains to the antioxidant effects of various extracts of *R. ribes*, Tartik et al. evaluated the effect of an ethanol extract of *R. ribes* root (EERR) (500, 1000 μg/ml for 24 h) on human umbilical vein endothelial cells (HUVECs) and prostate cancer cells (PC3) in vitro. It was demonstrated that the production of hydrogen peroxide (H_2O_2)-induced ROS and lipid peroxidation (LPO) levels decreased following treatment of HUVECs with EERR. However, the production of ROS and malondialdehyde (MDA) in PC3 cells increased with exposure to EERR, although cell viability significantly decreased. Thus, EERR can exert both prooxidant and antioxidant activities on different types of cells [23].

In a different study, the effect of various extracts of *R. ribes* flowers was investigated for its capacity to scavenge ROS. Different concentrations of a *R. ribes* flower extract (100, 150, 200, 250, and 300 ppm) were assessed with regard to inhibiting free radical formation using the Folin-Ciocalteu method and the DPPH (2,2-diphenyl-1-picrylhydrazyl) test. It was shown that the highest free radical scavenging power of the extract occurred at a concentration of 300 ppm. The inhibitory effect was also dose-dependent [36].

Others have also investigated the antioxidant effects of specific extracts of *R. ribes*. For example, Yildirim et al. studied the antioxidant activity of methanolic extracts of *R. ribes* fruits and seeds to inhibit protein oxidation and lipid peroxidation using different assays, such as a standard method employing copper (II) chloride (extract concentration: 50, 100, 250, and 500 ppm), as well as the ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] method (extract concentration: 10, 20, 30, 40, and 50 mg/ml). It was shown using

Table 2 Summary of results reporting antioxidant effects of *R. ribes*

Dose	Experimental model	Effect	References
500, 1000 µg/ml, in vitro	Human umbilical vein endothelial cells (HUVECs) and prostate cancer cells (PC3)	Reduced the reactive oxygen species (ROS) and lipid peroxidation (LPO) in HUVECs, increased the ROS and malondialdehyde (MDA) in PC3 cells	[23]
100, 150, 200, 250, and 300 ppm, in vitro	Folin-Ciocalteu method and DPPH test	Free radical scavenging activity increased as dose- dependent	[36]
Different doses on the basis of method, in vitro	Cupric and ABTS method	Powerful free radical scavenging activity	[37]
Different doses on the basis of method, in vitro	Different antioxidant tests, for example, cupric and ABTS method	Chloroform extract of the roots has better antioxidant activity	[38]
Essential oil 100 µL, in vitro	DPPH test	Hexane extract has the most powerful antioxidant effect	[22]
Hexane, 0–1200 µg/ml, in vitro	ABTS and DPPH methods	The effective antioxidant dose was 200 µg/ml	[35]
1333.33 µg/ml to 2.60 µg/ml, in vitro	DPPH method	Potent antioxidant activity	[39]
1.5 mL, in vitro	DPPH and FRAP methods	Ethanol extract has the highest antioxidant activity	[40]

the cupric method that the antioxidant activity of the extract of *R. ribes* fruits was 2.44 ± 0.212 mmol/g, while the extract of the *R. ribes* seeds had an antioxidant capacity of 5.30 ± 0.245 mmol/g [37].

In yet another study by Ozturk et al. to assess the antioxidant activity of *R. ribes*, chloroform and methanol extracts of the stem and roots of *R. ribes* were evaluated using different antioxidant tests, such as total antioxidant activity (i.e., inhibition of lipid peroxidation), DPPH radical scavenging, superoxide anion radical scavenging, ferric reducing potential, cupric reducing potential (CUPRAC), and metal-chelating activities. Results reported by these investigators demonstrated that the chloroform and methanol extracts of the roots had higher antioxidant activity, with 93.1% and 84.1% inhibition, when compared to the chloroform and methanol extracts of the stems, with 82.2% and 82.0% inhibition, respectively. Additionally, of the six analytical methods used to determine antioxidant activity, inhibition of lipid peroxidation in a β-carotene linoleic acid system exhibited the highest activity by all extracts of *R. ribes*. Interestingly, the chloroform extract of the roots exhibited the highest antioxidant capacity when compared to standard quercetin, which may be due to a greater content of flavonoids in the extract [38]. Finally, the antioxidant activity associated with essential oil and the hexane extract of *R. ribes* flowers has also been assessed using the DPPH method mentioned above. The hexane extract of *R. ribes* flowers had very significant antioxidant activity, which was shown to be dose-dependent [22].

Silver NPs produced using waste extract of *R. ribes* (0–1200 µg/ml) also have potent antioxidant activity as assessed using methods employing ABTS and DPPH. A dose of 200 µg/ml of Ag NPs had significant antioxidant activity that was concentration-dependent, as determined using both ABTS and DPPH [35]. Additionally, others have demonstrated that ethyl acetate extracts of *R. ribes* shoot and root (ranging from 1333.33 µg/ml to 2.60 µg/ml) showed a potent antioxidant activity as assessed by the scavenging of DPPH radicals (i.e., IC_{50} values of 206.28 µg/ml for shoot and 10.92 µg/ml for root) [39] (Table 2).

Lastly, it should be emphasized that the solvent used for the extraction process (four different solvents including 50% methanol, 70% ethanol, 80% acetonitrile, and petroleum ether) also has an

effect on the antioxidant activity measured by DPPH, as well as the method known as ferric reducing antioxidant power (FRAP). In one study conducted by Alkaya et al., the highest antioxidant activity was shown when ethanol was used as the solvent to extract active compounds from the plant. Interestingly, ultrasound- and microwave-based extraction techniques yield the highest values for antioxidant activity when compared to other solvent-based extraction methods [40]

5 Hypoglycemic Effects

Diabetes mellitus is one of the most prevalent endocrine disorders in almost all countries and a major cause of morbidity and mortality in the United States [41]. Diabetes mellitus is a group of chronic metabolic diseases characterized by hyperglycemia, which is caused by defective insulin action (insulin resistance), insulin secretion, or a combination of both. Prolonged persistence of elevated blood glucose levels eventually leads to damage of multiple organ systems [42].

Although there are conventional antidiabetic drugs with which to treat diabetes, its treatment with medicinal plants is often very successful, especially as it pertains to type 2 diabetes mellitus. In fact, medicinal plants possess insignificant toxicity and virtually no side effects, so they represent a therapeutic option for the treatment of this disease [43].

The effects of an aqueous extract of *R. ribes* on cellular pancreatic insulin secretion, expansion, and possible extrapancreatic effects on cell-free in vitro systems of carbohydrate absorption have been evaluated using the MIN6 cell line as the cellular model. Results demonstrated that at 48-h post-seeding, *R. ribes* (0.1, 10, and 25 mg/ml) significantly expanded the proliferation of pancreatic β-cells (MIN6) when compared to control (untreated cells) as assessed using the MTT assay. Furthermore, *Rheum ribes* (0.01, 0.1, 0.5, 1, 10, and 25 mg/ml) was shown to significantly increase glucose-stimulated insulin secretion (GSIS) in pancreatic MIN6 cells following a 1-h incubation, compared to untreated control cells. The protective effect of *R. ribes* on pancreatic MIN6 cells was related to its active components, specifically, compounds such as flavonoids, phenolics, and coumarins [44].

Using an experimental animal model, a hydroethanolic extract of *R. ribes* root (75 and 150 mg/kg, orally) was evaluated for its effects on alloxan monohydrate-induced diabetes in female rats for 4 weeks. Findings of this study demonstrated that *R. ribes* could decrease the serum levels of glucose, cholesterol, triglyceride, urea, and creatinine and increase the serum levels of HDL. Additionally, this study showed that treatment of diabetic rats with the *R. ribes* hydroethanolic root extract also improved various histopathological changes seen in the diabetic rats, for example, less thickening of the basement membrane, decreased atrophy of glomerular capillaries with a reduction in the Bowman's space, and partial reversal of acute tubular necrosis when compared to these same parameters in the control (non-diabetic) rats. These authors suggested that it was the presence of polyphenols and flavonoids in *R. ribes* that might be responsible for the nephron-protective activity in the diabetic rats [45].

Similar to the findings in the experimental animal model of diabetes described above, a clinical study determined the effect of a hydroethanolic extract of *R. ribes* root in 80 patients with type 2 diabetes mellitus and showed that treatment (3 capsules daily, 400 mg/capsule) for 30 days significantly reduced HbA1C and fasting blood glucose (FBS). These authors proposed that the protective effects of the *R. ribes* root extract on diabetes is potentially associated with its antioxidant properties and the presence of flavonoids, such as quercetin, in the plant [46]. In a similar study, the effects of *R. ribes* root (3 capsules daily, 350 mg/capsule) on type 2 diabetes mellitus was studied in a 120 patients for 12 weeks. Their results indicated that treatment with *R. ribes* root significantly reduced the levels of blood glucose. Similar to previous investigators investigating the effects of *R. ribes* root extract on diabetes, these authors suggested that the therapeutic effects of *R. ribes* may possibly be due to the presence of alkaloids, tannins, flavonoids, anthraquinones, and quinones contained in the plant [47].

Returning to preclinical investigations that have been conducted in experimental animal models, another study evaluated the effects of a hydroethanolic extract of *R. ribes* root in healthy mice. Animals were fasted for 5 h prior to *R. ribes* administration (50 mg/kg). Blood glucose levels determined in blood samples collected at 1, 2, 4, and 24 h after treatment showed that blood glucose was significantly reduced. Furthermore, an aqueous extract of *R. ribes* (0, 1, 10, and 100 μg/ml) significantly increased insulin release in INS-1E cells in vitro at stimulatory (20 mM) and non-stimulatory (1 mM) glucose concentrations. These authors, based on their findings using thin-layer chromatography of the *R. ribes* extract, identified the presence of aloe emodin, emodin, physcion, and chrysophanol and suggested that the hypoglycemic activity of the extract may occur via increased insulin release from pancreatic β-cells, as well as the release of intracellular Ca^{2+} [3].

Lastly, administration of aqueous and ethanolic extracts of *R. ribes* (450 mg, three times daily for 6 weeks) in 60 type 2 diabetes mellitus patients significantly decreased the serum levels of insulin, apolipoprotein B (ApoB), and the ratio of ApoB to apolipoprotein A1 (ApoA1) [i.e., the ApoB/ApoA1 ratio], as well as increased serum ApoA1. Moreover, using a well-established method for assessing β-cell function (B) and insulin resistance (IR) from basal (fasting) glucose and insulin, specifically "homeostatic model assessment" (HOMA), it was found that the aqueous and ethanolic extracts of *R. ribes* also significantly decreased HOMA-IR and HOMA-B (i.e., HOMA associated with β-cell function). These authors suggested that the protective effects of *R. ribes* in diabetes was related to the active components within the extracts, such as flavonoids rhapontigenin, desoxyrhapontigenin, rhaponticin, desoxyrhaponticin, piceatannol, and resveratrol, all of which possess potent antioxidant activity [48].

6 Nephroprotective Effects

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is an extremely effective chemotherapy drug for certain cancers. However, its clinical use is limited due to severe side effects, including notably nephrotoxicity in the kidneys and acute kidney injury [49].

Various experimental animal studies with *R. ribes* and cisplatin have demonstrated the benefits of various *R. ribes* extracts to lessen kidney damage caused by cisplatin. For example, the protective effects of an aqueous extract of *R. ribes* (150 mg/kg, orally) in cisplatin-induced nephrotoxicity in rats were investigated over the course of 6 weeks. Results showed that *R. ribes* significantly reduced the serum levels of blood urea nitrogen (BUN), creatinine, cholesterol, and glucose. The proposed mechanism for the beneficial effects of the extract of *R. ribes* was suggested to be associated with its inhibition of oxidative stress, as well as the presence of flavonoids, stilbenes, and anthraquinones in the plant [50].

In a different animal study, Asgharian et al. have reported that the oral administration of a hydroethanolic extract of *R. ribes* (200 and 400 mg/kg) daily for 10 days to rats with lead acetate-induced nephrotoxicity resulted in marked protective effects on oxidative stress and a reduction in degeneration, vacuolization, flattening, and cell destruction within kidney tissue, as well as an enhancement in serum antioxidant capacity [51].

7 Neuropharmacological Effects

Mental disorders are a mental pattern of behavior(s), which greatly impact multiple facets of an individual's life. This results in a "psychological syndrome" with accompanying personality, mind, and/or emotional disorganization, which tends to disrupt normal social function and lead to an increased risk of suffering, pain, disability, and even death [52].

The effect of various extracts of *R. ribes* on mental illness has been evaluated in a limited number of clinical studies. For instance, the therapeutic role of a hydroethanolic extract of *R. ribes* (400 mg, three times daily for 6 weeks) was evaluated in patients suffering from major depres-

sive disorder (MDD) by Sayyah et al. In this study, all of the patients were assessed before the study using the Hamilton Rating Scale for Depression (HAM-D) and then again at weeks 1, 2, 4, and 6. A total of 33 patients with MDD were randomized in a double-blind manner and received capsules containing 400 mg of *R. ribes* three times per day (1200 mg/kg/day) by oral administration. The results demonstrated a significant decrease in depressive symptoms as assessed using the HAM-D in those patients with MDD who received *R. ribes*. These authors suggested that the beneficial effects of *R. ribes* on neurobehavioral function were related to the active chemical compounds contained in *R. ribes*, such as flavonoids, as well as their antioxidant activity [53]. Similarly, the administration of a hydroethanolic extract of *R. ribes* (400 mg, three times daily for 8 weeks) reduced the symptoms of obsession and compulsion in 56 patients with obsessive-compulsive disorder (OCD) as assessed by the score using the Yale-Brown scale (Y-BOCS) [54].

Switching to preclinical, experimental animal models, *R. ribes* has also been evaluated for its neuropharmacological effects in rats. An aqueous extract of *R. ribes* (250 and 500 mg/kg, i.p., 20 days) was evaluated after the induction of Alzheimer's disease (AD) by nucleus basalis of Meynert lesions (NBML) in a rat model of AD. The results of this study indicated that *R. ribes* significantly increased the time spent in the target quadrant in the Morris water maze test and the initial latency and step-through latency time in the passive avoidance test. It should be mentioned for completeness that the Morris water maze assesses impairments in visual short-term memory and visual-spatial abilities, while the "initial" and "step-through" latency times are used as an index of the ability of an animal to learn and remember the association between an aversive stimulus and a specific environmental context. Nevertheless, the authors suggested that the potential mechanism of action associated with the protective effects of *R. ribes* might possibly be related to flavonoids, antioxidants, and other "actives" contained in *R. ribes* that possess free radical scavenging activity [55].

Overall, the results of these previous studies, as it pertains to AD, suggest that the possible mechanisms associated with the beneficial therapeutic effects of *R. ribes* may conceivably be due to inhibition of oxidative stress and acetylcholinesterase, as well as the presence of flavonoids (which are potent antioxidants) contained in the plant [55, 56].

8 Anti-trichomonas Effects

Trichomoniasis is a very common sexually transmitted disease. It is caused by infection with a protozoan parasite called *Trichomonas vaginalis* (*T. vaginalis*) [57]. The anti-trichomonas activity of a hydroethanolic extract of *R. ribes* (100–300 μg/ml) was evaluated in vitro using TYI-S-33 medium for cultivation of *T. vaginalis*. The results of this in vitro study demonstrated that *R. ribes* inhibited growth by 97.8% and 100% after 24 and 48 h, respectively, following incubation with *T. vaginalis* [58].

In another study, the effect of extracts using different portions of the *R. ribes* plant (flowers, leaf, and stem), as well as the use of various extraction solvents (water, dichloromethane, hexane, and methanol), was evaluated using an in vitro assay. The results of this study showed that the aqueous fraction (extract) of the flowers of *R. ribes* elicited the greatest percent growth inhibition of *T. vaginalis* following 24 h of co-culture/exposure [59] (Table 3).

9 Conclusions

There continues to be an increasing interest worldwide in determining the pharmacological effects of bioactive components contained in medicinal plants, as well as the underlying mechanisms of action, to better treat various diseases/ailments. *R. ribes* is a versatile plant with a plethora of medicinal properties that could be utilized for the prevention and/or treatment of various diseases. This review has highlighted the diverse pharmacological effects of *R. ribes* that have been reported in numerous, previously published

Table 3 Summary of results reporting effects of *R. ribes* on diabetes, kidney, nervous, and trichomonas diseases

Dose	Experimental model	Effect	References
0.1, 10, and 25 mg/ml, in vitro 0.01, 0.1, 0.5, 1, 10, and 25 mg/ml, in vitro	MIN6 cell line	Expanded the proliferation of pancreatic β-cells MIN6 Augmented the GSIS in pancreatic MIN6	[44]
75 and 150 mg/kg, orally	Alloxan monohydrate-induced diabetes in female rats	Reduced the level of glucose, cholesterol, triglyceride, urea, and creatinine Increased the level of HDL improved histopathological changes	[45]
1200 mg/kg, orally	Diabetic patients	Reduced HbA1C and FBS	[46]
1050 mg/kg, orally	Diabetic patients	Reduced the level of blood glucose	[47]
50 mg/kg, orally 0, 1, 10, and 100 µg/ml, in vitro	Healthy mice INS-1E cell line	Reduced the level of blood glucose Increased the insulin release	[3]
1350 mg/kg, orally	Diabetic patients	Reduced serum levels of insulin, HOMA-IR, HOMA-B, ApoB, and ApoB/ApoA1 Increased ApoA1	[48]
150 mg/kg, orally	Cisplatin-induced nephrotoxicity in rat	Reduced BUN, creatinine, cholesterol, and glucose	[50]
200 and 400 mg/kg, orally	Lead acetate-induced nephrotoxicity in rats	Improved histopathological changes Increased the serum antioxidant capacity	[51]
1200 mg/kg, orally	Patients with mild to moderate major depression	Reduced depression symptoms on score of HAM-D	[53]
1200 mg/kg, orally	Patients with obsessive compulsive disorder	Reduced obsession and compulsion symptoms on score of Y-BOCS	[54]
250 and 500 mg/kg, i.p	NBML-induced Alzheimer's disease in rat	Increased the time spent in target quadrant and step-through latency	[55]
100–300 µg/ml, in vitro	TYIS33 culture media	Inhibited the growth of *T. vaginalis*	[58]
0.125, 0.25, 5, and 10 mg/ml, in vitro	TYIS33 culture media	Inhibited the growth of *T. vaginalis*	[59]

articles. However, the present authors would highly recommend that additional research be conducted on various extracts of *R. ribes* to explore their full therapeutic benefits. Following identification of the precise chemical compounds contained in the extract that are responsible for specific pharmacological actions and an elucidation of techniques needed for purification, stability testing, optimal formulation, and "scale-up," pharmacokinetic studies may then be conducted to determine optimal doses and dosing frequency. Once these parameters have been established, the therapeutic "actives" contained in *R. ribes* can then be fully harnessed to treat human disease alongside, or complementary to, conventional/traditional drug compounds.

Conflict of Interests None.

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Antitumor and Protective Effects of Melatonin: The Potential Roles of MicroRNAs

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Abstract

MicroRNAs (miRNAs) are endogenous short noncoding RNAs with approximately 22 nucleotides. The primary function of miRNAs is the negative regulation of target gene expression via mRNA degradation or translation inhibition. During recent years, much attention has been made toward miRNAs' role in different disorders; particularly cancer and compounds with modulatory effects on miRNAs are of interest. Melatonin is one of these compounds which is secreted by the pineal gland. Also, melatonin is present in the leaves, fruits, and seeds of plants. Melatonin has several valuable biological activities such as antioxidant, anti-inflammation, antitumor, and antiaging activities. This important agent is extensively used to treat different disorders such as cancer and neurodegenerative and cardiovascular diseases. This review aims to describe the modulatory effect of melatonin on miRNAs as novel targets.

Keywords

Melatonin · Cancer · MicroRNAs · Noncoding RNAs · Therapeutic

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1 Introduction

Next-generation sequencing has expanded our understanding of genome, and genome is mainly transcribed into RNAs [1, 2]. There are two distinct types of RNAs, (a) RNAs with the coding ability and (b) RNAs without coding ability, which is known as noncoding RNAs (ncRNAs) [3, 4]. It has been shown that ncRNAs compose more than 70% of the human genome and just 1–2% of RNAs can code proteins [5, 6]. ncRNAs are divided into two major categories: short and long noncoding RNAs [6]. A large body of data shows the critical role of these ncRNAs in processes responsible for cellular development, physiology, and pathology [5]. It has been reported that the level of ncRNAs is associated with the complexity of the organism so that more complex organisms have higher levels of ncRNAs [7]. MicroRNAs are short noncoding RNAs with approximately 20 nucleotides that involve negative modulation of gene expression [8]. So far, thousands of miRNAs have been recognized [9]. According to the role of miRNAs in cellular biological processes, studies have focused on finding compounds that affect the expression profile of miRNAs [8].

miRNAs are endogenous short noncoding RNAs (about 22 nucleotides) associated with negative modulation of expression of target genes through mRNA degradation and inhibition of translation [10, 11]. It has been shown that this negative effect on gene transcription is triggered via binding to the 3′ untranslated region (UTR) of mRNAs [12, 13]. The biogenesis of mRNA is as follows: in the nucleus, RNA polymerase II transcribes a full-length transcript, known as primary RNA (pri-RNA), and then it produces precursor miRNA (pre-miRNA) through the action of a complex including the double-stranded RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCR8) and the RNase II endonuclease Drosha [13]. Next, pre-miRNA (a fragment containing approximately 60–70 bp) enters the cytoplasm by crossing the nuclear pore via exportin-5 [13]. Then, in collaboration with trans-activation response RNA-binding protein (TRBP), Dicer enzyme generates mature miRNA [8, 14].

Of course, there is an alternative pathway where this pathway synthesizes just a few miRNAs. In this pathway, miRNAs are produced from short hairpin introns, known as mirtrons [15, 16]. miRNAs can repress target genes. There are several mechanisms for target repression. One of them is the binding of miRNAs to the complement (target) through seed region (nucleotides 2–8 of the miRNA) which results in decomposition of mRNA [14, 17–19]. Finding targets is performed by the seed region of miRNA, a region containing nucleotides 2–8 located at the 5′ end of miRNA [20–24]. The problem in using miRNAs is the various functions in different organs and tissues [25–27]. For instance, in hepatocellular, breast, and lung cancers, the expression level of miR-125b decreases, while in colorectal, pancreatic, gastric, and some leukemias, its expression level increases [25]. A number of mechanisms for regulation of miRNAs include transcriptional activation or inhibition, epigenetic repression, and controlled degradation rates [28]. This study aims to describe the modulatory effect of melatonin on miRNAs.

