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Review Article

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The Effect of Saffron and its Active Compounds on Oxidative Stress Markers