2 Melatonin: Physiology and Importance

Melatonin (N-acetyl-5-methoxy tryptamine) was first introduced in 1958 [29–31]. It was isolated from the bovine pineal gland. Melatonin is found in a number of sources such as the retina, gut, skin, platelets, and bone marrow, but pineal gland is the main secretion site of this hormone [32–36]. This compound is synthesized from serotonin. Despite the general belief about the animal origin of melatonin, it has also been found in the leaves, fruits, and higher plants [37]. Besides, melatonin is present in bacteria, fungi, and insects. Melatonin can scavenge reactive oxygen species [3, 38], modulate the immune system [39], have an antiaging effect, exert antitumor effects [40], protect neuron cells [41], and exert protective effects on cardiovascular disease [42],

diabetes [43], and obesity [44]. Furthermore, it has been shown that melatonin is associated with modulation of mood, sexual maturation, and body temperature. Also, it is beneficial in periodontology [45]. The interplay between melatonin and ROS is oxidized into N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), which has high antioxidant activity [46]. The liver is responsible for excretion of more than 90% of circulating melatonin [47]. The production level of melatonin is regulated by an endogenous clock in the suprachiasmatic nuclei (SCN) of the hypothalamus [48]. Melatonin has been on focus in recent years due to its valuable biological activities and health-promoting effects. It was found that consumption of foods containing high melatonin levels enhances the serum concentration of melatonin [49]. These foods include animal and plant sources. Animal foods, such as meat, fish, chicken, egg, milk, and dairy products, and plant foods, such as cereals, fruits, legumes, and seeds, as well as nuts are potential sources of melatonin. They can be considered as potential nutraceuticals [50].

Notably, there are studies which show the efficacy of melatonin in clinical trials. Zhao et al. examined the protective effects of melatonin on brain ischemia and reperfusion (I/R) in humans [40]. This double-blind, randomized clinical trial included 60 patients, and they took 6 mg/g melatonin orally from 3 days before surgery to 3 days after surgery. The blood samples were obtained at the following times: baseline, preanesthesia, carotid reconstruction completion, and 6, 24, and 72 h after carotid endarterectomy (CEA). It was found that melatonin significantly reduces the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and S100 calcium-binding protein β (S100β) compared to the oral placebo treatment. On the other hand, melatonin enhanced the expression of Nrf2, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in patients after CEA, showing the potential of melatonin in ameliorating brain I/R injury after CEA which is attributed to the antioxidant and anti-inflammatory effects of melatonin. Drake et al. designed a randomized, double-blind, placebo-controlled, crossover trial to investigate the effects of melatonin on nocturia in adults with multiple sclerosis (MS) [51]. Thirty-four patients with nocturia secondary to MS underwent a 4-day pretreatment monitoring phase. The patients were divided into two groups: (1) receiving 2 mg per night of capsulated sustained-release melatonin or (2) receiving 1 placebo capsule for 6 weeks. This study demonstrated that low doses of melatonin taken at bedtime have no remarkable effect on the mean number of nocturia episodes on bladder diaries, quality of life, and sleep quality. Chojnacki et al. investigated the impact of long-term supplementation of melatonin on psychosomatic disorders in postmenopausal women [52]. In this study, 60 postmenopausal women, aged 51–64 years, participated and were randomly divided into two equal groups: group I received placebo (2*1 tablet) and group II received melatonin (3 mg at the morning and 5 mg at the bedtime) for 12 months. The following indexes were determined before the start and at 12 months after placebo or melatonin administration: 17 β-estradiol, follicle-stimulating hormone (FSH), melatonin and urinary 6-sulfatoxymelatonin (aMT6s) excretion, and Kupperman index (KI) as well as body mass index (BMI). The only alteration in group I was the decreased KI. In group II, KI and MBI significantly reduced. Also, melatonin supplementation had no significant effect on the serum concentration of female reproductive hormones, 17β-estradiol, and FSH, showing the positive effect of melatonin on postmenopausal psychosomatic symptoms in women. Varoni et al. designed a triple-blind, placebo-controlled, crossover randomized clinical trial to examine the impacts of melatonin supplementation in patients with burning mouth syndrome (BMS) [53]. Twenty BMS patients, aged 35–82 years, received melatonin (12 mg/day) or placebo for 8 weeks. Then alterations in pain, sleep quality, and anxiety were evaluated. Melatonin demonstrated no greater effect than placebo in decreasing pain. Also, melatonin remarkably promoted anxiety scores and slightly increased the number of hours slept, whereas sleep quality showed no

remarkable change during the trial. Grima et al. performed a randomized controlled trial to assess the potential of melatonin for sleep disturbance following traumatic brain injury (TBI) [54]. Thirty-three patients with mild to severe TBI and sleep disturbances post-injury, with mean age of 37 years, participated and were given sustained-release melatonin formulation (2 mg) and placebo capsules for 4 weeks. The results were exciting, and it was found that melatonin significantly improves sleep quality compared to the placebo, increases sleep efficiency, and decreases anxiety. At the same time, it does not affect daytime sleepiness.

3 Melatonin and MicroRNAs

3.1 Protective Effects of Melatonin Mediated by MicroRNAs

Melatonin has the potential of modulating the expression of miRNAs to exert its protective effects (Table 1, Fig. 1). In a study, the effect of N-acetyl cysteine and melatonin in regulating miRNAs during oxidative stress-induced cardiac hypertrophy was investigated [55]. Oxidative stress increased the expression profile of miR-152 and miR-212/131. In contrast, it decreased the expression of miR-142-3p during the hypertrophic condition. It was found that melatonin and N-acetyl cysteine as antioxidants reversed the expression profile of miRNAs compared to the hypertrophic condition, showing oxidative stress in regulating anti-hypertrophy pathway elements through miRNAs and potentially protective role of melatonin and N-acetyl cysteine [55]. Liu et al. examined the impact of melatonin on endothelial-to-mesenchymal transition (EndMT) of glomerular endothelial cells (GEnCs) in diabetic nephropathy [56]. It was shown that melatonin decreases the expression of ROCK1 and ROCK2 and the activity of TGF-β2-stimulated GEnCs via increasing the expression of miR-497 to attenuate the EndMT in GEnCs in diabetic rats [56]. Ma et al. showed the role of melatonin in enhancing the therapeutic efficacy of cardiac progenitor cells (CPCs) for myocardial infarction [57]. H_2O_2 stimulated proliferation reduction and apoptosis in CPCs by enhancing the expression level of miR-98, and melatonin inhibited the increase of this miRNA by H_2O_2 in CPCs, showing a potential new strategy in improving CPC-based therapy. Meng et al. investigated the role of miR-590-3p in melatonin-induced cell apoptosis in the human osteoblast cell line [58]. It was found that miR-590-3p targets the association between septin 7 (SEPT7) to stimulate the proapoptotic effect of this miRNA

Table 1 Studies supporting the protective effects of melatonin mediated by microRNAs

In vitro/in vivo/ clinical trial	Cell line/animal model	Major outcomes	References
In vivo	High-fat diet (HFD)-treated ApoE$^-$ mice	Inhibition of endothelial cell pyroptosis through regulation of miR-223	[65]
Clinical trial	Patients with autism	Impaired levels of miR-451 levels due to the lack of melatonin synthesis	[66]
In vivo	Alcohol-fed mice	Amelioration of alcohol-induced bile synthesis through increasing miR-497 expression	[67]
In vitro	GC-1 spg cells	Induction of cell growth in the mouse-derived spermatogonial cell line via miR-16	[68]
In vitro	The rat model of brain inflammation	Modulation of neonatal brain inflammation by miR-24a, miR-14a, and miR-126	[69]
In vitro	Cardiac progenitor cells	Inhibition of premature senescence of e-kit(+) cardiac progenitor cells by promoting miR-675	[70]
In vitro	Hepatocytes	Amelioration of ER stress-mediated hepatic steatosis by miR-23a	[71]
In vivo	The rat model of amnesia	Attenuation of scopolamine-induced memory/synaptic disorder via rescuing EPACs/miR-124/EGr1 pathway	[72]

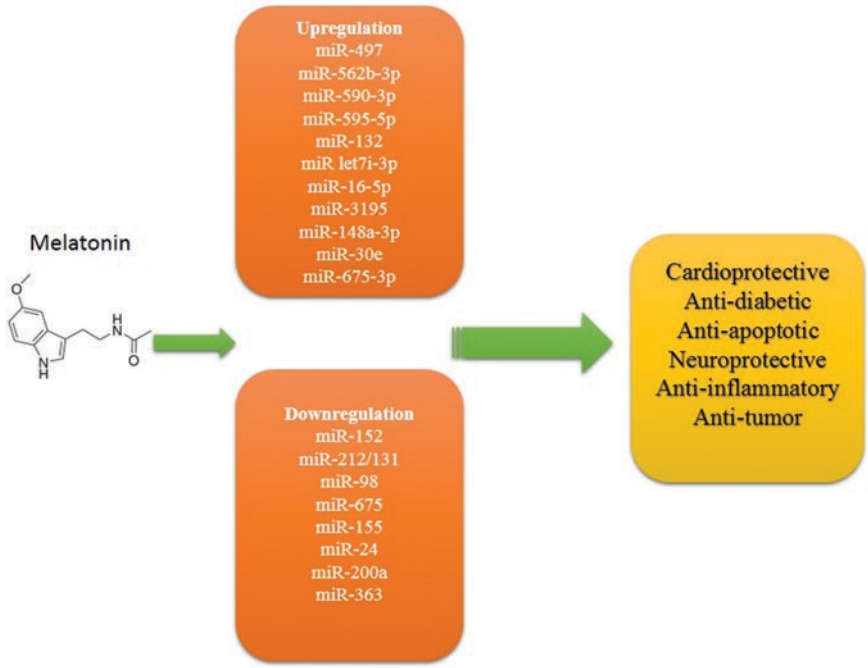

Fig. 1 Valuable therapeutic and biological activities of melatonin mediated by microRNA modulation

in human osteoblasts and higher concentrations of melatonin lead to the inhibition of miR-590-3p expression [58]. Wu et al. indicated the effect of melatonin in increasing the chondrogenic differentiation of human mesenchymal stem cells [59]. It was found that melatonin positively affects miR-526b-3p and miR-595-5p expression. Subsequently, these miRNAs increase the SMAD1 phosphorylation by targeting SMAD7, resulting in the chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells [59]. Yang et al. demonstrated the protective effect of melatonin against early brain injury (EBI) after subarachnoid hemorrhage [60]. It was shown that melatonin treatment decreases the expression of H19, miR-675, and neural growth factor (NGF), resulting in the attenuation of neurological deficits and reduction in brain swelling [60]. Zhao et al. examined the protective effect of melatonin against Aβ-induced neurotoxicity in primary neurons [61]. Melatonin increased the expression level of miR-132 and downregulated PTEN and FOXO3a and subsequently inhibited the nuclear translocation of FOXO3a and suppressed its proapoptotic pathways, resulting in the neuroprotective effects of melatonin [61].

In a study conducted by Wu and colleagues, the ameliorative effect of melatonin on radiation-induced lung injury was evaluated [62]. It was found that melatonin significantly attenuates oxidative stress, infiltration of macrophages, and neutrophils and suppresses NLRP3 inflammasome. Mechanistically, these protective effects are mediated by upregulation of miR-30e [62]. Besides, melatonin has demonstrated great potential in treating pulmonary arterial hypertension (PAH) [63]. Melatonin remarkably alleviates the systolic pulmonary artery pressure (SPAP), the ratio of medical thickening, and the weight of right ventricle (RV), left ventricle (LV), and interventricular septum (IVS). Mechanistically, it was found that melatonin directly upregulates the expression of miR-0675-3p and indirectly downregulates the expression of miR-200a by H19 to exert its protective effect [63]. Interestingly, melatonin is also an efficient candidate in treating vitamin A deficiency (VAD)-associated deformities [64]. It was found that VAD rats have an increased level of

whole-embryo expression of miR-363. Furthermore, miR-363 diminishes proliferation and neuronal differentiation via notch1 inhibition, resulting in spinal deformities. It was demonstrated that melatonin inhibits the expression of miR-363 to suppress spinal deformities [64].

3.2 Antitumor Effects of Melatonin Mediated by MicroRNAs

Gu et al. examined the inhibitory effect of melatonin on the proliferation and invasion of glioma cells [73]. In this study, human glioma cell lines U87, U373, and U257 were used, and it was found that melatonin decreases the expression level of miR-155 to inhibit the proliferation and invasion of glioma cells [73]. Mori et al. investigated the antitumor activity of melatonin on HCT116 and MCF-7 cells [74]. It was shown that long-term treatment with melatonin could reduce miR-24 levels posttranscriptionally, resulting in decreased survival of colon and breast cancer cells [74]. Lee et al. indicated the anticancer property of melatonin in human breast cancer cell lines [75]. They showed that melatonin changes the expression profile of miRNAs (has-miR-362-3p and has-miR-1207-3p) to inhibit breast cancer cells [75]. In another study, Wang et al. showed the antitumor activity of melatonin against hepatocellular carcinoma [76]. It was demonstrated that melatonin treatment remarkably prevented the proliferation, migration, and invasion capacities of Huh7 and HepG2 cell line via stimulating the expression of miRNA let7i-3p in cells. Zhu et al. examined the antiproliferation effect of melatonin on gastric cancer cells [77]. It was found that melatonin increases the expression of miR-16-5p, and subsequently, this miRNA negatively affects the Smad3 pathway, leading to the inhibitory effect on gastric cancer cells [77]. Sohn et al. showed the antiangiogenic effect of melatonin in hypoxia PC-3 prostate cancer cells [71]. It demonstrated that melatonin enhances the expression level of miR-3195 and miRNA-374b, resulting in inhibition of typical angiogenic protein VEGF at the protein level and induction of VEGF production [71]. Lacerda and coworkers assessed the antitumor effect of melatonin in breast cancer cells [78]. In this study, MDA-MB-231 cells were used, and it was found that melatonin effectively suppresses the proliferation, migration, and invasion of breast cancer cells through upregulation of miR-148a-3p [78].

4 Conclusion

MicroRNAs, as significant modulators of genes, significantly affect a number of cellular processes. This review focused on the modulatory effect of melatonin on microRNAs and exhibited how melatonin affects microRNAs to exert its therapeutic and biological activities. Cardioprotective, antidiabetic, antiapoptotic, neuroprotective, anti-inflammatory, and antitumor are important effects of melatonin resulting from microRNA modulation. It was shown that melatonin upregulates/downregulates microRNAs in various conditions to exert its activities. Still, in terms of antitumor effect, it mainly enhances the expression profile of microRNAs. However, more studies are needed to describe the impacts of melatonin on microRNAs in detail.

Conflict of Interest The authors declare no conflict of interest.

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Antioxidant Effects of Trehalose in an Experimental Model of Type 2 Diabetes

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Abstract

Background: Oxidative stress that occurs as a consequence of the imbalance between antioxidant activity and free radicals can contribute in the pathogenesis of metabolic disorders, such as type 2 diabetes mellitus (T2DM). Antioxidant therapies have been proposed as possible approaches to treat and attenuate diabetic complications. The purpose of this study was to evaluate potential antioxidant effects of trehalose on oxidative indices in a streptozotocin (STZ)-induced diabetic rat model.

Methods: Diabetic rats were divided randomly into five treatment groups (six rats per group). One test group received 45 mg/kg/day trehalose via intraperitoneal injection, and another received 1.5 mg/kg/day trehalose via oral gavage for 4 weeks. Three control groups were also tested including nondiabetic rats as a normal control (NC), a nontreated diabetic control (DC), and a positive control given 200 mg/kg/day metformin. Levels of thiol groups (-SH), and serum total antioxidant capacity were measured between control and test groups. In addition, superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities were assessed.

Results: In both oral and injection trehalose-treated groups, a marked increase was observed in serum total antioxidant capacity (TAC) ($p > 0.05$) and thiol groups (-SH) ($p < 0.05$). Also, SOD and GPx activities were increased after 4 weeks of treatment with trehalose.

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Conclusion: In conclusion, the present results indicate ameliorative effects of trehalose on oxidative stress, with increase antioxidant enzyme activities in STZ-induced diabetic rats.

Keywords

Trehalose · Diabetes mellitus · Oxidative stress · Total antioxidant capacity · Malondialdehyde · Superoxide dismutase

1 Introduction

Type 2 diabetes mellitus (T2DM) is defined as a permanent condition of hyperglycemia with predominant impacts on multiple metabolic pathways and physiologic functions of organs, caused by beta-cell dysfunction and insulin deficiency, tissue insulin resistance, or other metabolic alterations such as disruption of the redox balance and stress [1–3]. Oxidative stress has the potential to induce cell death mechanisms associated with tissue damage and multiple diabetic complications, including diabetic cardiomyopathy, retinopathy, and nephropathy [4]. This can occur via activation of nuclear factor kappa B (NF-κB), p38 MAPK, and c-jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK) signaling pathways [5]. Indeed, there is an association between hyperglycemia-induced oxidative stress and local or systemic inflammation via increased pro-inflammatory cytokine production and macrophage infiltration [6]. Due to the deleterious outcomes of oxidative stress on diabetes complications, application of antioxidant therapies has been considered as a potential means of reducing T2DM pathogenesis through a decrease in free radicals and an increase in antioxidant enzyme activities [7–9].

Trehalose (mycose) is a carbohydrate with a disaccharide structure naturally produced by a wide range of organisms from prokaryotes to plants, except humans [10]. This sweetener molecule is frequently applied in food and drug industries and has been found to exert important biological impacts and modulate several metabolic pathways after consumption [11–14]. Experimental studies have indicated trehalose functions as an antioxidant, anti-inflammatory, and autophagy enhancer, which suppresses oxidative stress, inflammation, and autophagy-related disorders such as diabetes [15–17], atherosclerosis [18, 19], and Parkinson [20], Alzheimer [21, 22], and Huntington [23] diseases. Antidiabetic effects of trehalose can be linked to improving pathophysiological mechanisms such as inflammation and oxidative stress, pancreatic islet function, and lipid profile correction [24]. The role of trehalose as a natural antioxidant has been reported in in vitro and in vivo studies [25–28]. Here, we have attempted to determine the antioxidant effects of intraperitoneal (IP) and oral trehalose administration on total antioxidant capacity (TAC) and total thiols, along with the activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) as markers of oxidative stress in a streptozotocin (STZ)-induced diabetes rat model. In addition, antioxidant effects of trehalose were compared to those of the standard T2DM medication, metformin. The results showed that both oral and IP routes of trehalose administration suppressed oxidative stress, confirming the trehalose therapeutic potential in controlling oxidative stress-induced complications of diabetes in animal models.

2 Material and Methods

2.1 Animal

Male Wistar albino rats (8 weeks old, 180–200 g) were bred and housed in the Laboratory Animal Research Center of Medicine Faculty, Mashhad University of Medical Sciences, Mashhad, Iran. All animal experiments were approved by the Institutional Ethics Committee and Research Advisory Committee of the Mashhad University of Medical Sciences and the National Institute for Medical Research Development (NIMAD). The animals were maintained using a 12:12-h day-night cycle, at a constant 22 ± 2 °C, and

humidity of 45–64%. Over the entire experimental procedure, the rats were fed with a standard rodent diet and water ad libitum. All rats were anesthetized with IP injections of thiopental sodium and blood samples collected after 4 weeks of treatment at study termination.

2.2 Induction of Rat T2DM Model

Non-insulin-dependent diabetes mellitus was induced by intravenous injection of single 60 mg/kg dose of streptozotocin in overnight-fasted rats (Masiello et al., 1998). STZ was dissolved in citrate-buffered saline (0.1 M, pH 4.5). Hyperglycemia was confirmed with blood glucose levels >180 mg/dL, determined at 72 h and then on day 7 after injection, and diabetic rats were included in this study. Two groups of diabetic rats (six rats per group) were treated daily with 45 mg/kg/day trehalose via i.p. injection and 1.5 g/kg/day via oral gavage for 4 weeks. Nondiabetic rats (n = 6) were used as the normal control (NC) group that received citrate buffer (i.p.). The diabetic (DC) and positive control groups received saline buffer and metformin (200 mg/kg/day), respectively.

2.3 Total Thiol (-SH) Group

Total thiol groups (-SH) were measured using the Kiazist kit according to the manufacturer's instructions. In this assay, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) reacts with reduced sulfhydryl (-SH) groups in the serum, resulting in a yellow-colored complex, which is detectable at 405 nm.

2.4 Total Antioxidant Capacity (TAC)

The potential of samples for reducing ferric (Fe^{+3}) to the ferrous form (Fe^{+2}) was considered as the total antioxidant capacity (TAC) and measured by a colorimetric method. For this assay, 150 μL Kiazist TAC reagent was added to 30 μL sample or standard and incubated at room temperature for 45 min. The absorbance was read in 450 nm.

2.5 Antioxidant Enzyme Activity Assay

The levels of antiperoxidative enzymes, including GPx and SOD, were determined in the serum of diabetic rats using specific assay kits (Kiazist, Iran). The measurement of SOD and GPx activities was based on reducing free radicals produced by the xanthine/xanthine oxidase system and conversion of hydrogen peroxide to water, accompanied by glutathione oxidation, respectively.

2.6 Statistical Analysis

Statistical analysis was performed with Microsoft Excel (2019) and GraphPad Prism version 8 software. The results were analyzed using one-way analysis of variance (ANOVA) and the Tukey's multiple comparison posttest to evaluate the significance of differences between treatment groups. Results with $p < 0.05$ were considered as statistically significant.

3 Results

3.1 Evaluation of Reduced (Free) Thiol (-SH) Groups and Total Antioxidant Capacity

IP and oral administration of trehalose led to an increase in TAC and thiols, with lower levels in diabetic rats than the healthy control group (nondiabetic). Although TAC alterations did not reach statistical significance (Fig. 1), total thiol groups were increased significantly ($p < 0.05$) in treated groups compared to nontreated diabetic control, and the effect of IP trehalose administration was more potent than the oral route (Fig. 2).

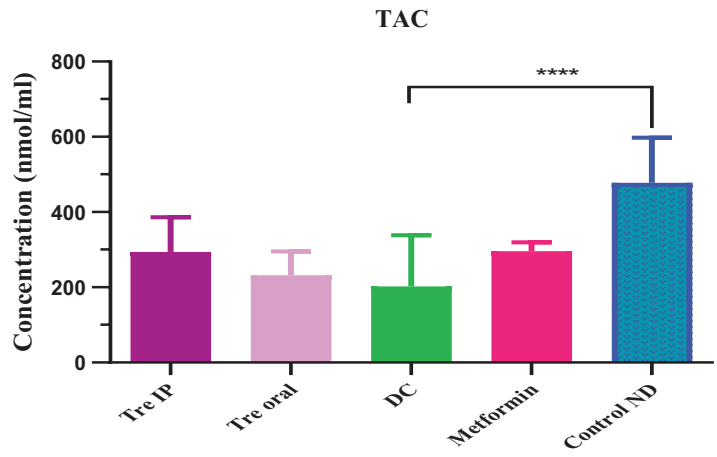

Fig. 1 Antioxidant effect of trehalose on total antioxidant capacity (TAC) in five groups. Data are given as the mean ± standard error of the mean. *P < 0.05, ** P < 0.01, ***P < 0.001, and ****P < 0.0001

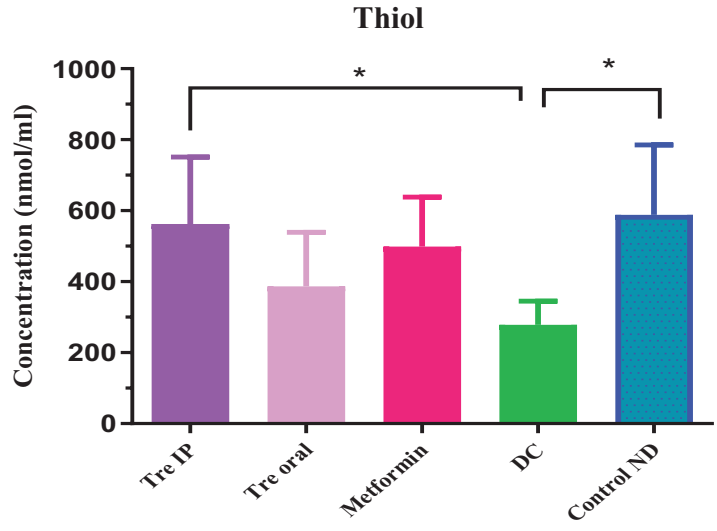

Fig. 2 Antioxidant effect of trehalose on total thiol groups (-SH) in five groups. Data are the mean ± standard error of the mean. *P < 0.05, ** P < 0.01, ***P < 0.001, and ****P < 0.0001

3.2 Evaluation of SOD and GPx Antioxidant Enzyme Activities

For studying the effect of trehalose to induce enzymes that counteract free radical production, we measured the activities of SOD and GPx. These enzymes were increased in both the IP and oral trehalose-treated groups with a stronger effect of oral trehalose when compared with diabetic control rats. Differences in oral (P = 0.07) and IP trehalose (P = 0.89) groups were not significant for SOD (Fig. 3), whereas a significant increase was observed in GPx activity (P < 0.05) (Fig. 4).

4 Discussion

Diabetes is a chronic disease characterized by hyperglycemia resulting from deficiency of insulin secretion or insulin resistance, leading to microvascular and macrovascular complications that can damage different organs and tissues [29]. Hyperglycemia causes oxidative stress through multiple pathways, which is considered as a trigger for developing vascular complications of T2DM [30, 31]. High glucose levels promote the activity of some enzymes, including protein kinase C and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to aug-

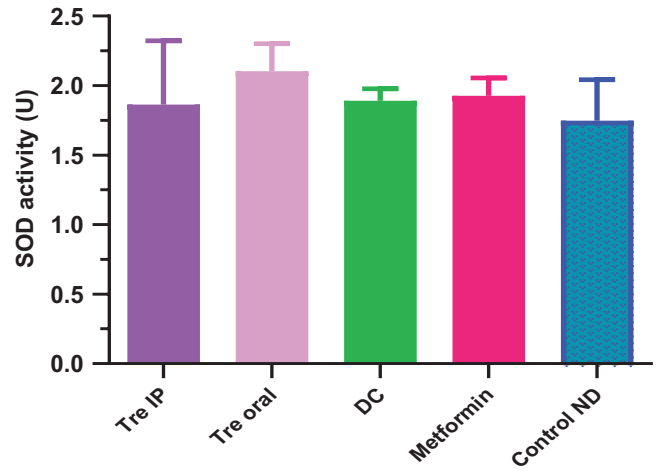

Fig. 3 Antioxidant effect of trehalose on SOD activity

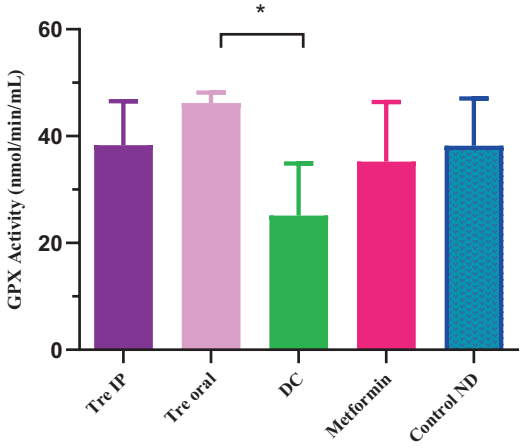

Fig. 4 Antioxidant effect of trehalose on GPx activity. *P < 0.005

mentation of reactive oxygen species (ROS) and oxidative stress, which in turn promote cell damage and tissue injuries [15]. Free radicals may attack cell membranes resulting in lipid peroxidation and an increase in MDA as a sensitive index of the systemic redox status and potential disease progression [32]. Besides lipid oxidation effects, ROS can oxidize free thiols and decrease circulating sulfhydryl (SH) concentrations, leading to a reduction in total antioxidant capacity [33]. Moreover, the alterations of antioxidant enzyme patterns are a characteristic feature of the uncontrolled diabetic state associated with a higher incidence of diabetic complications [34]. Since oxidative stress is a critical pathogenic factor for secondary complications of diabetes, the antioxidant therapy approach may be a useful strategy to treat diabetes by controlling free radical production; increasing intracellular antioxidant defenses, along with protective mechanisms against oxidative stress-induced apoptosis; and preserving β-cell function [35–37]. This study aimed to evaluate the antioxidant effects of trehalose as a natural antioxidant compound in T2DM. The changes in antioxidant markers such as serum thiol levels, and TAC, as well as the activity of GPx and SOD, were determined following 4 weeks of trehalose administration in STZ-induced diabetic rats.

Trehalose is a nonreducing disaccharide consisting of two glucose units in an α,α-1,1-glycosidic linkage, synthesized in numerous organisms from plants and bacteria to invertebrates and yeast [38]. Recent studies indicate that trehalose may decrease blood glucose and ameliorate insulin sensitivity and, thereby, may serve as a potential non-pharmacological agent for the management of diabetes [24]. We evaluated this possibility in our previous animal study and confirmed trehalose antidiabetic effects in a rat model of type 2 diabetes. The antioxidant effects of trehalose have also been assessed in different in vitro and in vivo studies [39, 40]. Treatment with trehalose in preclinical studies revealed that this antioxidant molecule significantly decreased the amount of ROS and H_2O_2 levels in a dose-dependent manner [15, 25] and upregulated anti-

oxidant gene expression of SOD, glutathione (GSH), and catalase (CAT) via promotion of nuclear translocation of Nrf2 [25, 41]. Although antioxidant enzyme-dependent defenses play a crucial role in scavenging free radicals produced under oxidative stress [42, 43], there have been conflicting reports on SOD and GPx activity in diabetes mellitus. Both increased and decreased antioxidant enzyme activities have been reported [44–49], while some studies have shown no change in comparison to nondiabetic healthy controls [50, 51]. In diabetes, impaired pancreatic β-cells may express low physiological levels of the antioxidant enzymes SOD and GPx [52–54]. On the other hand, elevated ROS levels and increased production of O2− may increase the total antioxidant enzyme activity, suggesting a possible adaptive response to oxidative status [55]. Our results indicated a marked decrease in GPX activity in the diabetic rats, whereas this activity was significantly increased in both trehalose-treated groups compared with the DC group. A similar trend was found for SOD activity after 4 weeks of trehalose intervention, though the differences were not statistically significant. Experimental models have determined that antioxidant compounds can change TAC in serum or plasma; therefore, monitoring plasma TAC may be a valuable index for oxidative burden [56, 57]. However, no prior study has investigated the effects of trehalose on plasma TAC levels; our research reported that TAC and the amount of free thiol increased during the treatment process. Differences in TAC marker was significant between the IP-treated trehalose group and DC group. Intraperitoneal administration of trehalose had greater potential efficacy than oral administration, which could be due to the higher bioavailability of trehalose in the IP route.

As mentioned earlier, previous studies displayed in vitro antioxidant activities of trehalose, and here we carried out the in vivo experimental study to support an antioxidant effect of trehalose in T2DM model during 4 weeks of treatment. The obtained results suggest that trehalose might be regarded as a safe antioxidant supplement for diabetic subjects in clinical studies over a longer timeframe.

In conclusion, regarding the importance of oxidative stress in activating intracellular signaling pathways and the pathogenesis of multiple disorders, natural antioxidant products could be a potential therapeutic strategy to manage and reduce oxidative damage. The findings of our study demonstrated that trehalose administration could enhance antioxidant capacities, and protect antioxidant enzyme activity slightly; however, a clear and comprehensive understanding of the effect of trehalose on antioxidant enzymes needs further investigation.

Conflict of Interests None.

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Investigation of the Effects of Trehalose on Glycemic Indices in Streptozotocin-Induced Diabetic Rats

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Abstract

Background and Aim: Diabetes is a chronic metabolic disorder with considerable morbidity and mortality because of its associated complications that has become a challenging health problem worldwide. Trehalose (mycose) is a nonreducing disaccharide with a unique therapeutic potency without adverse effects, which has been found to improve glucose metabolism and homeostasis in different diabetes models. We hypothesized that trehalose can reduce blood glucose and improve insulin sensitivity. We have conducted this study to evaluate the effect of trehalose on glycemic indices in streptozotocin (STZ)-induced diabetic rats.

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Method: Fourteen diabetic rats were randomly assigned in two treatment groups (seven rats per group) that received trehalose at a dose of 1.5 g/kg/day via oral gavage and a dose of 45 mg/kg/day via intraperitoneal (i.p.) injection. Three control groups, including a positive control, diabetic control (DC), and nondiabetic rats as a normal control group (NC), received metformin (200 mg/kg/day), normal saline, and citrate buffer, respectively. The levels of fasting blood glucose (FBG) were measured at baseline (week 0) and after 4 weeks of treatment. Moreover, an oral glucose tolerance test (OGTT) was performed at the end of the study to determine glucose tolerance.

Results: The results showed that FBG levels were significantly decreased by −66% (−221 ± 65 mg/dL, p = 0.01), −40% (−114 ± 46 mg/dL, p = 0.02), and − 72% (−191 ± 68 mg/dL, p = 0.01) in trehalose-oral, trehalose-i.p., and metformin groups, respectively, after 4 weeks of administration. Evaluating the results of glucose tolerance test and analysis of corresponding areas under the glucose curve ($AUC_{glucose}$) over 180 min indicated that glucose tolerance was significantly improved in the trehalose-i.p. group (p = 0.03) compared to DC group.

Conclusion: Our findings suggested that trehalose administered via i.p. route might reduce FBG levels and improve glycemic control in STZ-induced diabetic rats.

Keywords

Diabetes · Trehalose (mycose) · Oral glucose tolerance test · Insulin tolerance test · Streptozotocin

1 Introduction

The prevalence of diabetes mellitus (DM) is growing exponentially globally [1]. DM is a chronic disorder affecting various metabolic pathways as well as physiologic functions of most organs resulting in diabetic complications [2, 3]. Despite the growth of various pharmacological agents for the management of diabetes, millions of deaths were directly caused by diabetes [4]. DM can result in the development of various forms of diabetic complications through destruction of pancreatic beta-cells (type1 diabetes), lack of sufficient insulin receptors (IR) on the cell membranes, glucose transporter deficiency, and insulin resistance (type 2 diabetes) [5]. It can also result in various other metabolic changes such as fibrosis and apoptosis, oxidative stress, lipid metabolism disruption, and inflammation. Since high blood glucose can damage tissues and organs, various natural or synthetic pharmaceutical compounds have been developed to normalize blood glucose levels and improve diabetic complications.

Trehalose (also known as mycose) is a nonreducing disaccharide composed of two D-glucose linking by the reducing end α-1,1. It is present in various organisms and plants as a source of energy [6]. In nature, the α-α form is the dominant isomer than α-β and β-β forms [7]. Glycosidic bond demonstrates stability and is resistant to acid and α-glycosidase cleavage [8]. Trehalose is also distinguished from other disaccharides by the high number of OH groups [9] and intramolecular hydrogen bonds resulting in

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stronger water interactions in the solution [10, 11]. This sweetener molecule is frequently used as an additive in the food industry and as a moisturizing agent in various cosmetic products. Also, trehalose is used as one of the components for stabilizing commercialized pharmaceutical products such as antibodies, enzymes, liposomes, and the genomic material [12]. Besides these current applications, trehalose is proposed as a promising therapeutic candidate for various disease states. There is evidence indicating that it can normalize glucose metabolism and enhance insulin response in patients with diabetes, leading to an improvement in glycemia [13, 14]. Trehalose can modulate glucose homeostasis and improve hyperglycemia via ameliorating various pathophysiological mechanisms such as improving beta-cell function, oxidative stress, and inflammation, as well as by upregulating the expression of IRS-1 (insulin receptor substrates-1) and IRS-2 [15] and normalizing lipid profiles [16].

In the current in vivo study, we attempt to determine the ameliorative effects of trehalose on hyperglycemia.

2 Material and Methods

2.1 Animal

Thirty-five male Wistar albino rats (179 ± 5.5 g) were obtained from the laboratory animal research center of medicine faculty, Mashhad University of Medical Sciences, Mashhad, Iran. Animal welfare protocol was approved by the Institutional Ethics Committee and Research Advisory Committee of the Mashhad University of Medical Sciences. Animals were housed in a specific pathogen-free environment in positive pressure rooms at a constant temperature of 22 ± 2 °C with a standard 12-h day/12-h night cycle and fed a standard rodent diet and water ad libitum. All efforts were made to minimize suffering, and all animals were euthanized by intraperitoneal injection of thiopental sodium at the end of the study [17, 18].

2.2 Induction of Rat T2DM Model

Diabetes was induced in the overnight fasted (12 h) rats by i.p. injection of a single dose (60 mg/kg) of streptozotocin (STZ; Sigma-Aldrich) freshly dissolved in citrate-buffered saline (0.1 M, pH 4.5). On the third and seventh days after STZ injection, fasting blood glucose (FBG) levels were measured, and rats with blood glucose levels >180 mg/dL were subjected to the study. Two groups of diabetic rats (seven rats per group) were treated daily by trehalose at the dose of 1.5 g/kg/day via oral gavage and 45 mg/kg/day via i.p. injection for 4 weeks. The positive control group and diabetic control (DC) group received metformin (200 mg/kg/day) and saline buffer, respectively. Nondiabetic rats (n = 7) acted as normal control (NC) group receiving citrate buffer, intraperitoneally. Before (week 0) and after 4 weeks of treatment, the tail vein bleeding was performed to measure the levels of FBG.

2.3 Oral Glucose Tolerance Test (OGTT)

To measure the glucose tolerance of treated animals, an oral glucose tolerance test (OGTT) was conducted on overnight fasted rats gavaged with glucose at the dose of 2 g/kg after 4 weeks of treatment. Briefly, glucose solution was orally given, and blood glucose levels were measured by a glucometer (EasyGluco, South Korea) at time point 0 min (before glucose load), 30, 60, 90, 120, 150, and 180 min after oral glucose load [19]. The results were analyzed as the integrated area under the curve for glucose ($AUC_{glucose}$), calculated by trapezoid rule using GraphPad Prism version 7.04.

2.4 Statistical Analysis

Statistical analysis was performed by SPSS Statistics version 20 software and GraphPad Prism version 7.04 software. The results were analyzed using one-way ANOVA and Dunnett's post hoc multiple comparison tests to evaluate

the significance of differences between animal groups. Values were expressed as mean ± SD and lower-upper 95% confidence interval of the mean. Results with $p < 0.05$ were regarded as statistically significant.

3 Results

3.1 Effect of 4-Week Treatment on FBG Levels

Measuring the FBG levels before (week 0) and after (week 4) treatment showed that FBG levels were significantly decreased by −66% (−221 ± 65 mg/dL. p = 0.01), −40% (−114 ± 46 mg/dL, p = 0.02), and −72% (−191 ± 68 mg/dL, p = 0.01) in trehalose-oral, trehalose-i.p., and metformin groups, respectively, while there were no significant changes in FBG levels of control groups NC and DC (Fig. 1).

3.2 Oral Glucose Tolerance Test (OGTT)

An OGTT was performed to evaluate glucose tolerance in treated diabetic rats. Oral administration of glucose (2 g/kg) in the DC rats showed a significant increase in blood glucose levels (after 60 min) and exhibited a significant impairment in glucose tolerance to exogenously administered glucose compared to the NC rats. The trehalose (i.p.)-treated diabetic rats recorded a significant reduction in blood glucose levels over 180 min compared to the DC rats (Fig. 2a). The integrated areas under the glucose curve ($AUC_{glucose}$) over 180 min of the trehalose (i.p.)-treated diabetic rats were significantly ($p < 0.001$) higher than the NC rats. Analyzing AUC values demonstrated that blood glucose levels were significantly ($p = 0.03$) decreased by 8.5% in the trehalose-ip group in comparison with the DC group. However, com-

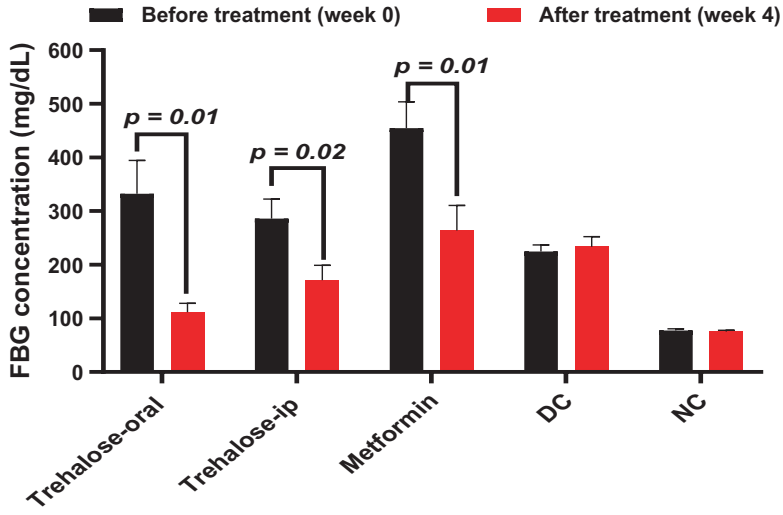

Fig. 1 Analysis of fasting blood glucose (FBG) before (week 0) and after (week 4) treatment. The results showed that trehalose-oral (1.5 g/kg/day), trehalose-i.p. (45 mg/kg/day), and metformin as the positive control (orally, 200 mg/kg/day) could significantly decrease FBG levels after 4 weeks of administration by −66% (−221 ± 65 mg/dL. p = 0.01), −40% (−114 ± 46 mg/dL, p = 0.02), and −72% (−191 ± 68 mg/dL, p = 0.01), respectively, while there were no significant changes in FBG levels of both normal control (NC) and diabetic control (DC) groups Values are expressed as mean ± SEM. The results were analyzed using the paired two-tailed t-test to evaluate the significance of the differences. P-values < 0.05 were statistically considered significant

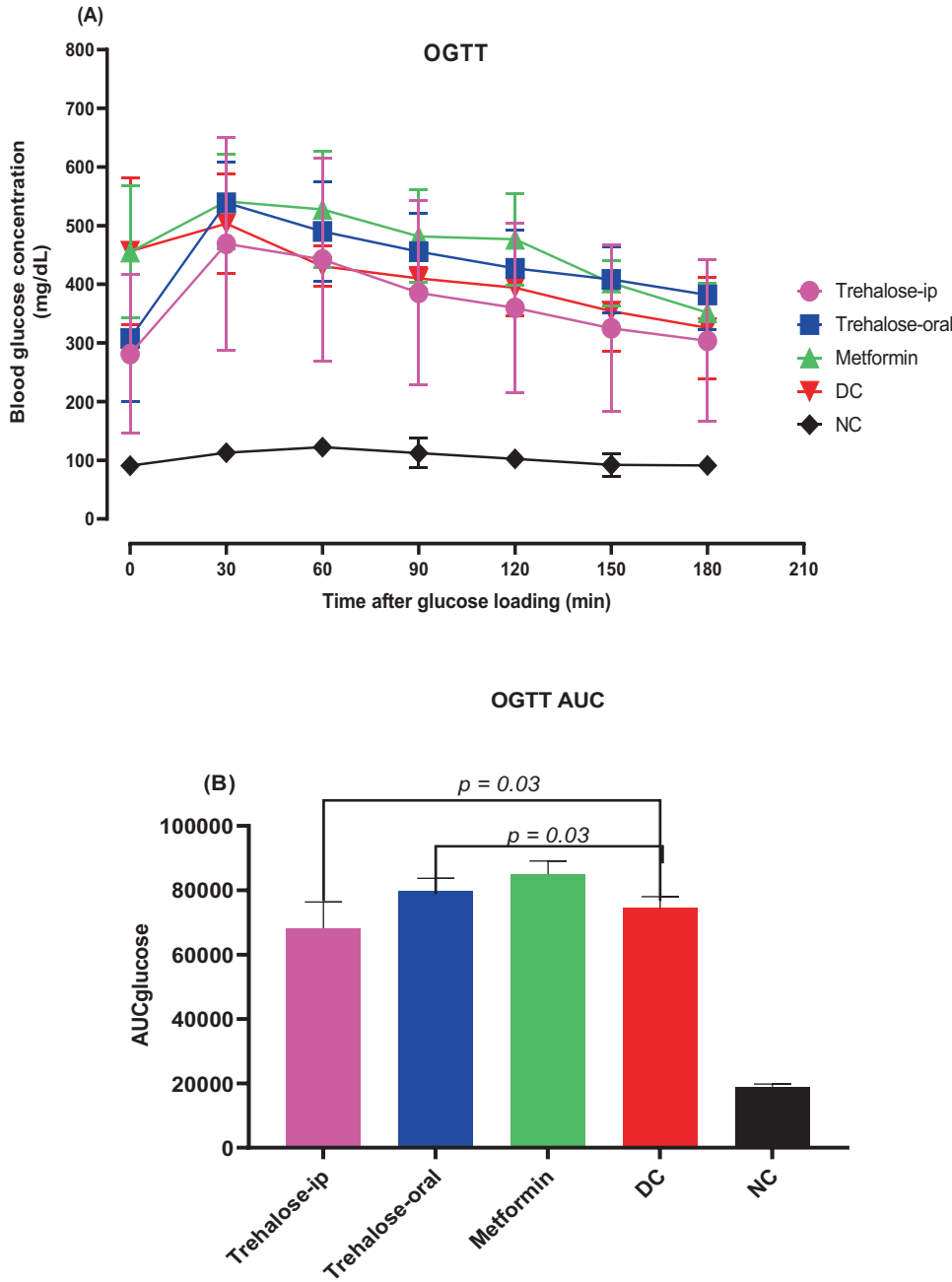

Fig. 2 Evaluating glucose sensitivity via (**A**) oral glucose tolerance test (OGTT) and (**B**) analysis of corresponding areas under the glucose curve ($AUC_{glucose}$). The results revealed that glucose tolerance was significantly improved in trehalose-i.p. group, while it was significantly diminished in trehalose-oral group. Measurement of the integrated areas under the glucose curve ($AUC_{glucose}$) over 180 min demonstrated that blood glucose levels were significantly (p = 0.03) decreased by 8.5% in the trehalose-i.p. group in comparison with DC group. However, a comparison of AUC value in trehalose-oral and DC groups showed that blood glucose levels were significantly increased in trehalose-oral group (p = 0.03) Values are expressed as mean ± SEM. The results were analyzed using one-way ANOVA, followed by Dunnett's post hoc multiple comparison tests to evaluate the significance of the differences between groups. P-values < 0.05 were statistically considered significant

parison of AUC value in trehalose-oral and DC groups showed that blood glucose levels were increased in trehalose-oral group ($p = 0.03$) (Fig. 2b).

4 Discussion

The main purpose of the present study was the evaluation of glycemic and insulinemic responses following trehalose treatment in rat model of type 2 diabetes. Although there is evidence showing that trehalose can modulate insulin sensitivity and glucose metabolism [13, 14], to the best of our knowledge, there is still scant information on the antihyperglycemic effects of trehalose for the management of diabetes. In the current investigation, i.p. administration of trehalose effectively ameliorated the increased FBG levels and glucose tolerance in STZ-induced diabetic rats. Analysis of the data between treatment and control groups showed that after 4 weeks of daily trehalose treatment, FBG levels were significantly decreased. Glucose tolerance was also significantly improved in the trehalose (i.p.)-treated groups, which can be due to more bioavailability of trehalose in i.p. route. This result is according to the other preclinical research investigations in which consuming parenteral trehalose resulted in improved glucose metabolism [19].

Several experimental studies have demonstrated the beneficial effects of trehalose in the modulation of glucose metabolism via different pathways. Pancreatic beta-cell dysfunction in producing insulin is directly linked to the onset and development of DM. Emerging evidence indicates that trehalose, particularly after long-term consumption, can improve pancreatic islet function and efficiency [20] by suppressing apoptosis [21, 22], modulating the autophagy process [23], and ameliorating islet amyloid polypeptide (IAPP) synthesis. Abnormal aggregation of (IAPP) into amyloid fibrils has been implicated in the beta-cell dysfunction and type 2 diabetes, and trehalose can prevent protein misfolding or aggregation via chaperone-like activity and contributing to the removal of accumulated proteins [24]. Trehalose efficiently reverses high glucose-suppressed autophagy and induces autophagosome complexes to remove either damaged cellular organelles or protein aggregates in both in vitro and in vivo diabetes models [23, 25, 26]. Moreover, trehalose can correct the lipid profile closely linked to insulin resistance disorders. Indeed, trehalose effectively improves insulin sensitivity and glucose homeostasis by modulating the secretion of adipokines and increasing the adiponectin release [27]. Antidiabetic effects of trehalose may also stem from its anti-inflammatory effect by reducing inflammatory cytokines such as TNF-α (tumor necrosis factor-alpha), MCP-1 (monocyte chemotactic protein-1), and PAI-1 (plasminogen activator inhibitor-1) [28]. Upregulation of the expression of insulin receptor substrates-1 (IRS-1) and IRS-2 [15] that have an important role in insulin response may be another effective mechanism by which trehalose improves insulin resistance and the effects on glycemic indices. Besides preclinical research, it was further supported by clinical trials that showed consumption of trehalose could effectively decrease levels of blood glucose in healthy subjects and patients with impaired glucose tolerance [29, 30]. In a placebo-controlled, double-blind trial in 34 subjects with body mass index (BMI) > 23 kg/m^2 classified as high risk for metabolic syndrome and type 2 diabetes, ingesting 10 g/day of trehalose with meals for 12 weeks reduced blood glucose concentration and contributed to reducing risk factors for lifestyle-related diseases [31]. These findings suggest that trehalose can be proposed as a suitable sugar substitute for patients with diabetes not only for lowering sweetness than sucrose (45% relative sweetness) [32] but also for the beneficial effects on glucose control and insulin sensitivity.

In conclusion, we have demonstrated that trehalose i.p. administration could effectively ameliorate the increased FBG levels and glucose tolerance in streptozotocin-treated rat model of type 2 diabetes. This suggests that trehalose has

the potential to be a non-pharmacological compound for the management of hyperglycemia in patients with diabetes; however, future clinical trials are required to evaluate this further.

Conflict of Interests None.

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Hepatoprotective Effect of Trehalose: Insight into Its Mechanisms of Action

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Abstract

Trehalose is a nonreducing disaccharide formed by two glucose molecules. It has been shown that trehalose can protect proteins and cellular membranes against the adverse effects of different types of stress, such as dehydration, cold, heat, and oxidation. Chronic liver disease has emerged as an important cause of morbidity and mortality throughout the world. This disaccharide has received attention for its hepatoprotective activities against liver damage. The main mechanisms underlying the hepatoprotective action of trehalose are reducing inflammatory signaling, enhancing antioxidant defense, and induction of autophagy.

Keywords

Trehalose · Hepatoprotection · End-stage liver disease · Cirrhosis

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1 Introduction

Trehalose, also known as mycose, is a nonreducing disaccharide formed by two molecules of glucose [1]. In the mid-nineteenth century, the French chemist Marcellin Berthelot isolated trehalose from *Trehala manna*, a sweet substance isolated from the nests and cocoons of the Syrian coleopterous insect (*Larinus maculatus*, *Larinus nidificans*) which feeds on the foliage of various thistles [2, 3]. Trehalose is present in most organisms except for vertebrates [4]. High hydrophilicity, chemical stability, and strong resistance to acid hydrolysis and cleavage by glucosidases are conferred due to its nonreducing property. Trehalose was shown to act as a molecular chap-

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erone and prevent proteins from denaturing [5, 6], as well as against the adverse effects of stresses, such as desiccation, dehydration, cold, heat, and anoxia [1]. Many studies have been done on the biological and chemical properties of trehalose and its role in living organisms, as well as on its protective effects in many diseases [2, 3, 7–11]. In short, trehalose treatment prevented inflammation and oxidative stress, improved dopaminergic and tau pathology in parkin-deleted/tau-overexpressing mice via autophagy activation [12], improved vasospasm following experimental subarachnoid hemorrhage in rabbits [13], induced chaperone molecules accompanied by autophagy in a mouse model of Lewy body disease [14], alleviated polyglutamine-induced protein aggregation in a mouse model of Huntington disease [15], slowed down amyotrophic lateral sclerosis progression by enhancing autophagy in motor neurons [16], and increased progranulin expression in human and mouse models of progranulin haploinsufficiency [17], reduced hepatic endoplasmic reticulum stress and inflammatory signaling in aged mice [18], attenuated hepatic steatosis [19], and protected against liver and lung injury in the endotoxic shock rat model [20].

The liver is an important organ that is responsible for all metabolic processes and physiological processes such as bile production, energy generation, vitamin storage, and the metabolism of carbohydrates, proteins, and lipids. The blood becomes rich in nutrients and xenobiotics following complete intestinal absorption. The blood is then transported to the portal vein and then into the liver [21–23]. Consequently, the liver is particularly susceptible to toxicity and damage. Liver injury is recognized as a global public health problem [21, 24]. Pathologies including hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma are caused from dysregulation of the liver function [25, 26]. Usually, the histological and biochemical conditions most commonly associated with liver disease are hepatocyte death, hepatic stellate cell (HSC) activation, Kupffer cell (KC) activation, peripheral inflammatory cell infiltration and activation, free radical generation, proinflammatory cytokine production, and extracellular matrix protein expression and deposition to irreversible cirrhosis and hepatocellular carcinoma (HCC) [27–29]. Numerous factors such as ethanol and drug abuse, inadequate nutrition, viral infection, xenobiotic exposure, and metabolic disorders may affect the stage of liver diseases [27, 30]. However, it has been documented that the main causal agents of liver failure in a particular area depend on the prevalent hepatotropic virus infections and patterns of drug use [30–32].

Cholestasis has relatively high morbidity and mortality rates in the world and may occur if there is impaired formation of bile and/or bile flow [33, 34]. The most chronic cholestatic diseases can occur simply as a result of physical obstruction of the small intrahepatic bile ducts or hepatocellular functional defects [34]. On the other hand, hepatitis A, B, C, D, and E are viral liver diseases that can cause mild to severe illness, and high incidence of hepatitis A infection has been linked with poor hygiene and sanitation [35]. However, drug-induced liver damage which is the second major cause of acute liver failure is common in the developed world [36]. Several drugs can cause severe liver damage such as some anti-inflammatory, anti-infective and anticonvulsant drugs. Acetaminophen overdose-induced hepatotoxicity is the most common example [37, 38]. Fibrogenesis is a normal physiological repair process after injury or inflammation, while fibrosis only becomes clinically relevant when it alters tissue structure and disturbs normal tissue functioning [37, 39–41].

Consumption of alcohol has also been linked to liver disease (alcoholic liver disease, ALD) that includes a broad spectrum of disorders, including an acute (alcoholic hepatitis) or chronic (steatosis, steatohepatitis, fibrosis, and cirrhosis) form [42]. Oxidative stress [43] and changes in lipid metabolism [44] that cause damage in cell membranes and organelles (especially mitochondria) are implicated in alcoholic liver disease (ALD). Individual susceptibility, any other liver involvement such as viral hepatitis [45], obesity, and metabolic syndrome [46] are also contributing factors. However, the mechanisms that are involved in fibrogenesis in

ALD are alcohol metabolism, oxidative stress, methionine metabolism abnormalities, hepatocyte apoptosis, and increased serum lipopolysaccharide (LPS) level that activates KC [47]. Lipogenesis during the early stages of ALD has been implicated as a risk factor for the progression of cirrhosis. This makes newer mechanisms involve stimulation of lipogenesis and inhibition of fatty acid oxidation, osteopontin, IL-1 signaling, and genetic variations [47]. The spectra of disorders included in ALD are asymptomatic fatty liver, steatohepatitis, progressive fibrosis, end-stage cirrhosis, and HCC [48, 49]. HCC is one of the most common and lethal cancers in the world [48, 49]. Inflammation is strongly linked to carcinogenesis [50] as exemplified by HCC [51], and both chemically and genetically induced HCC depend on inflammatory signaling [52]. Multiple signaling pathways are involved in these processes. Out of these signaling pathways, inhibitor of κB kinase β (IKKβ)-dependent classical nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling and signal transducer and activator of transcription 3 (STAT3) were found to be important for compensatory liver regeneration and chemically induced HCC development [53]. Unfortunately, the treatment options for liver diseases are controversial, as drugs for the treatment either are having serious side effects or are not effective [54, 55]. Review of the literature revealed that trehalose unexplored for numerous of its claimed hepatoprotective effects. Hence, the present study aimed to review the hepatoprotective effect of trehalose.

2 Methodology

To meet the purposes of current search, the databases of Web of Science, PubMed (NLM), Open Access Journals, LISTA (EBSCO), and Google Scholar were searched for the articles published as late as 31 October 2020, using the following keywords: trehalose, hepatoprotective, oxidative stress, autophagy, end-stage liver disease, and cirrhosis.

For a summary of the selected in vitro and in vivo studies, see Tables 1 and 2.

3 In Vitro Studies

3.1 The Benefit of Trehalose as an Additional Cryoprotective Agent

Cryopreservation of hepatocytes is an essential step for preservation of cells and tissues for treating certain liver diseases [56]. A study was designed to determine the effect of different natural cryoprotectant disaccharides (sucrose, glucose, and trehalose) on the cryopreservation of rat hepatocytes. Liver cells were frozen in freezing solutions in the presence or absence of low concentrations of Me_2SO (5% Me_2SO), and supplemented with varying concentrations of the mentioned disaccharides. After 7 days of cryopreservation, hepatocyte viability was determined by exclusion of trypan blue and by the MTT technique, as well as by measuring albumin production. Among the investigated disaccharides and concentrations, hepatocytes cryopreserved in 0.2 M trehalose showed the best overall outcome, and improvement in post-thaw cell viability over Me_2SO alone was found [57]. In another study, trehalose-containing organ preservation solution, namely, ET-Kyoto (ETK) solution, showed beneficial effect for cryopreservation of human hepatocytes [58].

In one study, the effect of trehalose on the cryopreservation of human hepatocytes was evaluated. For analysis, liver cells were frozen in culture medium containing 10% dimethyl sulfoxide (DMSO) that was supplemented with different concentrations of trehalose. During the post-thawing culture period, parameters including viability, plating efficiency, total protein, cell proliferation, enzyme leakage, and albumin and urea formation, along with phase I and II metabolism, were examined. They found that 0.2 M trehalose showed the best overall outcome. In the trehalose group, significant improvement in post-thaw cell viability and plating efficiency was observed in comparison to the use of DMSO alone.

Table 1 Experimental model studies showing the impact of trehalose on hepatoprotection

Dose and route of administration	Model of diseases	Major outcomes	References
2 g/kg body weight) for 8 weeks (oral)	Cd-induced hepatic injury in rat	Ameliorated Cd-mediated elevation serum hepatic enzymes and liver pathological changes Improved Cd-mediated oxidative stress and antioxidant status in serum, indicating its antioxidant action for the whole body Inhibited Nrf2 nuclear translocation and subsequent elevated expression of Nrf2-downstream targets in the rat liver induced by Cd Ameliorated Cd-mediated elevation protein levels of hepatic antioxidant enzymes Cd-induced autophagy inhibition in liver tissues was noticeably restored by trehalose Ameliorated Cd-induced apoptosis in hepatic tissues through inhibiting caspase-dependent apoptotic pathway	[63]
2 g/kg for 8 weeks (oral)	Cd-induced hepatic injury in rat	Decreased splenic pathological changes, apoptosis, and spleen tissue oxidative stress induced by Cd exposure Suppressed Cd-induced Nrf2 and upregulated the protein expression of nuclear Nrf2 Decreased the protein expression of sequestosome 1 (p62/SQSTM1) and microtubule-associated protein LC-3II	[64]
2.5 g/kg/day for 16 weeks (oral)	Apolipoprotein E knockout mice	Inhibited atherosclerosis and decreased hepatic steatosis Increased the liver protein expression of beclin 1, LC3, LC3-II, and APG7	[66]
2% trehalose (in drinking water for 4 weeks)	Liver injury in old mice	Decreased age-associated activation of the ER UPR and inflammatory signaling, in addition to markers of liver injury	[13]
3% trehalose (in drinking water for 48 h)	Hepatic steatosis model in mice	Attenuated hepatic steatosis via the autophagy-mediated inhibition of AMPK	[14]
1 g/kg/i.p.	Endotoxic shock model in rat	Decreased hypotension, NF-κB binding activity, IκBα protein loss, TLR-4 activation, as well as TNF-alpha, IL-1, IL-6, and MDA levels Protected against liver and lung injury in the endotoxic shock model of rat	[15]

Cd cadmium, *Nrf2* nuclear factor erythroid 2-related factor 2, *LC-3II* light chain 3II, *ER UPR* endoplasmic reticulum unfolded protein response, *AMPK* adenosine 5′-monophosphate-activated protein kinase, *NF-κB* nuclear factor kappa B, *IκBα* inhibitor of nuclear factor kappa B, *TLR-4* toll-like receptor 4, *TNF alpha* tumor necrosis factor alpha, *IL* interleukin, *MDA* malondialdehyde

The addition of trehalose to the cryopreserved human hepatocytes resulted in a significantly increased total protein level in the attached cells, a higher secretion of albumin, and a lower aspartate aminotransferase (AST) level after thawing [59]. Stokich et al. reported that trehalose incubation facilitates preservation of hepatocyte (HepG2) cells in monolayer format [60]. One study showed that di-rhamnolipids, which are non-toxic, effective, and commercially available, improved the effect of trehalose against hypothermic or cryopreservation cell damage [61].

Table 2 In vitro studies showing the impact of trehalose on hepatoprotection

	Cell type	Major outcomes	References
Trehalose (0–1000 μM)	Hepatocellular carcinoma (Huh7 and Hep3B), hepatoblastoma (HepG2), and a highly differentiated immortalized human hepatocyte (OUMS29)	Activated autophagy in an mTOR-independent manner Reduced abnormal proteins and protected liver-derived cultured cells from ER stress and apoptosis	[57]
Trehalose (0–0.2 mM)	HepG2 cells	Reduced the amount of LDH in palmitate-induced toxicity in HepG2 cells Reduced the H2O2 release in the presence of palmitate in HepG2 cells Interacted with the plasma membrane to protect HepG2 cells from palmitate-induced changes in membrane fluidity	[58]
Novel liposomes composed of L-α-dimyristoylphosphatidylcholine and trehalose surfactant Trehalose surfactant (L-α-dimyristoylphosphatidylcholine $=1.0 \times 10^{-4}$ M, TreCn = $0.1–2.3 \times 10^{-4}$ M)	Human hepatocellular carcinoma (Hep-G2 and HuH-7) cells	Caspase-3, caspase-8, and caspase-9 were activated by trehalose surfactant in Hep-G2 and HuH-7 cells BAX activation and cytochrome c release were recorded	[59]
Trehalose (100 mM)	Primary hepatocytes and HepG2 cells	Inhibited the SLC2A or GLUT family Attenuated hepatic steatosis via the AMPK-dependent autophagy-mediated inhibition Reduced the accumulation of lipid droplets in primary murine hepatocyte cultures	[14]
Trehalose (100 mM)	HepG2	Attenuated mTORC1 signaling	[83]

mTOR mammalian target of rapamycin, *ER* endoplasmic reticulum, *mTORC1* mammalian target of rapamycin complex 1, *AMPK* adenosine 5′-monophosphate-activated protein kinase, *GLUT* glucose transporter

3.2 In Vitro Studies Regarding the Protective Effects of Trehalose in Hepatocytes

Trehalose-activated autophagy is accompanied by increments in LC3-II levels and the LC3-II/LC3-I ratio, the number of GFP-LC3 puncta structures, and beclin 1, and reduced p62, abnormal proteins, and cytoplasmic inclusion body formation, as well as induced p70 S6 kinase, that indicate the autophagy induction is in an mTOR-independent manner. Furthermore, treatment of trehalose protected liver-derived cultured cells from ER stress and apoptosis [62]. Trehalose reduced the amount of LDH in palmitate-induced toxicity in HepG2 cells and H_2O_2 release in the presence of palmitate in HepG2 cells. Trehalose interacted with the plasma membrane to protect

HepG2 cells from palmitate-induced changes in membrane fluidity [63].

Matsumoto et al. showed that novel liposomes composed of L-α-dimyristoylphosphatidylcholine (DMPC) and trehalose surfactant (DMTreCn) have been produced by using sonication in buffer solution. The thickness of fixed aqueous layer of DMTreCn was larger than that of DMPC liposomes and increased dose dependently. The augmented apoptotic effect of DMTreCn on human hepatocellular carcinoma (HCC) (Hep-G2 and HuH-7) cells, but not on normal cells, was obtained. Caspase-3, caspase-8, and caspase-9 were activated by DMTreCn in Hep-G2 and HuH-7 cells. BAX activation and cytochrome c release were recorded, indicating that DMTreCn induced apoptosis of Hep-G2 and HuH-7 cells through the mitochondrial pathway via BAX [64]. Rat liver mitochondrial alanine aminotransferase (mALT) is an unstable enzyme. Mukorah et al. investigated the possibility of stabilizing mALT with ethanol, trehalose, and protease inhibitors. In the presence of ethanol, the mALT inactivation was decreased and its half-life increased from 1 to 4 h. The stability of mALT was significantly enhanced by trehalose in a dose-dependent manner. In the presence of 36.5% trehalose, the half-life of mALT was 85 h. Among the protease inhibitors tested, only antipain and chymostatin lead to reduction in the inactivation of mALT, but only within the first 24 h following preparation of the crude enzyme. The authors suggested that the inclusion of ethanol and trehalose in purification protocols could aid the purification of the enzyme [65].

In a study conducted by DeBosch et al., trehalose inhibited the SLC2A or GLUT family. It attenuated hepatic steatosis via the AMPK (adenosine 5′-monophosphate-activated protein kinase)-dependent autophagy-mediated inhibition, and it reduced the accumulation of lipid droplets in primary murine hepatocyte cultures [19]. Another study showed that, although trehalose profoundly attenuated mTORC1 signaling, AMPK or GLUT8 is required for trehalose-induced mTORC1 suppression. Strikingly, transient, heterologous *Tret1* overexpression reconstituted autophagic flux and AMPK signaling defects in GLUT8-deficient hepatocyte cultures [66]. Introduction of trehalose into the matrix of isolated mitochondria improves inner membrane integrity than those without trehalose loading. These findings suggest the presence of trehalose in the mitochondrial matrix affords improved desiccation tolerance to the isolated mitochondria [67].

4 In Vivo Studies

One study was conducted to examine the protective effect of trehalose on cadmium (Cd)-induced hepatic injury in rats. Trehalose treatment ameliorated Cd-mediated elevation serum hepatic enzymes and liver pathological changes. Also, trehalose significantly improved Cd-mediated oxidative stress and antioxidant status in serum, indicating its antioxidant action for the whole body. In addition, trehalose inhibited nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear translocation and subsequent elevated expression of Nrf2-downstream targets in the rat liver induced by Cd. Simultaneously, trehalose ameliorated Cd-mediated elevation protein levels of hepatic antioxidant enzymes. Furthermore, Cd-induced autophagy inhibition in liver tissues was noticeably restored by trehalose, evidenced by immunohistochemical analysis and immunoblot assays. Additionally, trehalose treatment significantly ameliorated Cd-induced apoptosis in hepatic tissues through inhibiting caspase-dependent apoptotic pathway [68].

Another study showed that trehalose decreased splenic pathological changes, apoptosis, and spleen tissue oxidative stress induced by Cd exposure. Besides, trehalose suppressed Cd-induced Nrf2 and upregulated the protein expression of nuclear Nrf2. Moreover, trehalose decreased the protein expression of p62/SQSTM1 and microtubule-associated protein light chain 3II (LC-3II) to restore autophagy inhibition induced by Cd exposure [69].

Pagliassotti et al. examined the unfolded protein response (UPR) and inflammatory signaling in the liver of young (~6 months) and old (~28 months) mice (n = 8/group) and the ability

of trehalose to counteract age-induced effects on these systems. Adding trehalose to drinking water was done for 4 weeks. Activation of the UPR increased inflammatory signaling, and indices of liver injury in old mice were noticeably restored by trehalose. Decreases in proteins involved in autophagy and proteasome activity found in old mice were restored after trehalose treatment. An increment in the autophagy marker LC3B-II under trehalose treatment in old mice was found. Metabolomics analyses revealed that reductions in hexosamine biosynthetic pathway metabolites and nicotinamide in old mice were restored by trehalose. Trehalose appears to be an effective intervention to reduce age-associated liver injury and alleviate the need for activation of quality control systems that respond to disruption of proteostasis [18]. Trehalose was shown to inhibit members of the GLUT family of glucose transporters. Trehalose attenuated hepatic steatosis via the autophagy-mediated inhibition of AMPK (adenosine 5′-monophosphate-activated protein kinase) in vivo and decreased accumulation of lipid droplets in primary murine hepatocyte cultures. The authors suggested that trehalose triggers beneficial cellular autophagy by inhibiting glucose transport [19]. Trehalose decreased hypotension, NF-κB binding activity, IκBα protein loss, TLR-4 activation, as well as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and malondialdehyde (MDA) levels. Trehalose also protects against liver and lung injury in the endotoxic shock model of rat. Quasi-elastic neutron scattering measurements showed that trehalose also possesses a high "switching off" capability. Sucrose did not modify endotoxic shock-induced sequelae. Trehalose inhibited inflammatory cascade triggered by endotoxin shock, stabilizing the biomembranes and switching off the water diffusive dynamics [20]. Zhang et al. demonstrated that trehalose induced hepatocyte TFEB (transcription factor EB)-dependent thermogenesis in vivo, besides increased hepatic and white adipose expression of UCP1 (uncoupling protein 1 [mitochondrial, protein carrier]).

Hepatocyte fasting transcriptional and metabolic responses depend upon PPARGC1A (peroxisome proliferative activated receptor, gamma, coactivator 1 alpha), TFEB, and FGF21 (fibroblast growth factor 21) signaling. Selective knockdown of hepatocyte TFEB abrogated trehalose induction of thermogenesis and upregulation of white adipose tissue UCP1 in vivo. In contrast, the trehalose effect on thermogenesis was independent of LEP (leptin) and the autophagy pathway, as there was robust thermogenic induction in trehalose-treated ob/ob, Becn1, Atg16l1, and Epg5 mutant mice. The authors suggested that trehalose induced favorable metabolic effects in whole body thermogenesis by mechanisms such as hepatocyte-centered fasting-like mechanisms, which seem to be independent of the autophagic flux [70]. Prolonged treatment with trehalose (given orally for a 16-week period) inhibited atherosclerosis and attenuated hepatic steatosis in apolipoprotein E knockout mice. Besides, trehalose treatment significantly increased the protein expression of beclin 1, LC3, LC3-II, and APG7 in the liver of apolipoprotein E knockout mice [71].

5 Possible Mechanism of Hepatoprotective Activity of Trehalose

An imbalance between oxidants and antioxidants in favor of the former oxidants is termed oxidative stress that potentially leads to cell death. This process involves the formation of ROS/reactive nitrogen species by multiple injury mechanisms, including mitochondrial inhibition, Ca^{2+} overload, and inflammation [72, 73]. In some of the articles highlighted above, trehalose induced liver protection through antioxidant effects. Trehalose was previously reported to suppress ROS-induced lipid peroxidation in yeast cells [74]. There is considerable evidence from both in vitro and in vivo experiments that trehalose protected membranes against lipid peroxidation and thereby suppressed radical oxidation of unsaturated fatty acids [75]. Nrf2-Keap1 signaling pathway has been recognized as the main cellular defense mechanism [76, 77]. Its function confers cellular protection to oxidative stress, [77]. It has been postulated that during oxidative stress, disruption in the Keap1-Nrf2 interaction in the cytoplasm occurred [78]. Nrf2 is translocated into the nucleus and interacts with the ARE,

which results in induction of several cellular defense gene transcriptions, such as phase II detoxification enzymes HO-1, NQO1, and direct reactive oxygen species (ROS) scavenging proteins (GPx, SOD, CAT).

Autophagy is a lysosomal degradation pathway that the cell adapts to stressful conditions [79]. Autophagy and oxidative stress are reciprocally linked. This suggests that in this context, autophagy enhancement could be one of the hepatoprotective effects of trehalose. Constitutive autophagic activity contributes to downregulate ROS production, while excessive oxidative stress contributes to autophagy inhibition [80]. In addition to the effects on Keap1, levels of active Nrf2 are regulated by autophagy [81]. The p62 protein (SQSTM1) is commonly used as a marker to study autophagic activity. This protein accumulates when autophagy is inhibited, whereas it is degraded when autophagy is induced [82]. During oxidative stress conditions, p62 upregulation with resultant sequestration of Keap1 resulted in Nrf2 activation that subsequently led to Nrf2-dependent antioxidant defense gene expression [81]. In another proposed mechanism, trehalose activated mTOR-independent autophagy in hepatocytes, as was the case in nerve cells in some reports [83]. The increase of beclin 1, a mammalian ortholog of the yeast autophagy-related gene 6 (Atg6) [84, 85], was one of the mechanisms of trehalose-mediated autophagy activation in hepatocytes. This contrasts with another study indicating that trehalose induced autophagy via inhibition of mTOR pathway [19]. Therefore, the effects of trehalose on autophagy in hepatocytes are complex, and further studies are needed to accurately identify the mechanisms. Further research on the role of trehalose has indicated that it induces autophagy as it causes glucose starvation state and mitigates hepatic steatosis in an SLC2A (solute carrier 2A)- and AMPK-dependent manner. Furthermore, it reduces triglyceride accumulation in cultured hepatocytes [19].

The glucose transporter (GLUT) family of proteins belongs to the Major Facilitator Superfamily (MFS) of membrane transporters [19, 86]. Trehalose is rapidly transported into hepatocytes in a GLUT8-dependent manner, a homolog of the trehalose transporter-1 (Tret1). More specifically, the amino acids involved in trehalose binding display the highly conserved residues Gln162, Gln267, Gln268, Asn273, Gly390, and Asn 417, which constitute a hydrogen bond network at the glucose binding site [87] to form polar interactions between the ligand and GLUT8 [88]. A signaling pathway called the endoplasmic reticulum unfolded protein response (ER UPR) contributes to hepatic steatosis in liver aging [89, 90]. ER UPR disturbed protein folding process in the ER and initiates inflammation [91]. It is mainly responsible for upregulation of gene targets related to protein folding and ER-associated degradation [18, 92, 93]. Trehalose reduced ER UPR activation, inflammatory signaling, and liver damage [18].

6 Conclusions

Trehalose is one of the major osmoprotectants found in nature, and its biosynthesis capacity is present in most organisms, except vertebrates. Herein, we elaborated on the potential application of trehalose in protecting the liver against various types of insults and injuries. Being a safe agent, trehalose can exert its hepatoprotective effects through a variety of mechanisms (Fig. 1). The suggested hepatoprotective mechanisms of trehalose are reduction of ER UPR activation, inflammatory signaling, and liver damage and activation of autophagy by enhancing in an mTOR-independent autophagy in hepatocytes, besides trehalose-induced liver protection through antioxidant action.

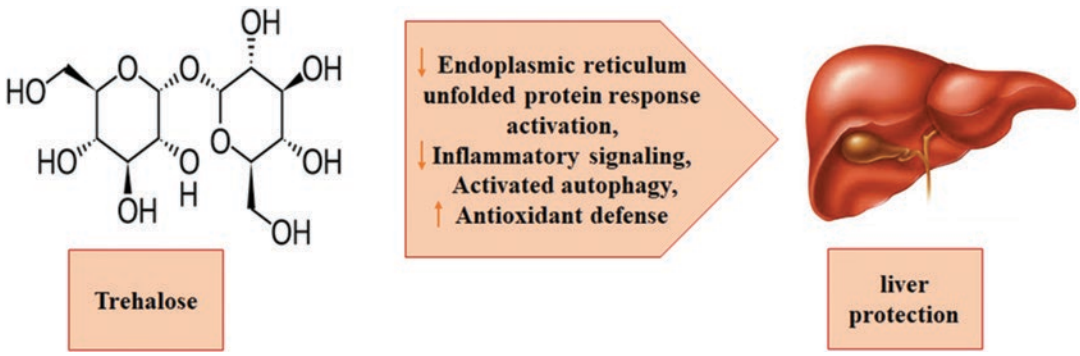

Fig. 1 Hepatoprotective effects of trehalose

Conflict of Interest None.

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Effect of Vitamin D Supplementation on the Regulation of Blood Pressure in Iranian Patients with Essential Hypertension: A Clinical Trial

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Abstract

Background: Low serum vitamin D level is associated with both high blood pressure and incidence of primary hypertension. Experimental studies suggest that vitamin D supplements may reduce blood pressure.

Objective: The aim of this study was to investigate whether vitamin D supplementation reduces systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in Iranian patients with essential hypertension.

Method: A total of 173 patients with essential hypertension participated in this open-label clinical trial. SBP, DBP, and serum vitamin D levels were measured at baseline and at the end of the study. Vitamin D was administered at a dose of 50,000 IU/week, and 1000 IU/day in patients with serum vitamin D levels

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<20 ng/mL and 20–30 ng/mL, respectively, for 8 weeks.

Results: Based on serum vitamin D levels, 45.1%, 17.3%, and 29.5% of patients were deficient, insufficient, and sufficient for vitamin D intake, respectively. Baseline serum levels of vitamin D were not correlated with SBP, DBP, and MAP at the beginning of the study (p = ns). Multiple logistic regression analysis revealed that the risk of vitamin D deficiency was 2.5-fold times higher in women than in men ($p = 0.03$). After 8 weeks of supplementation with vitamin D, mean SBP and MAP were significantly reduced by 5.5 ± 16.16 ($p = 0.01$) and 3.7 ± 9.24 ($p = 0.004$) mmHg, respectively. Neither sex nor age could significantly predict BP response to vitamin D supplementation.

Conclusion: Vitamin D supplementation may significantly reduce SBP and MAP but not DBP in patients with essential hypertension.

Keywords

Essential hypertension · Vitamin D · Blood pressure · SBP · DBP · MAP

1 Introduction

Vitamin D deficiency is a common problem with serious implications for human health [1–3]. Several studies revealed that low 25-hydroxy (25-OH) vitamin D levels are strongly associated with cardiovascular diseases including higher blood pressure and a higher rate of hypertension [4–9].

Previous observational studies and meta-analyses of vitamin D intervention suggest that vitamin D supplementation may decrease blood pressure in selected patient groups and populations. For instance, in a recent meta-analysis of observational studies, every 16 ng/mL reduction in serum vitamin D levels was associated with a 16% increase in the risk of hypertension [10]. Moreover, low serum vitamin D levels in normotensive individuals have been reported to predispose to future hypertension [4].

Vitamin D receptor (VDR) is a transcription factor belonging to the nuclear receptor family. Vitamin D receptor is highly expressed on the vascular smooth muscle endothelium and cardiomyocytes [11, 12]. Sufficient levels of vitamin D prevent contraction of venous smooth muscle cells and increase arterial compliance. Downregulation of vitamin D receptor in animal models led to elevation of blood pressure [13], which suggest that it can be amended with vitamin D oral supplementation.

Molecular events of vitamin D–vitamin D receptor ligation, including suppression of the renin–angiotensin–aldosterone system (RAAS), nephroprotective actions, or induction of endothelial/vascular function, suggest an antihypertensive properties of vitamin D [14, 15]. Vitamin D plays several roles in vascular structure and functions such as reducing the expression of thrombogenic genes, increasing vasodilation-related genes, and upregulation of prostacyclin, the latter being a vasodilator [16, 17].

However, several randomized controlled trials (RCTs) on vitamin D supplementation in hypertensive patients have shown disappointing results with most reports showing no beneficial effects [18–25]. For instance, Witham et al. reported that supplementation with 100,000 IU of oral vitamin D every 3 months for 1 year causes no appreciable change in blood pressure compared with placebo [26]. These findings have argued the value of vitamin D as a powerful treatment for hypertension.

Despite a wide range of antihypertensive drugs now being approved and available with various mechanism of actions, treatment of refractory hypertensive patients – the blood pressure remains above the goal despite use of three different classes of hypertensive agents – remains challenging, with numerous subjects experiencing treatment-limiting side effect [27]. Although recent strategies have focused on invasive approaches, such as renal denervation therapy with debatable result [28], the large burden of resistant hypertension at the population level means that low-cost, easy-to-apply interventions to mitigate the problem are still required.

However, it does not seem likely that vitamin D administration will have an effective and uni-

formly lowering effect on blood pressure across all populations and races. In this light, we conducted an open-label trial to investigate the effect of vitamin D on blood pressure in Iranian patients with essential hypertension.

2 Materials and Methods

2.1 Study Design

The present study was an open-label clinical trial. The study enrolled 173 patients, between 18 and 65 years old (mean age = 57.6 ± 9.3 years) who had an averaged SBP ≥ 140 or DBP ≥ 90 mm Hg based on Joint National Committee 7 (JNC7) criteria [29], or participants were eligible for inclusion if they had received ≥1 antihypertensive medication and recruited at endocrine disease clinic in Baqiyatallah Hospital, affiliated Tehran, Iran. Mean arterial pressure (MAP) was calculated based on systolic and diastolic blood pressure (SBP and DBP) using the formula below:

$$\text{Mean arterial pressure}(\text{mmHg}) = (\text{SBP} \times 1/3) + (\text{DBP} \times 2/3)$$

Enrollment began in October 2014, and the final follow-up visit was carried out in July 2015. Information regarding type and dose of antihypertensive drugs were documented. Exclusion criteria were as follows: patients with known cardiovascular disease (left ventricular ejection fraction (LVEF) ≤ 45%, as prior myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass, or stroke), renal disease (serum creatinine ≥1.5 mg/dl) and liver disorders (subjects with the liver enzyme alanine aminotransferase (ALT) ≥3 folds of upper limit of normal value range), secondary hypertension, and chronic obstructive pulmonary disorder. Individuals were also excluded if they had used any kind of vitamin D supplementation in the past 3 months. This study was performed in accordance with the Declaration of Helsinki guidelines, and the study protocol was approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences, Tehran, Iran (IR.RMSU.REC.1394.31). Written informed consent was obtained from all participants. The trial was registered at IRCT.ir (IRCT20080901001165N57). At the first screening visit, the blood pressure was measured by a physician three times using standard sphygmomanometer [30].

2.2 Vitamin D Supplementation and Blood Pressure Monitoring

Following participants enrolling, vein blood was drawn from patients and serum level of 25-OH vitamin D was measured in the medical laboratory of Baqiyatallah Hospital. The patients were classified in three groups as vitamin D deficient (<20 ng/ml), insufficient (20–30 ng/ml), and sufficient (>30 ng/ml) (to convert to nanomoles per liter, multiply by 2.496) [31]. 50,000 U weekly and 1000 U daily vitamin D (pearl vitamin D3, Zahravi Pharma Co, Tehran, Iran) were administrated in vitamin D-deficient and vitamin D-insufficient patients, respectively. Vitamin D administration was continued for 8 weeks and endpoint blood pressure was measured. At week 8, serum level and relation of 25-OH vitamin D status to change in blood pressures were also determined. Outcomes, including 25-OH vitamin D serum levels and endpoint blood pressure, were all measured on the same day. Patients were not administrated with calcium supplementation; however, they were informed by lifestyle changes with advice on optimal calcium consumption. All patients were followed during the 8 weeks of intervention to ensure regular administration of vitamin D and to monitor suspected medication adverse effects (Fig. 1).

2.3 Statistical Methods

Sample size was calculated based on power (1-β probability error) = 80% and α = 0.05 using G Power version 3.1. We estimated the size of the sample for this study based on data from the previous study [32]. There was a significant inverse correlation between serum level of 25-OH vitamin D and blood pressure in our study. A power

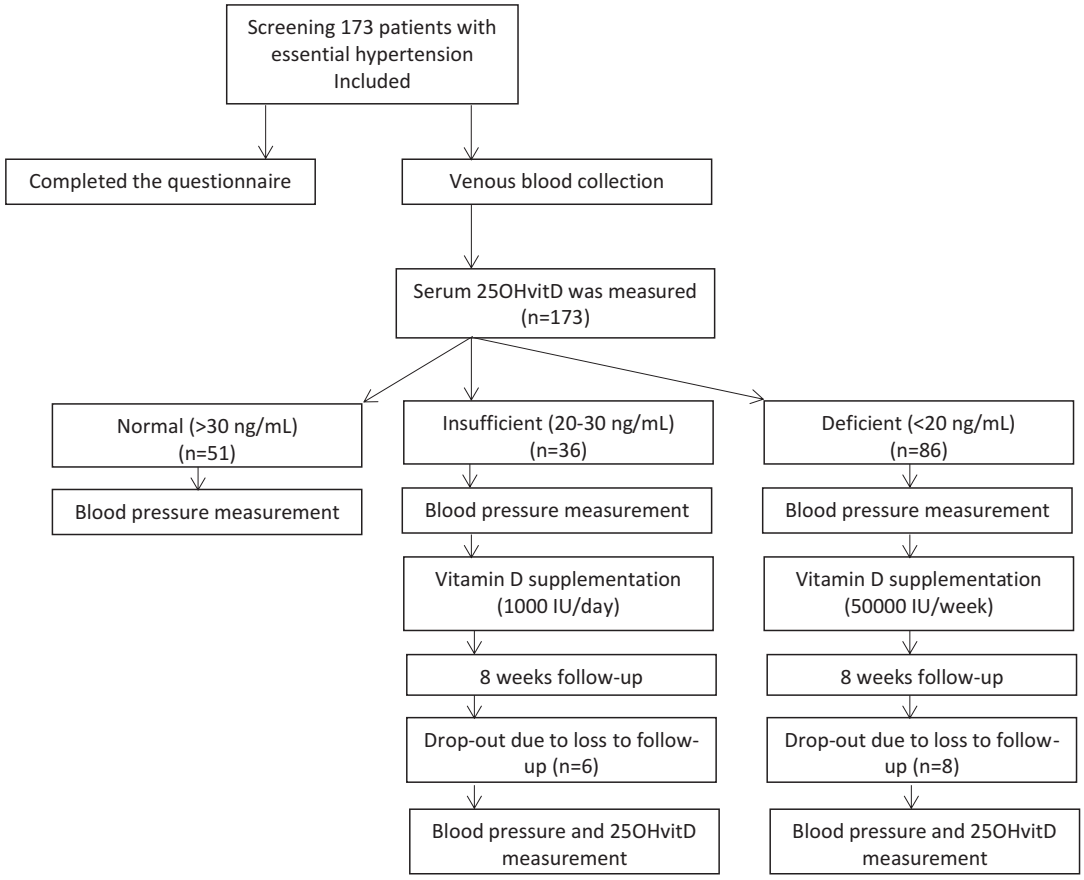

Fig. 1 Flowchart of the trial. 173 eligible patients with essential hypertension participated in the study. 159 patients could be completely followed up

analysis using G*power software revealed that 52 subjects were required for our study to detect a significant difference between the groups.

Qualitative and quantitative data are demonstrated by percentage and mean ± standard deviation of three independent measurements. The Kolmogorov–Smirnov test was used to assess the normal distribution of data. The statistical analysis was only performed on the treated group. The correlation between serum level of 25-OH vitamin D and SBP, DBP, and MAP was determined using Spearman correlation test. The number of the antihypertensive drug regimens was compared between normal and vitamin D-deficient groups using chi-square test (patients were divided into two: deficient and sufficient groups). Multivariate logistic regression method was applied to determine the potential effect of risk factors (sex, age, and body mass index (BMI)) on the goal of therapy, and in this regard, patients were classified in groups with or without age (≥55 for men, ≥65 for women) and body mass index (≥30 kg/m2) risk factors. The patients were classified as deficient and sufficient groups (because of low number of patients in insufficient group) for chi-square and logistic regression tests. SPSS 20 (SPSS Inc., Chicago, Illinois) software used to run statistical data analysis and P values of less than 0.05 were regarded as statistically significant.

3 Results

3.1 Demographic Data

In the present study, 173 patients with essential hypertension were evaluated. Representative data of sex, age, BMI, and clinical features of participants flow through the trial is shown in Table 1. The antihypertensive drug regimens were not changed during the present study. Hypertensive patients were classified in three groups of sufficient (51 patients), insufficient (36 patients), and deficient (86 patients) based on serum level of 25-OH vitamin D. No significant correlation was observed between baseline 25-OH vitamin D serum level and SBP, DBP, and MAP of 155 patients (Table 2). Fourteen patients were dropped out due to loss to follow up.

3.2 Effect of Intervention on 25-OH Vitamin D Serum Level

At the first visit screening, 86 (49.7%), 36 (20.8%), and 51 (29.5%) patients were characterized in deficient, insufficient, and sufficient groups regarding serum level of 25-OH vitamin D, respectively. After 8 weeks, mean 25-OH vitamin D serum levels significantly increased following supplementation from a baseline level in insufficient group from 25.3 ± 3.0 ng/ml to 35.1 ± 9.1 ng/mL and deficient group from 9.9 ± 5.0 ng/ml to 30.2 ± 10.6 ng/mL ($P < 0.0001$).

3.3 Effect of Vitamin D Supplementation on Systolic, Diastolic, and Mean Arterial Pressure

No significant correlation was observed between SBP, DBP, and MAP with 25-OH vitamin D serum level. However, vitamin D supplementation concomitant with conventional antihypertensive drug regimens caused a statistically significant 5.5 ± 16.2 mm Hg decrease of overall SBP ($p = 0.01$, 95% confidence interval = 1.3, 9.6). Vitamin D intervention decreased overall DBP by mean of 1.4 ± 12.4 mm Hg ($p = 0.3$, 95% confidence interval = −1.7, 4.6). Moreover, the

Table 1 Demographic characteristic and clinical information of participants

			Serum 25-OH vitamin D		
		Total	Deficient	Insufficient	Normal
Patients *N* (%)		173 (100)	86 (49.71)	36 (20.81)	51 (29.48)
Age (year) Mean ± SD		57.94 ± 9.00	55.66 ± 10.18	59.58 ± 7.58	60.47 ± 8.62
Blood pressure Systolic/diastolic mean ± SD (mmHg)		128.82 ± 21.95/ 80.32 ± 10.47	130.65 ± 22.72/ 82.35 ± 10.85	126.08 ± 26.07/ 81.46 ± 10.81	127.78 ± 18.05/ 76.70 ± 8.83
Sex	Female *N* (%)	111 (64.20)	46 (41.44)	24 (21.62)	41 (36.94)
	Male *N* (%)	62 (35.80)	37 (59.68)	11 (17.74)	14 (22.58)
BMI (kg/m^2), mean ± SD		27.30 ± 3.66	27.17 ± 3.88	28.05 ± 3.99	27.05 ± 3.30
Hyperlipidemia (LDL-C ≥ 100 mg/dL) (%)		98 (56.65)	52 (53.06)	22 (22.45)	24 (24.49)
Diabetes mellitus (%)		137 (79.19)	67 (48.90)	24 (17.52)	46 (33.58)

Table 2 Correlation between 25-OH vitamin D serum level and systolic, diastolic, and mean arterial blood pressures ($N = 155$)

		Baseline SBP	Baseline DBP	Baseline MAP
Baseline 25-OH vitamin D level	Pearson correlation (*r*)	0.015	−0.156	−0.078
	P value	0.857	0.052	0.338
	Number of patients	155	155	155

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure

Table 3 The effect of vitamin D intervention on systolic blood pressure, diastolic blood pressure, and mean arterial pressure ($N = 155$)

Blood pressure (mmHg)	Before vitamin D therapy	After vitamin D therapy	95% confidence interval of the difference	*P* value
SBP mean ± SD (mmHg)	133.2 ± 17.81	127.2 ± 14.54	1.3, 9.6	0.010
DBP mean ± SD (mmHg)	82.6 ± 13.40	81.2 ± 10.38	−1.7, 4.6	0.369
MAP mean ± SD (mmHg)	99.29 ± 17.81	95.57 ± 9.72	1.2, 6.2	0.004

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure

intervention led to 3.7 ± 9.2 mmHg decrease of mean arterial pressure (MAP) ($p = 0.004$, 95% confidence interval = 1.2–6.2) (Table 3). In the present study, 58.4% of patients were receiving at least one RAAS blockers. The vitamin D supplementation in this group leads to a statistically significant reduction in SBP ($p = 0.028$, 95% confidence interval = 0.7–11.9) and MAP ($p = 0.02$, 95% confidence interval = −0.9-8.0) and a marginally nonsignificant reduction in DBP ($p = 0.055$, 95% confidence interval = 0.9–8.0).

3.4 The Relation of Antihypertensive Drug Regimens and 25-OH Vitamin D Serum Level

To investigate if 25-OH vitamin D serum level is in relation with the number of antihypertensive drug regimens, the number of administrated antihypertensive drugs was compared with vitamin D-sufficient and vitamin D-deficient patients using chi-square test. In the present set of analysis, the patients were categorized as vitamin D sufficient (≥30 ng/ml) and deficient (<30 ng/ml). There were no significant differences in the number of antihypertensive drugs that patients are taking in the vitamin D-sufficient and vitamin D-deficient groups according to the chi-square test ($p > 0.05$) (Table 4).

3.5 Effect of Sex, Age, and Body Mass Index on Clinical Response of Vitamin D Treatment in Patients with Hypertension

Multiple logistic regression analysis showed a significant association between sex but not age and BMI with 25-OH vitamin D serum level ($p = 0.03$) (Table 5). This test was also employed to analyze association of sex, age, and BMI risk factors and clinical response of vitamin D intervention (Table 6). In accordance with JNC7 guideline, the goal of therapy was determined as systolic/diastolic blood pressure <130/80 mmHg and <140/90 for diabetic and nondiabetic patient groups, respectively [29]. Comparison of risk factors between patients whose blood pressure reached the goal of therapy and not showed that neither sex nor age had significant association with goal of therapy in both groups of patients. However, a marginally significant (*p* value: 0.053) and negative correlation was seen between BMI and goal of therapy (Table 6).

4 Discussion

Hypertension has recently emerged as an important risk factor for the public health burden. Recently, several preventive and protective effects of vitamin D are found in a wide range of diseases including cancer, autoimmune disease, diabetes, infections, depression, osteoporosis, and cardiovascular diseases [33]. It is of interest to investigate if vitamin D supplementation has a uniformly lowering effect on blood pressure to clarify the beneficial effect of vitamin D for public health [34].

There are consistent epidemiological evidences linking low vitamin D status to a higher risk of hypertension. However, the findings of randomized controlled trials investigating the effects of vitamin D supplementation on blood pressure have not been fully conclusive, though a trend toward a modest reduction in blood pressure could be implied [35]. Several genetic and environmental factors can influence the conse-

Table 4 The number of vitamin D-deficient and vitamin D-sufficient individuals in patient groups using one, two, and three antihypertensive drugs. The comparison between deficient and sufficient groups and groups receiving one ($p = 0.47$), two ($p = 0.18$), and three ($p = 0.22$) hypertensive drugs using chi-square test revealed no significant differences

	No. of anti-hypertensive drugs			
Cross tabulation	One	Two	Three	Total
Deficient no. (% within D/S)	41 (54.7)	18 (24.0)	16 (21.3)	75 (100)
Sufficient no. (% within D/S)	22 (53.7)	13 (31.7)	6 (14.6)	41 (100)
Total no. (% within D/S)	63 (54.3)	31 (26.7)	22 (19.0)	116 (100)

S sufficient, *D* deficient

Table 5 Multiple logistic regression analysis of the effect of sex, age, and body mass index on vitamin D-deficient and vitamin D-sufficient patient groups ($N = 173$)

		95% CI for odds ratio		
Risk factor	Odds ratio	Lower	Upper	*P* value
BMI	0.77	0.35	1.7	0.53
Age	1.10	0.48	2.55	0.81
Sex	2.54	1.09	5.89	0.03

Table 6 Association between sex, age, and body mass index and clinical response of patients with hypertension treated with vitamin D ($N = 155$)

		95% CI for odds ratio		
Risk factor	Odds ratio	Lower	Upper	*P* value
Age	1.24	0.38	3.9	0.75
BMI	3.1	0.98	10.3	0.053
Sex	0.93	0.31	2.73	0.89

quence of vitamin D supplementation on blood pressure. These underlying factors include the vitamin D baseline status, the vitamin D dose, the dose–response relation between vitamin D and parathyroid hormone, calcium intake, and other factors, such as age, sex, BMI, genetics, and races [26]. It is important to put these variations in context of other evidence. One important aspect of the potential vitamin D–blood pressure relationship to consider is the population and biological variation [36].

In the present study, we conducted an open-label trial to investigate the effect of vitamin D on 173 Iranian patients with essential hypertension. Based on baseline 25-OH vitamin D serum level, 49.7%, 20.8%, and 29.5% of patients were classified as vitamin D deficient, insufficient, and normal, respectively. Mean 25-OH vitamin D serum level of patients was 24.0 ± 18.1 ng/ml. The data implies higher incidence (70.5%) of vitamin D shortage in the hypertensive patients in comparison to incidence of vitamin D deficiency of the general population in Iran (51%) [37]. In accordance with our finding, 81.3% and 72% of essential hypertensive patients were found with vitamin D deficiency in previous studies [38, 39].

No significant correlation was observed between SBP, MAP, and DBP with 25-OH vitamin D serum level. A similar study on 251 patients also showed no significant association of high blood pressure and 25-OH vitamin D serum level [40]. However, Vimaleswaran et al. showed a significant association between increased 25-OH vitamin D level and decreased SBP and odds of hypertension of 49,363 patients [41]. In a cross-sectional study, a higher vitamin D level was associated with lower blood pressure [42]. Several possibilities merit discussion to explain these controversial findings. The geographical and seasonal differences and smaller sample size of our study may explain the discrepancy. However, some aspect like the dose–response relation between 25-OH vitamin D and parathyroid hormone, calcium intake, and underlying population factors such as age, sex, BMI, genetics, and medications can be considered in the potential vitamin D–blood pressure relationship [26]. All participants were resident of Tehran and all examinations and measurements were carried out in spring.

Eight weeks of oral vitamin D supplementation concomitant with conventional antihypertensive drug regimens caused 5.5 ± 16.2 mm Hg, 1.4 ± 12.6 mm Hg, and 3.7 ± 9.2 mm Hg decrease of overall SBP, DBP, and MAP, respectively. Altogether, the present intervention significantly reduced SBP and MAP in patients with essential hypertension.

The previous study using similar dose of vitamin D has shown significant reductions in blood pressure in patients with type 2 diabetes mellitus

and low vitamin D levels [43]. However, in a recent study, a total of 100,000 U of oral cholecalciferol every 3 months for 1 year did not improve blood pressure or markers of vascular health in patients with hypertension [44]. 100,000 U of vitamin D every 2 months for 6 months did not reduce 24-hour ambulatory and office blood pressure in patients with resistant hypertensive patients [35]. Some possibilities merit discussion to enlighten these controversial findings. It is possible that the dose of vitamin D was inadequate to reach clinical response. Moreover, regional variation may influence mean of serum level of vitamin D and the therapeutic effect of vitamin D intervention [36]. Finally, a wide range of antihypertensive agents have been used by subjects; thus, many of the available molecular pathways of blood pressure may have already been engaged. Vitamin D exerts antihypertensive effects through renin–angiotensin–aldosterone system [13]. A high proportion of hypertensive patients are regularly taking angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists that may obviate any further therapeutic benefit of vitamin D intervention. In the present study, 58.4% of patients were taking RAAS blockers. In this group, vitamin D supplementation could lead to a significant decrease in SBP and MAP. This effect may be caused by a synergistic effect of vitamin D supplementation and RAAS blockers, which led to achieving the goal of therapy in hypertensive patients.

We hypothesized that 25-OH vitamin D serum level may be in relation with the number of antihypertensive drug regimens. So, in the next set of analysis, the number of administrated antihypertensive drugs was compared in vitamin D-sufficient and vitamin D-deficient groups. This study failed to prove the hypothesis, and no significant differences was found in the number of antihypertensive drugs that patients administrated in vitamin D-sufficient and vitamin D-deficient groups.

Multiple logistic regression analysis in our study showed that risk of vitamin D deficiency was 2.5-fold times higher in women subjects. Women dress code (Hijab) in Islamic regions as sunlight barrier may explain lower vitamin D in women participants [45]. This finding emphasizes the necessity of vitamin D fortification of food in countries where some people may not be exposed to sunlight because of cultural and religious dress styles. Moreover, neither sex nor age showed significant association with goal of therapy. However, a marginally nonsignificant and negative correlation was seen between BMI and goal of therapy. In Multi-Ethnic Study of Atherosclerosis, a cohort and prospective study, on 3002 subjects free of prevalent cardiovascular disease and hypertension, lower serum 25-OH vitamin D categories were associated with higher unadjusted incident hypertension, but after adjustment for potential confounders such as BMI (as continuous variable) and kidney function, the association was no longer significant [46]. On the other hand, in meta-analysis of 108,173 subjects of 35 studies, increased 25-OH levels were associated with reduced SBP but not DBP and this association was not altered after adjustment for age, sex, method of blood pressure measurement, geographical region, and BMI [41]. To the best of our knowledge, until now, no study was found that investigate the effect of age, sex, or BMI on the goal of therapy after vitamin D supplementation.

One limitation of the current study was lack of placebo control group in the trial, which might have caused an overestimation of the effects of vitamin D supplementation. Also the present study was not blinded, and therefore, the results, though objective in nature, might have been biased due to potential placebo effects.

In conclusion, the present results suggested that vitamin D supplementation decreases SBP and MAP but not DBP in patients with essential hypertension on antihypertensive treatment regimens. Further investigations could explore if higher doses of vitamin D at longer treatment durations may be more effective, or whether selected patients (e.g., those not taking renin–angiotensin–aldosterone system inhibitors) may elicit differential blood pressure response to vitamin D supplementation. Molecular experiments on vitamin D receptor expression levels and polymorphisms of patients who reached treat-

ment goal would shed more light on the underlying mechanism action of vitamin D on blood pressure.

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Conflict of Interest None.

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Ethics Approval and Consent to Participate This study was performed in accordance with the Declaration of Helsinki guidelines, and the study protocol was approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences, Tehran, Iran (IR.RMSU.REC.1394.31).

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Boosting GLP-1 by Natural Products

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Abstract

The prevalence of diabetes mellitus is growing rapidly. Diabetes is the underlying cause of many metabolic and tissue dysfunctions, and, therefore, many therapeutic agents have been developed to regulate the glycemic profile. Glucagon-like peptide-1 (GLP-1) receptor agonists are a newly developed class of anti-diabetic drugs that have potent hypoglycemic effects via several molecular pathways. In addition to synthetic GLP-1 receptor agonists, some evidence suggests that natural products may have modulatory effects on GLP-1 expression and secretion. In the current study, we conclude that certain herbal-based constituents, such as berberine, tea, curcumin, cinnamon, wheat, soybean, resveratrol, and gardenia, can exert an influence on GLP-1 release.

Keywords

GLP-1 agonist · Diabetes mellitus · Berberine · Curcumin · Cinnamon · Resveratrol

1 Introduction

The global occurrence of diabetes mellitus is growing rapidly [1]. This chronic disorder affects many metabolic processes and results in well-

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recognized diabetic complications [2]. Chronic uncontrolled hyperglycemia induces a series of pathophysiological pathways, such as inflammation, oxidative stress, apoptosis, and fibrosis, leading to metabolic perturbation and tissue dysfunction, notably in the kidneys, heart, liver, retina, and nervous system [3–5]. Thus, many therapeutic agents have been developed to regulate blood glucose and prevent diabetic complications [6, 7].

Glucagon-like peptide-1 (GLP-1) agonists are a newly introduced class of antidiabetic drugs which exert their hypoglycemic effects via suppression of glucagon secretion and induction of insulin release [8, 9]. These antidiabetic agents have a minor risk of hypoglycemia and are therefore important additions to the antidiabetic armamentarium [10]. Several synthetic forms of this class have been developed [11]. However, emerging evidence indicates that some herbal-based components and/or natural products have similar stimulatory effects on GLP-1 and can modulate its activation [12–14]. In the current study, we present current information about the GLP-1 modulatory effects of natural compounds and draw conclusions about their possible beneficial activity in regulating blood glucose.

2 Diabetes Mellitus Classification

Diabetes mellitus (DM) is commonly categorized into three main forms: type 1, type 2, and gestational diabetes [15]. Type 1 diabetes (T1DM) or insulin-dependent diabetes mellitus (IDDM) accounts for approximately 5–10% of all diabetic subjects and is caused by autoimmune-mediated β-cell destruction resulting in insufficient circulating levels of insulin [15]. Type 2 diabetes (T2DM) or non-insulin-dependent diabetes mellitus (NIDDM) is the most common form of diabetes accounting for about 90–95% of all cases of diabetes and is due to a combination of beta cell loss and insulin resistance in peripheral tissues [15].

Gestational diabetes is another type of DM which occurs during pregnancy, largely due to the hormonal variations of pregnancy [16]. However, another form of diabetes, known as "latent autoimmune diabetes in adults" (LADA), has been described and is primarily considered as a subtype of TIDM but with certain distinct features [17].

3 GLP-1 Receptor Agonists

GLP-1 receptor agonists are inducers of the GLP-1 receptors and act as incretin mimetic agents; they were approved in 2010 by the FDA for treatment of diabetic patients [18, 19]. Incretins are a family of metabolic hormones that include intestinal GLP-1 and gastric inhibitory peptide (GIP), which lower postprandial blood glucose by inhibition of glucagon release from alpha cells and stimulation of insulin secretion from beta cells in a glucose-dependent manner [18, 20, 21]. Additional effects of incretins include delaying gastric emptying, suppression of appetite, decreasing nutrient absorption from the gut, improving lipid metabolism, inhibition of pancreatic β-cell apoptosis, and induction of beta cell neogenesis [20, 22, 23].

These hypoglycemic effects of GLP-1 agonists are initiated by linking the GLP-1 agonist to its specific receptors (GLP-1Rs) which are predominantly located in pancreatic beta cells [21]. The GLP-1R is a member of the G-protein-coupled family of receptors, and its activation induces cAMP (cyclic adenosine monophosphate) generation with beta cell depolarization and augmentation of intracellular calcium concentration, leading to insulin secretion [21, 24]. While endogenous GLP-1 has a half-life of about 2–3 min, due to rapid cleavage by the dipeptidyl peptidase-4 (DPP-4) enzyme, synthetic GLP-1 agonists such as dulaglutide, albiglutide, and liraglutide have a prolonged half-life of up to several days and thus provide effective sustained glucose modulation in subjects with diabetes [20, 23, 25].

Although these antidiabetic drugs have a lower risk of hypoglycemia when compared with insulin, sulfonylureas, and meglitinides, they do harbor some adverse effects such as nausea, vomiting, diarrhea, injection-site inflammation, pan-

creatitis, formation of antibodies against the peptide, and induction of heart rate elevation [20, 26, 27]. Exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, and semaglutide are approved synthetic forms of GLP-1 agonists [8]. In addition to intrinsic and synthetic GLP-1 agonists, some natural-based products may show similar hypoglycemic effects [12, 13].

4 Natural-Based Agents Modulating GLP-1 Secretion

Some herbal-based agents demonstrate GLP-1 secretagogue activity [13, 19, 28]. In the following sections, we consider the GLP-1 modulatory effects of medicinal plants.

4.1 Berberine

Berberine is an alkaloid with hypoglycemic action that is found in the roots, rhizomes, stems, and bark of certain plants such as *Berberis* [29]. Lu et al. in 2009 demonstrated that berberine lowers blood glucose by increasing mRNA expression and secretion of GLP-1 in an animal model of streptozotocin (STZ)-induced diabetes [30]. Yu and coworkers in 2010 reported compelling in vivo and in vitro evidence indicating that berberine increases GLP-1 levels via promoting GLP-1 biosynthesis and release in both nondiabetic and diabetic states [31]. Zhang et al. in 2014 demonstrated that 8 weeks of berberine therapy ameliorated blood glucose by improving GLP-1 mRNA expression and enhancing GLP-1 secretion via MAPK (mitogen-activated protein kinase) and GLP-1-GnRH (gonadotropin-releasing hormone) signaling pathways [32]. Yu et al. in 2015 found that berberine induced GLP-1 release via activation of gut-expressed bitter taste receptors in a phospholipase C-dependent pathway in human enterocyte NCI-H716 cells [33].

Some studies have also suggested that berberine may modulate GLP-1 release by improvement in mitochondrial function [34, 35]. Sun et al. in 2018 reported that berberine promoted GLP-1 secretion via improvement in mitochondrial stress in colon enterocytes of diet-induced obese mice [35]. Also, Ye and coworkers in 2018 found that berberine improved GLP-1 secretion via restoration of mitochondrial function and normalization of the misbalance between complexes I, II, and IV [34]. Improvement in mitochondrial function may therefore be a potential mechanism, whereby berberine modulates GLP-1 secretion.

4.2 Resveratrol

Resveratrol is a naturally occurring phenolic constituent found in certain plants such as blueberries, red wine grapes, raspberries, mulberries, and peanuts [36]. Some reports indicate that it can modulate GLP-1 physiology in the setting of diabetes [13]. Dao et al. in 2011 found that resveratrol normalized blood glucose by increasing proglucagon mRNA expression and improving GLP-1 secretion in an animal model of diabetes [13]. Brasnyó et al. in 2014 suggested that resveratrol increased GLP-1 levels by exerting an estrogen-like effect, or by stimulating PPAR-γ (peroxisome proliferator-activated receptor-γ) activity [37].

However, contradictory reports exist [38]. Thazhath et al. in 2015 published that 5 weeks of resveratrol therapy in type 2 diabetes mellitus (T2DM) patients had no significant effect on either GLP-1 levels or the glycemic profile [38]. Brasnyó et al. in 2011 demonstrated that resveratrol did not impact GLP-1 secretion in patients with T2DM [39].

4.3 Soybean

Park et al. in 2010 demonstrated that glyceollins derived from soybean normalize glucose homeostasis by induction of GLP-1 secretion [40]. Mietlicki-Baase et al. in 2017 suggested that dietary consumption of soybean protein induces GLP-1 signaling pathways, leading to improved glucose homeostasis [41]. Moreover, Watanabe and coworkers in 2018 established that dietary soybean protein improves the lipid profile by increasing GLP-1 expression and secretion [42]. This combined evidence strongly suggests that soybean protein exerts modulatory effects on GLP-1 secretion.

4.4 Wheat

Wheat fragment proteins may have modulatory effects on GLP-1 secretion [43]. Freeland et al. in 2008 demonstrated that an increased intake of wheat fibers increases GLP-1 secretion in hyperinsulinemic patients [43]. Also, they established in 2010 that wheat fiber induces GLP-1 secretion in hyperinsulinemic patients [44]. Similarly, Kato and colleagues in 2017 demonstrated that protein fractions of wheat improve glucose homeostasis by induction of insulin secretion and an increase of GLP-1 release via the Ca^{2+}/calmodulin-dependent kinase II pathway [45]. Additionally, Eelderink and coworkers in 2017 showed that wheat bread markedly increased the postprandial GLP-1 response in healthy men [46].

4.5 Gardenia

Gardenia-derived products may also potentiate GLP-1 secretion [47]. Liu et al. in 2007 demonstrated that geniposide, derived from the gardenia plant, is a potent agonist for the GLP-1 receptor, increasing its activation and preventing PC12 cells from oxidative damage via MAPK signaling pathway [48]. Yin et al. in 2010 found that GLP-1 receptors play an important role in mediating the effects of geniposide in PC12 cells [49]. Shin et al. in 2014 demonstrated that gardenia consumption improved lipid metabolism by modulating GLP-1 secretion in obese women [47]. Also, Liu et al. in 2012 established that geniposide induced glucose-dependent insulin release by activating GLP-1 receptors in INS-1 cells (a pancreatic beta cell line) [50].

4.6 Cinnamon

Cinnamon is a herbal ingredient with potent hypoglycemic effects [51]. Hlebowicz et al. in 2009 demonstrated that cinnamon increases postprandial GLP-1 levels in healthy men [51]. Also, Plexopathy and coworkers in 2009 showed a dose-dependent increase of postprandial GLP-1 activity mediated by cinnamon [52]. Moreover, Vallianou et al. in 2014 suggested that the glucose-lowering effects of cinnamon may be related to GLP-1 induction [53]. Further investigations are, however, needed.

4.7 Tea

Tea drinking may modulate GLP-1 levels [54]. Planes-Muñoz et al. in 2018 demonstrated that green tea induces GLP-1 secretion in the STC-1 cell line [54]. Also, Liu and colleagues in 2014 showed that green tea extract improved insulin resistance and increased GLP-1 levels in patients with T2DM and impaired lipid profiles [55]. Furthermore, Hussein et al. in 2011 reported that mate tea (a traditional drink in South America) induces GLP-1 secretion in mice [56]. This evidence implies that tea drinking may regulate GLP-1 secretion.

4.8 Curcumin

Curcumin is a yellow-colored constituent derived from the rhizomes of *Curcuma longa* that possesses several health benefits including immunomodulatory, anti-oxidative, and anti-inflammatory properties [57–63] and can also lower blood glucose [54, 64] and exert insulin-sensitizing and antidiabetic effects [65–69]. Kato et al. in 2017 demonstrated that curcumin improves glucose homeostasis by induction of GLP-1 secretion via a G-protein-dependent pathway [64]. Takikawa and coworkers in 2013 found that curcumin increases GLP-1 secretion via the Ca^{2+}/calmodulin-dependent kinase II signaling pathway in GLUTag cells [70]. Thota et al. in 2018 demonstrated that curcumin reduces postprandial blood glucose, probably via GLP-1 induction in healthy subjects [71]. Curcumin, therefore, has shown potential for induction of GLP-1 activity.

4.9 Quercetin and Its Glycosides

Quercetin is a naturally occurring flavonoid found in many plant products, such as fruits, leaves, grains, and vegetables, and primarily acts as a polar auxin

transport inhibitor [24]. Some evidence suggests that quercetin and its glycoside compounds, such as rutin, can induce insulin sensitivity and improve the glycemic profile [72, 73]. Some studies have suggested that these hypoglycemic effects are modulated via GLP-1 secretion [24, 74]. Gaballah et al. in 2017 demonstrated that combination therapy with quercetin and liraglutide, a GLP-1 analogue, intensifies the hypoglycemic effects of the GLP-1 receptor agonist in diabetic rats [75]. Wootten et al. in 2011 reported that quercetin has potential effects on GLP-1 receptor activation via intracellular Ca^{2+} signaling modulation [24]. Also, Phuwamongkolwiwat and coworkers in 2014 demonstrated that oral daily consumption of quercetin glycosides for 48 days markedly increased GLP-1 secretion in STZ-induced diabetic rats [73]. Moreover, Koole et al. in 2010 found that quercetin is a potent and selective inducer for GLP-1R activation by increasing intracellular Ca^{2+} concentration [76].

4.10 Ginger

Ginger (or so-called *Zingiber*) is a well-known medicinal plant widely used as a food additive [77]. Ginger has historically been prescribed for diabetic patients, and recent studies indicate that it, along with gingerol (a chemical compound found with ginger), may exert this hypoglycemic effect via induction of GLP-1 secretion [78, 79]. Samad et al. in 2017 demonstrated that the hypoglycemic effects of ginger are mediated by GLP-1 induction in type 2 diabetic mice and therefore suggested that ginger is a potent inducer of GLP-1 secretion [79]. Also, Emery and colleagues in 2015 suggested that ginger may facilitate GLP-1 secretion by modulating transient receptor potential in enterocyte generating GLP-1 [80].

4.11 Ginseng

Ginseng and its active component, ginsenoside, have historically used for medicinal purposes [85]. Some evidence suggests that they may modulate GLP-1 homeostasis and induce its secretion [81]. Liu et al. in 2013 provided in vitro and in vivo evidence indicating that ginsenosides, derived from ginseng, induce GLP-1 expression and secretion by increasing the ATP/ADP ratio in diabetic rats [81]. Similarly, Kim and coworkers in 2014 demonstrated that ginsenosides increase GLP-1 secretion via an increase in Ca^{2+} and cAMP levels in NCI-H716 cells [82]. Kim and coworkers reported that a ginsenoside metabolite, Rg3, induces GLP-1 release in an animal model of T2DM [83]. Moreover, Liu et al. in 2014 demonstrated that ginsenosides potentially ameliorate hyperglycemia and hyperlipidemia via induction of GLP-1 secretion in an animal model of diabetes [84]. This evidence strongly suggests that ginseng plant extract can improve GLP-1 secretion in the diabetic state [84].

4.12 Other Candidates

In addition to the natural products already discussed, some herbal-derived constituents such as little dragon, bitter melon, yacon, blueberry, glutamine, garlic and allicin, naringenin, monounsaturated fatty acids, mango, and pygeum may provide modulatory effects on GLP-1 secretion [28, 80, 86–91]. Current evidence concerning these agents is, however, very limited and further investigation is needed.

5 Conclusion

Modulation of GLP-1 expression/secretion is an effective method for normalizing blood glucose. While synthetic forms of GLP-1 agonists may have adverse effects, some natural-based nutraceuticals have modulatory effects on GLP-1 activity by enhancement of expression and induction of secretion with fewer side effects (Table 1). Compelling data suggests that berberine, quercetin, ginseng, ginger, gardenia, tea, wheat, soybean, curcumin, cinnamon, and resveratrol each have potent effects on GLP-1 activity. Other candidates may show similar effects, but need further investigation. The use of herbal-based GLP-1 agonists should be considered as a new therapeutic strategy to normalize blood glucose.

Table 1 Natural products with potential to modulate GLP-1 secretion

Natural product	Effects on GLP-1 secretion	References
Berberine	Increases GLP-1 biosynthesis and release in both nondiabetic and diabetic states via MAPK and GLP-1-GnRH signaling pathways with improvement in mitochondrial function	[30–35]
Resveratrol	Increases proglucagon mRNA expression and improves GLP-1 secretion in an animal model of diabetes, increases GLP-1 levels by exerting an estrogen-like effect, or by stimulating PPAR-γ activity	[13, 37]
Soybean	Induces and increases GLP-1 signaling pathways	[40–42]
Wheat	Induces GLP-1 secretion in hyperinsulinemic patients, increases GLP-1 release via the Ca2+/calmodulin-dependent kinase II signaling pathway	[43–46]
Gardenia	Stimulates GLP1-expression and release via the MAPK signaling pathway in diabetic/obese human	[47–50]
Cinnamon	Induces GLP-1 release in both nondiabetic and diabetic states	[51–53]
Tea	Green/mate tea increases GLP-1 secretion	[54–56]
Curcumin	Induces GLP-1 release via the G-protein-dependent pathway or the Ca2+/calmodulin-dependent kinase II signaling pathway	[54, 64, 70, 71]
Quercetin	Increases GLP-1 release by increasing intracellular Ca2+ levels	[73, 75, 76]
Ginger	Induces GLP-1 secretion by modulating TRP	[78–80]
Ginseng	Increases GLP-1 secretion via an increase in intracellular Ca^{2+} and cAMP levels	[81–84]

GLP-1 glucagon-like peptide-1, *PPAR-γ* peroxisome proliferator-activated receptor-γ, *MAPK* mitogen-activated protein kinase, *GnRH* gonadotropin-releasing hormone, *TRP* transient receptor potential, *cAMP* cyclic adenosine monophosphate

Conflict of Interests The authors declare no conflict of interest in this study.

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Naturally Occurring SGLT2 Inhibitors: A Review

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Abstract

With an increasing incidence of diabetes mellitus globally due to various factors, including unhealthy lifestyle, there is a need for developing novel drugs for the management of diabetes. This chronic metabolic disorder results in high blood glucose levels due to the body's inability to reduce the concentration of glucose. The decreased secretion of insulin and increased resistance to insulin action contribute to the development of diabetes mellitus. There have been efforts to target pathways involved in the metabolism of blood glucose. It seems that most of the currently applied antidiabetic medications are associated with unwanted side effects. Hence, it appears that plant-derived chemicals can be considered as potential candidates in the management of diabetes. Sodium-glucose cotransporter inhibitors (SGLT2i) are synthetic hypoglycemic medications approved for managing patients with diabetes in lowering blood glucose. SGLT2i reduces blood glucose concentration by enhancing its urinary excretion and inhibition of its absorption through the kidney. It has been demonstrated that some of the naturally occurring nutraceutical agents can imitate the action of SGLT2i and, consequently, diminish the level of blood glucose. At the present

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review, we have discussed the phytochemicals that act like SGLT2i to decrease blood glucose level.

Keywords

Diabetes · Glucose · SGLT2i · Herbal medicine · Antidiabetic medications

1 Introduction

The global prevalence of diabetes mellitus [1] is rising rapidly [2]. This chronic disorder negatively modulates most metabolic pathways throughout the body resulting in a wide range of diabetic complications [1, 3]. These diabetes-induced complications result in considerable morbidity and mortality [4, 5]. It has well confirmed that higher glycemia levels potentially induce various deleterious molecular mechanisms such as oxidative stress, inflammatory responses, apoptosis, and fibrosis, thereby acting as an effective upstream event for developing diabetic complications [3, 6, 7]. Various antidiabetic agents have shown to normalize blood glucose levels and related metabolic pathways leading to lowering the rate of diabetic complications and health costs [8, 9].

Sodium-glucose cotransporter inhibitors (SGLT2i) are one of these synthetic agents approved to manage patients with diabetes to lower blood glucose [10]. This class of antidiabetic agents potentially reduces blood glucose levels by inhibition of filtrated glucose reabsorption and induction of more glycosuria [10]. However, some recent studies have suggested SGLT2 inhibitory effects by natural-based agents and plants [11, 12]. They have shown that some herbal-based agents may provide antihyperglycemic outcomes in the same manner to SGLT2i [12, 13]. Since these findings are significant as they can open new ways for us to develop the next generation of more safe therapeutic strategies for patients with diabetes, we have reviewed these naturally occurring agents in the current review.

2 SGLT2 Inhibitors as a Novel Class of Antihyperglycemic Agents

SGLT2 inhibitors are a group of glucose-lowering agents that inhibit glucose tubular reabsorption and induce its urinary excretion by reducing the renal threshold for glucose excretion near to physiologic levels [14, 15]. Sodium-glucose cotransporters are two types of active imperative cotransporters (as 1 and 2) that are mainly located in S2 and S3 segments of proximal renal tubules (as well as in intestines) which potentially reabsorb the most amount of filtrated urinary glucose [10, 16] (Fig. 1). SGLT2i work completely independent of insulin, and their action is glucose-dependent so that they do not cause hypoglycemia [17]. Since discovering the phlorizin as the first SGLT2 inhibitor, several forms of these agents have been introduced [17, 18]. They all reduce the blood glucose near the level of nephrons' capacity for glucose reabsorption [19, 20].

Besides the potent hypoglycemic effects, they have other activities such as preventing gluconeogenesis, improving insulin sensitivity in peripheral tissues, enhancing glucagon response, and induction of insulin secretion from islets beta cells [21–24]. Canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin are examples of this class of antidiabetic medications [25]. However, using SGLT2i may be accompanied by side effects such as urinary tract infections, dehydration, dizziness, hypotension, and fainting [25].

3 Classification of Diabetes Mellitus

DM is mainly categorized into three types as TIDM, T2DM, and gestational diabetes [26]. T1DM (type 1 diabetes) is responsible for about 5–10% of all cases of diabetes and due to beta-cell dysfunction resulting in a reduction of insulin release [26]. T2DM (type 2 diabetes) is the most prevalent type of diabetes, which accounts

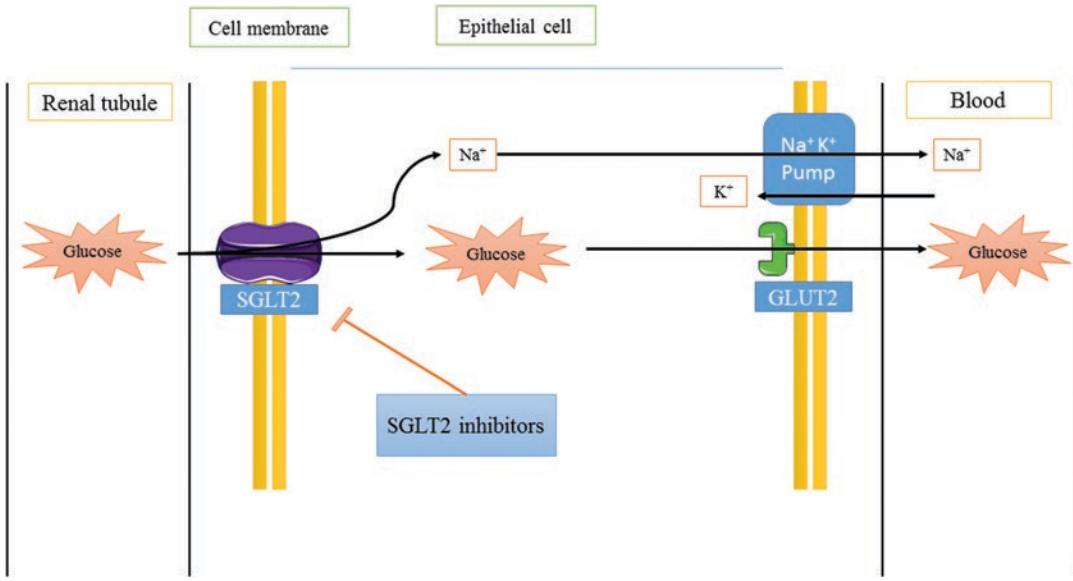

Fig. 1 A schematic representation of the function of SGLT2i

for about 90–95% of diabetic subjects and is mainly linked to inadequate insulin and insulin resistance [26]. Gestational diabetes is another type of DM found in pregnant women, mostly due to hormonal variations during pregnancy [27]. Other forms of diabetes include maturity-onset diabetes of young and secondary diabetes due to pancreatitis and steroids [28].

3.1 Natural SGLT2 Inhibitors

Phlorizin was the first discovered agent with SGLT2 inhibitory effects that reduce postprandial glucose which acts by competing with D-glucose to bind to the SGLT2 and thereby block its activity [29]. This phytochemical molecule is a naturally occurring glycoside that belongs to the polyphenol family. It may occur with other forms of polyphenols such as quercetin, procyanidins, catechin, and epicatechin in plants [30]. In the early 1970s, the relationships between active glucose transports in the brush border of proximal renal tubules were identified [31]. Phlorizin is hydrolyzed by lactase-phlorizin hydrolase (LPH), a glycoprotein with two catalytic sites, which is mainly expressed in the brush border of the small intestine [13]. This enzyme is also responsible for hydrolyzing the lactose [13].

It has been demonstrated that high doses of phlorizin inhibit renal SGLT2 activities and induce glycosuria [32, 33]. Phlorizin-induced glycosuria normalizes postprandial hyperglycemia in diabetic pancreatectomized rats [34]. Furthermore, evidence indicated that phlorizin decreases postprandial glucose and increases insulin sensitivity in diabetic milieu [34–36]. So, many fruits and plant-containing phlorizin have various degrees of SGLT2 inhibitory effects [13, 37–40]. Moreover, recent studies have found similar compounds to phlorizin plants such as sergliflozin, remogliflozin, canagliflozin, dapagliflozin, and empagliflozin which have the same inhibitory effects on SGLT (1/2) with varying strengths of potencies [11].

Several fruits and plants have varying degrees of SGLT2 inhibitory activities [13, 18, 40, 41]. Studies have demonstrated that the apple tree, quercetin, strawberry, rose hip, and pear have phenolic components with SGLT2 inhibitory effects. They can also reduce blood glucose by induction of glycosuria [11, 30, 37, 38, 42].

3.1.1 Apple Tree

We have some evidence indicating barks of apple tree have inhibitory effects on renal SGLT2 and thereby can make glycosuria and hypoglycemic effects [43]. As the first natural discovered SGLT2i, phlorizin was isolated for the first time from barks of an apple tree by French chemists in 1835 [29, 43]. They have considered it "glycoside from the bark of apple trees," and then it was used in clinical studies for more than 150 years [29, 43]. It has detected that it is mainly found in the young shoots, roots, leaves, and barks of apple tree, while in fruit, it is most abundant in the seeds [44]. Bailey et al. suggested that SGLT2 inhibition-dependent hypoglycemic effects of apple tree extract may be a novel therapeutic target in patients with diabetes [45]. Schulze et al. in 2014 reported the same hypoglycemic effects of apple tree phenolic extracts by inhibition of intestinal SGLT1 in human and mice model of diabetes [40].

Also, Johnston and coworkers in 2002 found that the phenolic compound of apple tree increases insulin secretion and induces insulin sensitivity and reduces postprandial glucose in patients with diabetes [41]. Similarly, Shirosaki et al. in 2012 demonstrated that apple leaf extract significantly reduces postprandial hyperglycemia in diabetic mice [46]. Moreover, Makarova and coworkers in 2015 have evaluated the hypoglycemic potencies of apple preparation and showed that it improves glycemic control in patients with diabetes [18]. This evidence strongly suggests that apple tree extract has significant hypoglycemic potencies using the same mechanisms as SGLT2i [18, 46].

3.1.2 Strawberry

Hilt and colleagues in 2003 detected the phlorizin compounds in strawberry by detailed spectroscopy [37]. They suggested that it can simulate SGLT2 inhibitory effects and reduces blood glucose [37]. Also, Ehrenkranz and coworkers in 2006 reported that phenolic compounds of phlorizin are found in strawberries and so can modulate renal SGLT2 activities [47]. Ehrenkranz et al. reported that phlorizin is found in all parts of the strawberry plant could have hypoglycemic effects by SGLT2 inhibition [48]. Moreover, Jugdé and coworkers in 2008 demonstrated the hypoglycemic outcomes of phlorizin-induced SGLT2 inhibition [49]. These studies strongly suggest that phlorizin is found in strawberry and stimulates SGLT2 inhibitory effects, thereby leading to lowering of blood glucose [48, 49].

3.1.3 Rose Hip

The extract of rose hip has some phenolic compounds with phlorizin-like properties [38]. Hvattum et al. in 2002 analyzed rose hip extract by liquid chromatography and found that it contained phenolic compounds of phlorizin by SGLT2 inhibitory effects [38]. More studies are needed to clarify these findings.

3.1.4 Pear

In the pear bark, phlorizin reduces the concentration of blood glucose by competitive inhibition of SGLT2 and SGLT1 and enhancing the urinary excretion of glucose [50]. Other studies have also confirmed these findings [51] but need further studies.

3.2 *Alstonia macrophylla*

Alstonia macrophylla is a tree of the Apocynaceae family mainly distributed in Southeast Asia [52]. Arai and coworkers in 2010 reported that it could exert potent SGLT2 inhibitory effects [53]. They have isolated three alkaloids as picraline, alstiphyllanines E–H, and ajmaline involved in SGLT2 inhibitory effects of these plants [53]. Also, Khyade et al. in 2014 reported that it has potent SGLT2 inhibitory effects and other therapeutic potentials [54]. However, more studies are still needed.

3.2.1 *Sophora flavescens*

Sophora flavescens is a plant of the Fabaceae family which is historically used in Chinese medicine and primarily cultivated in Asia, Oceania, and the Pacific islands [55, 56]. Some studies have confirmed its potent SGLT2 inhibitory effects [57]. Yang and colleagues in 2015 isolated nine flavonoids, which potentially inhibit SGLT2

Table 1 Main recognized plant with SGLT2 inhibitory effects

Plants	Possible SGLT2i effects	References
Apple tree	Have phenolic compounds which inhibit SGLT2	[18, 40, 41, 45, 46]
Strawberry	Have phlorizin compounds by SGLT2 inhibitory effects	[47–49]
Rose hip	Have phenolic compound with phlorizin effects	[38]
Pear	Have phlorizin with SGLT2i effects	[50, 51]
Alstonia macrophylla	Have alkaloids with SGLT2i effects	[53, 54]
Sophora flavescens	Have flavonoids with SGLT2i influences	[57, 58]

[57]. Also, Sato et al. in 2007 have demonstrated that *Sophora flavescens* has flavonoids which can significantly inhibit SGLTs with potent hypoglycemic effects [58] (Table 1).

3.2.2 Other Possible Sources Such as Vitexin and Quercetin

Vitexin is a well-known herb with a long story of application in Chinese traditional medicine due to its protective activities such as anti-inflammatory, antitumor, and antidiabetic effects [59]. A study in 2018 by Rezwendy and colleagues shed light on the antidiabetic effect of vitexin mediated by inhibition of SGLT2 and, consequently, improving blood glucose level. Cucumerin is another member of Indonesian herbs that has demonstrated great potential in reducing glucose level by targeting SGLT2 [60]. Quercetin is one of the critical members of the flavonoid family with tremendous biological and therapeutic effects such as antioxidant, anti-inflammatory, antitumor, hepatoprotective, and antidiabetic effects [61]. It seems that quercetin is absorbed by the intestine through sodium-glucose cotransporter-1 (SGLT-1). A study in 2001 has exhibited the inhibitory effect of quercetin on the SGLT-1 [62]. Ader et al. in 2001 reported that quercetin could inhibit intestinal SGLT1 [62]. We suggest that more studies on natural-based agents may lead us to discover new plants with SGLT2i effects.

4 Conclusion

In view of the increasing prevalence of diabetes mellitus, there is a growing need for more research into plant-based chemicals with less adverse effects to manage diabetes and its resulting complications. This has led to the discovery of naturally occurring nutraceutical compounds with the mechanism of action similar to SGLT2i mentioned in this review. However, we are at the beginning of this long road. More studies are needed to discover the plant-derived chemicals with the action similar to SGLT2i leading to new therapeutic agents' development with lesser side effects.

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Conflict of Interests The authors declare that they have no conflict of interest in this study.

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Renoprotective Roles of Curcumin

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Abstract

The use of herb-based therapies is increasing over the past decades. These agents have been reported to provide many beneficial effects in many experimental and clinical studies. Curcumin is one of these agents which has potent pharmacological effects enabling it for the prevent and treatment of many diseases and pathologies such as renal disorders, hyperglycemia, oxidative stress, hypertension, and dyslipidemia. However, the exact molecular mechanisms mediating these renoprotective effects of curcumin are not well established. So, in the current study, we surveyed for possible renoprotective roles of curcumin and concluded how curcumin protects against renal injuries.

Keywords

Diabetic nephropathy · Chronic kidney disease · Curcumin · Oxidative stress · Apoptosis · Inflammation

1 Introduction

The kidney plays a vital role in maintaining the extracellular electrolyte composition, fluid balance, and blood pressure homeostasis, which is

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critical for survival. The prevalence of chronic kidney disease is estimated to be 8–16% worldwide, and it is expected to grow with increase in chronic lifestyle diseases [1]. Injury to kidneys occurs due to the use of drugs including nonsteroidal anti-inflammatory drugs, antibiotics, antitumor drugs, and angiotensin-converting enzyme inhibitors, leading to acute kidney injury (AKI). Severe, long, and repeated episodes of acute kidney injury increase the risk of progression of chronic kidney disease [2].

Diabetic nephropathy (DN) is one of the main causes of end-stage renal disease. It is one of these complications which developed mainly due to uncontrolled diabetes and is damaging to renal microstructures [3]. Overall occurrence of diabetes mellitus (DM) is growing rapidly [4, 5]. This chronic disorder has adverse effects on most metabolic pathways and is a potent upstream event for a wide series of microvascular and macrovascular problems known as diabetic complications [5, 6]. These complications are responsible for many cases of disabilities or death in human [6]. So, prevention or treatment of them is a main issue of global health [6, 7]. It is commonly identified by failing in renal efficiency, microalbuminuria, and raised levels of creatinine in plasma [8]. DN is the leading cause of hemodialysis in patients with ESRD (end-stage renal disorder), and so, many therapeutic agents are introduced to prevent/treat it [8–11]. Despite these efforts, epidemiological evaluations still demonstrate a raising trend for its occurrence [3].

Curcumin is an herbal-based pharmaceutical compound which has been widely used in traditional medicine [12]. Recent evidences demonstrated that this phytochemical is safe and has potent pharmacological effects and modulates molecular pathways in biologic milieu [13–20]. Also, some evidences suggested that curcumin has obvious renoprotective properties and improves renal efficiency in diabetic milieu [21, 22]. So, in current review, we examined the possible molecular mechanisms by which curcumin ameliorates CKDs and improves renal functions.

2 Diabetic Nephropathy

DN is a main form of CKDs and the leading cause of ESRD worldwide [3, 23]. It has a high prevalence of up to 40% among diabetic patients which developed in approximately 15 years after onset of DM [24, 25]. DN has a very complex pathophysiology including many underlying molecular pathways [26–28]. Evidences demonstrated that different pathophysiologic pathways such as oxidative damages, inflammatory responses, apoptotic and necrotic processes, protein kinase c (PKC) isoforms, renin-angiotensin-aldosterone system (RAAS) activation, toll-like receptor (TLR) activation, transforming growth factor-β (TGF-β), nitric oxide (NO), tumor necrosis factor-α (TNF-α), death receptors, JAK/STAT (Janus kinase/signal transducers and activators of transcription) pathway, and adhesion molecule activation are closely involved in developing DN [26–29]. DN is commonly accompanied with higher levels of toxic by-products in body fluids and lower renal water excretion leading to water retention and edema [30]. Since kidneys have vital roles in body homeostasis, these conditions impose unusual status to the body and negatively affect on most physiologic systems as well as cardiovascular and central nervous systems [30].

3 Curcumin

Curcuminoids are aromatic pigments with three identified compounds as curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [31, 32]. Curcumin is a polyphenol compound with obvious pharmacological effects which mainly derived from the traditional medicine turmeric plant [33]. Turmeric is a flowering plant of the Zingiberaceae family mainly cultured in Southern Asia and Middle Eastern countries that its rhizomes annually gathered for fresh usage or dried for powdering [33]. Turmeric powder is widely consumed as a dietary supplement, food coloring, and food additive worldwide [34, 35]. Curcumin has the molecular formula of $C_{21}H_{20}O_6$ and a molecular weight of 368.37 [36]. It is a bioactive and unstable aromatic yellow pigment of

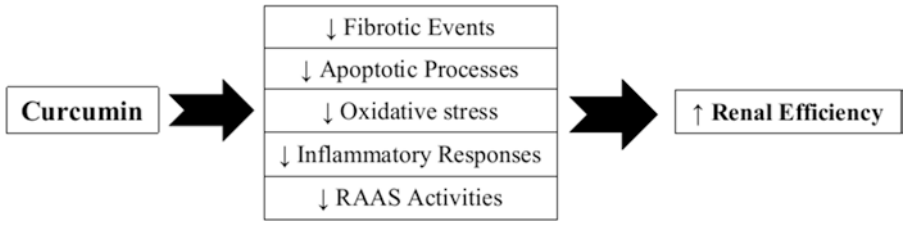

Fig. 1 Main possible pathways mediating renoprotective effects of curcumin

turmeric plant which has shown potent pharmacological effects in experimental and clinical evidences [20, 37–40]. These evidences implied that it has potent medicinal properties as antioxidant, anti-inflammatory effects, and modulatory impacts on intracellular molecular pathways as apoptotic events, necrotic processes, cell migration, cell growth, cell signaling, angiogenesis, and metabolic pathways [20, 37–41]. Therefore, it is now considered as a golden nutraceutical element with obvious pharmacological effects [34].

4 Curcumin and Chronic Kidney Diseases

CKDs are managed using synthetic pharmacological agents globally, which are frequently associated with undesirable adverse effects [42, 43]. Herbal-based agents commonly provide more safe preventive and/or therapeutic effects in biologic milieu [44, 45]. In this point, curcumin can be considered as an effective nutraceutical compound. Several studies have provided evidences indicating curcumin has potent renoprotective effects [36, 46, 47]. In the following sections, we conclude about these beneficial effects and related underlying molecular pathways (Fig. 1).

4.1 Antioxidative Effects

Oxidative stress has pivotal roles in the pathophysiology of many complications as well as CKDs [48, 49]. This state is developed when the amount of produced free radicals exceeds the physiologic levels and overcomes the antioxidative elements' potency [48]. Then, exceeded free radicals attack the biologic elements and macromolecules and interrupt their normal structure and functions [49]. Emerging evidences demonstrated that oxidative stress is a major upstream event in renal failure [26, 50]. It could induce CKDs via several mechanisms as PKC[1] isoform activation, hexosamine and polyol pathways, AGEs[2]-RAGEs[3] interactions, inflammatory responses, ANS[4] and RAS[5] activation, and direct attack to biologic molecules in renal tissues [26].

Curcumin has shown potent antioxidative effects in numerous experimental and clinical studies [51–54]. It is able to modulate free radical generation and neutralize their deleterious effects by its antioxidant activity at cellular or nuclear levels [54]. It can ameliorate oxidative damages via scavenging the free radicals and/or potentiating the intrinsic antioxidant defense system (ADS) [47, 55, 56]. Also, it can downregulate free radical generator enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [57]. Curcumin improves mitochondrial function and inhibits upcoming mitochondria-dependent free radical production [32]. It markedly reduces lipid peroxidation and decreases toxic by-products involved in oxidative damages [58]. Also it increases the total antioxidant capacity of the cells by upregulating nuclear mediators as Nrf2[6] or Sirt[7] [58–60]. Thus it is considered as a potent antioxidative herbal-based agent with

[1] Protein kinase C.
[2] Advanced glycation end products.
[3] Receptors for advanced glycation end products.
[4] Autonomic nervous system.
[5] Renin-angiotensin system.
[6] Nuclear factor erythroid 2-related factor 2.
[7] Sirtuin.

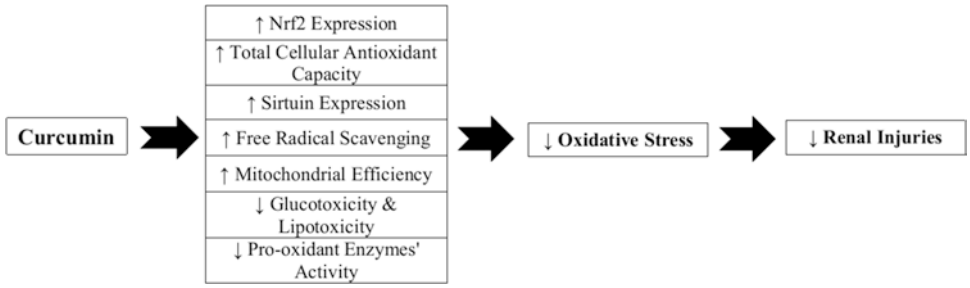

Fig. 2 Curcumin provides renoprotective effects via lowering oxidative damages thru several pathways (Nrf2 = nuclear factor erythroid 2-related factor 2)

pluripotent beneficial effects in tissues [32, 47, 55] (Fig. 2).

There are evidences indicating curcumin exerts renoprotective effects by attenuating oxidative stress [47, 55]. For example, Ugur et al. in 2015 have reported that curcumin improved renal function by ameliorating the oxidative stress and nephrotoxicity in rats [47]. They demonstrated that curcumin potentiates the ADS in kidneys by upregulation of SIRT (sirtuin) and NAMPT (nicotinamide phosphoribosyl transferase) as two main nuclear factor modulating cellular resistance to oxidative stress [47]. Similarly, Ali and coworkers in 2018 demonstrated that curcumin potentiates cellular ADS by inducing the Nrf2 and attenuates oxidative damages in renal tissues of rats [54]. Momeni et al. in 2017 reported that curcumin reduces histological injuries induced by oxidative stress in renal tissues [58]. Moreover, Al-Kuraishy et al. in 2019 found that curcumin reduces oxidative stress in gentamycin-dependent toxicity in kidneys of rats [55]. These findings strongly suggest that curcumin exerts antioxidative effects in renal tissues and thereby can be considered as new therapeutic agents against oxidative stress-induced CKDs.

4.2 Anti-inflammatory Effects

Inflammatory response has an essential role in pathophysiology of CKDs [61–63]. Strong evidence has well confirmed that different types of proinflammatory mediators such as IL[8]-1β, IL-6, IL-18, MCP-1,[9] TNF-α,[10] E-selectin, adipocytokines, PPAR[11]-α, PPAR-γ, PPAR-δ, leptin, various adhesion molecules, and matrix metalloproteinase-2 are closely associated with onset and progression of CKDs and DN [64–66]. So lowering inflammation is a main target in the prevention and/or treatment of these diseases [63].

Curcumin is known for its potent anti-inflammatory effects in renal tissues [67, 68]. For instance, Ghosh et al. in 2012 demonstrated that curcumin is effective to improving renal function via attenuating the inflammatory responses in rats [67]. They reported that curcumin prevents CKD by developing in 5/6 nephrectomized rats [67]. Also, Soetikno et al. in 2013 found that curcumin improved renal efficiency via attenuating the inflammation as well as oxidative damages in 5/6 nephrectomized rats [69]. They found that curcumin downregulates inflammatory mediators as TNF-α and Nf-κb[12] in kidney tissues of rats [69]. Moreover, Buyuklu and colleagues in 2014 expressed that curcumin therapy in rats protected kidneys against inflammation and so improved renal function [70]. Awad et al. in 2011 reported the same results implying curcumin alleviates inflammatory levels as TNF-α, IL-1β, IL-12, IL-18, and INF-γ[13] and reduces renal damages during ischemia reperfusion (I/R) in rats [68]. These experimental findings confirm that cur-

[8] Interleukin.

[9] Monocyte chemoattractant protein 1.

[10] Tumor necrosis factor-alpha.

[11] Peroxisome proliferator-activated receptor.

[12] Nuclear factor kappa b.

[13] Interferon gamma.

cumin has anti-inflammatory properties in renal tissues which may enable it to use as preventive or therapeutic agent in CKDs.

4.3 Antiapoptotic Effects

Apoptosis is a cellular process defined as programmed cell death which occurs in many biologic events as growth, migration, differentiation, proliferation, transition, and fetal development [71]. This event is a physiologic process that is regulated by a wide variety of stimuli such as survival factor deprivation, death receptor activation, mitochondrial injury, endoplasmic reticulum stress, lysosomal destabilization, and caspase cascade activation [71, 72]. But in uncontrolled or pathologic states, it will be a major cause of tissue damages and promotes undesirable cellular death leading to tissue failure [71, 73]. Apoptotic cell death has an essential role in renal injuries during the progression of CKDs [74–76]. It is naturally active in glomerular and tubular epithelium but hyperactivated in response to pathologic stimuli such as hyperglycemia and hypertension and develop CKDs dependent renal injuries [72].

Curcumin has modulatory effects on apoptotic events and cellular death [77]. Buyuklu et al. in 2014 found that curcumin reduces nephropathy-dependent renal injuries in the kidney of rats by suppressing apoptosis process [70]. Also, Wei et al. in 2017 demonstrated that curcumin inhibits apoptotic cell death in kidneys via PIP3−/Akt-dependent signaling pathway [78]. Similarly, Wu and colleagues in 2017 demonstrated that curcumin attenuated apoptotic cellular death via PIP3/Akt signaling pathway in renal tissues of rats with glycerol-induced nephropathy [79]. Moreover, Awad and coworkers in 2011 examined the antiapoptotic effects of curcumin in I/R--induced renal injury and found that it is able to suppress apoptosis process by a caspase-3 inhibitory-dependent mechanism [68]. Furthermore, Alkuraishy and coworkers in 2019 demonstrated that curcumin protects against apoptotic death in renal tubules by lowering the KIM-1[14] expression in nephrotoxic rats [80]. Fan and colleagues in 2017 provided further evidence demonstrating curcumin suppresses apoptotic events thru Akt- and APPL1[15]-dependent molecular mechanisms in rats with experimental acute kidney injuries [36]. These evidences highly suggest that curcumin has antiapoptotic properties and thereby protects renal tissues against damages in CKDs.

4.4 Modulatory Effects on RAAS

Renin-angiotensin-aldosterone system (RAAS) is a hormonal system responsible for body fluids and electrolyte homeostasis and vascular resistance [81]. It is triggered by releasing the prorenin from the renal juxtaglomerular cells and converting the angiotensinogen (produced in liver) into angiotensin I (Ang I), which subsequently converted to angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE) mainly on the surface of vascular endothelial cells of the lungs and renal proximal tubules [82]. Ang II is the final effectors of this system with potent vasoconstrictive effects and acts via binding with two types of receptors as type 1 (AT1) and type 2 (AT2) [83]. Also, it induces the release of aldosterone hormone from the adrenal gland (zona glomerulosa), and then, it controls the salt homeostasis [83]. While the ACE enzyme promotes RAAS activities, its other isoform of ACE2 counteracts the RAAS activity by catabolizing the Ang II and converting it to Ang 1–7, an isoform of angiotensin with opposed effects to those of Ang II [84].

The kidneys have their specific renin-angiotensin-aldosterone system as "intrarenal RAAS" with all required elements which not only regulate renal glomerular hemodynamics and tubular salt transport but are also able to activate a number of pathologic processes involved in diabetic and non-diabetic nephropathies [85]. Ang II and aldosterone promote inflammation,

[14] Kidney injury molecule 1.

[15] Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1.

oxidative stress, apoptosis, and fibrosis during the onset and progress of CKDs [85, 86]. Also, Ang II as the final effector of RAAS can induce renal hemodynamic changes, accumulation of extracellular matrix, productions of the cytokines, podocyte injury, proteinuria, and interstitial nephritis [87]. Moreover, patients with CKDs typically have higher activities of mineralocorticoid receptors that are probably driven by increased levels of circulating aldosterone due to higher RAAS activity [88]. Thus, modulating the RAAS activities is a main target in the prevention and treatment of CKDs [89–91].

Curcumin has been shown to have modulatory effects on RAAS activity [56, 57, 92, 93]. Fazal et al. in 2015 demonstrated that curcumin markedly reduces RAAS activity by suppressing ACE gene expression in rats [56]. Also, Abd and coworkers in 2015 found that curcumin has similar effects to captopril (an ACE inhibitor) and thereby is able to reduce RAAS activity in diabetic rats [92]. They suggested that this RAAS modulatory effect on curcumin is valuable against diabetic nephropathy [92]. Furthermore, Xu et al. in 2018 reported that curcumin derivative of B6 (isolated from turmeric) significantly reduced RAS activity and declined RAS-induced kidney injuries in diabetic rats [94]. They found that curcumin promotes Ang II conversion to Ang 1–7 [94]. These findings suggested that curcumin as a potent pharmaceutical may be able to prevent CKDs by modulating RAAS activity [56, 57, 92, 93].

4.5 Antifibrotic Effects

Fibrotic process has pivotal roles in some histopathological changes of the kidney during CKD development [95]. This is a pathological state in which normal parenchymal tissues replaced with fibroblasts and connective tissues and thereby converted to nonphysiologic and inactive tissue [95]. Higher expression of fibrotic molecules such as TGF-β, adhesion molecules, matrix metalloproteinase (MMPs), and MCP-1 is a main criteria in most cases of renal failure due to CKD [95–97]. So amelioration of fibrotic process has always been a main target to prevent tissue remodeling and associated disorders as well as CKDs [97, 98].

The antifibrotic properties of curcumin has been studied by several research groups [99, 100]. Zhang et al. in 2011 found that curcumin has potent antifibrotic effects and inhibits fibrosis thru upregulation of Cat[16] B and Cat L and downregulation of TGF-β in lung tissues [99]. They reported that curcumin declined migration and proliferation of fibroblasts in human and mouse cultured lung cells [99]. Moreover, Saidi et al. in 2019 found that curcumin exerts potent antifibrotic effects by inducing Cat B and Cat L expression and lowering TGF-β expression by a PPAR-γ-dependent mechanism in lung tissues [101]. Also, Smith and coworkers in 2010 demonstrated that curcumin attenuated collagen deposits and fibrotic processes in lungs of mice following bleomycin-induced lung injury [102]. They showed that curcumin markedly suppresses TGF-β expression and fibroblast migration in these tissues [102]. Similarly, Xu and colleagues in 2017 established that curcumin attenuated fibroblast migration and intestinal fibrosis by suppressing epithelial-to-mesenchymal transition and PPAR-γ-dependent TGF-β1/Smad pathway in rats [103]. Rodriguez and colleagues in 2019 provided further evidences suggesting curcumin attenuates fibrotic events in lungs via its antioxidative properties [104]. These evidences confirm that curcumin has potent antifibrotic properties. Curcumin is also reported to lower the pro-fibrotic cytokine release such as MCP-1 and MMPs from renal tubular and mesenchymal cells and thereby inhibit renal fibrosis in primary stages [105–107]. These effects are partly mediated to its anti-inflammatory effects [107] or via inducing HO-1[17] expression/activity [108, 109] (Table 1).

4.6 Other Possible Pathways

In addition to aforementioned molecular mechanisms, curcumin may be able to provide renopro-

[16] Cathepsine.

[17] Heme oxygenase 1.

Table 1 Molecular mechanisms mediating renoprotective effects of curcumin

Molecular mechanisms	Effects	References
Antioxidative effects	Reduces oxidative injuries in renal tissues via several pathways as free radicals scavenging and potentiating antioxidative defense system	[47, 54, 55]
Anti-inflammatory effects	Lowering the proinflammatory mediators as TNF-α, IL-1β, IL-12, IL-18, and INF-γ and reducing the rate of inflammatory responses in kidneys	[67–70]
Antiapoptotic effects	Preventing proapoptotic agent activity such as caspases and p53 and lowering the apoptosis in renal tissues	[68, 70, 77–80]
RAAS modulation	Making modulatory effects on RAAS and reducing the RAAS-dependent renal injuries	[56, 57, 92, 93]
Antifibrotic effects	Lowering the rate of fibrotic processes due to more Cat B and Cat L and lowering TGF-β, MCP-1, and MMP expressions	[101–107]

TNF-α tumor necrosis factor-alpha, *IL* interleukin, *INF-γ* interferon gamma, *RAAS* renin-angiotensin-aldosterone system, *Cat* cathepsine, *TGF-β* transforming growth factor beta, *MCP-1* monocyte chemoattractant protein 1, *MMP* matrix metalloproteinase

tective effects via other pathways, such as autophagy and hypoglycemic effects, and by alleviating dyslipidemia [110–113]. These pathways have important roles in the pathophysiology of CKDs and onset or progress of other pathologic mechanisms [110–113]. Hyperglycemia is a main upstream event triggering other pathophysiologic pathways involved in CKDs such as oxidative stress and inflammation [114]. So hypoglycemic effects of curcumin indirectly suppress downstream pathways induced by hyperglycemia [51, 115]. Similarly, evidences have indicated that curcumin positively modulates lipid metabolism and corrects lipid profile in experimental or clinical designs [116, 117]. Since dyslipidemia is a potent inducer for lipid accumulation and vascular complications of CKD and is able to induce or exaggerate oxidative damages, beneficial effects of curcumin on lipid metabolism may be other possible pathways providing renoprotective effects [113, 117]. Kim et al. in 2016 provided evidence showing curcumin plays renoprotective roles at least partly via improvement in lipid metabolism in animal model of diabetic nephropathy [113].

Autophagy is another molecular process which is involved in CKD development [118]. These mechanisms which refer to removing unnecessary or dysfunctional components of the cells are highly regulated by various factors [118]. But in pathophysiologic milieu such as hypertension, oxidative stress, or hyperglycemia, it will be uncontrolled and contributes in histopathological injuries in the tissues [118]. Some evidences suggested that curcumin regulates this process [70, 119, 120]. They reported that curcumin positively modulates autophagy process and reduces related histological damages [119, 120]. So, one can conclude that modulating the autophagy may be another route by which curcumin provides renoprotective effects [120]. However, more clinical studies are still required.

5 Clinical Evidences

Clinical evidences support the role of curcumin in renoprotection [121]. Moreillon et al. in 2013 conducted a clinical trial on 16 patients with CKD and found that curcumin is able to reduce the inflammatory cytokines and improve renal functions. This study showed a significant reduction of IL-6 in the treatment group and increase in the placebo group [121], which partially support anti-inflammatory effects of curcumin hypothesis. As impaired renal function partly results from adverse effects of persistent proteinuria, it is important to elucidate the effect of natural product such as dietary turmeric in ameliorating diabetic nephropathy and the renal lesions associated with it. Despite hyperglycemia and poor control of diabetes which make patients vulnerable to progression of renal lesions, a significant decrease in urinary protein excretion was

Table 2 Clinical evidences about renoprotective effects of curcumin

Treatment	Population of study	Dose/length of study	Effects	References
Curcumin	16 patients with CKDs	824 mg/twice daily/8 weeks	Attenuates inflammatory markers as TNFα, IL-6, and CRP	[121]
Turmeric	20 patients with DN and 20 healthy volunteers	500 mg/2 months	Lowering the TGF-β and IL-8 levels, reducing the albuminuria	[122]
Turmeric	24 patients with lupus nephritis	500 mg/day/3 months	Markedly reduces proteinuria and systolic blood pressure	[123]
Curcumin	414 participants	90 mg/day/6 months	In progress	[124]
Curcumin	18 patients with CKDs	8 weeks	Reduced inflammatory responses via lowering the PGE2 activity	[125]
Curcumin	101 patients with CKDs	320 mg/day/8 weeks	Improved oxidative stress in kidneys, has no significant effects on eGFR	[126]

eGFR estimated glomerular filtration rate, *PGE2* prostaglandin E2

found in Khajehdehi and colleagues' trial group comparing pre- and post-supplementation values, but there was no significant change in serum creatinine levels. They also found that turmeric reduces the levels of TGF-β and IL-8 [122]. Khajehdehi and coworkers in 2012 conducted another clinical study demonstrating curcumin reduces albuminuria and systolic blood pressure in patients with nephritis [123]. Considering that there is strong relationship between hypertension and CKD progression, turmeric supplementation might be beneficial to delay or even prevent CKD progression of CKD. However, long-term trials are needed to clarify the issue. Also, as inflammation is increased in early-stage CKD, Shelmadine et al. showed that curcumin supplementation reduces PGE2 in early-stage CKD. More clinical evidences are presented in Table 2.

6 Conclusion

Using herbal-based pharmacological agents has received a lot of attention in recent years. Curcumin is an inexpensive available pharmaceutical agent which has shown obvious pharmacological effects in many experimental and clinical studies. Curcumin is able to prevent or suppress many pathophysiologic pathways involved in various complications associated with kidney diseases. Curcumin can provide renoprotective effects via at least five molecular mechanisms. Clinical studies also provide evidence for the use of curcumin in management of kidney diseases. Further therapeutic studies with curcumin will be helpful in developing a strategy for the treatment of chronic kidney ailments using herbal medicines.

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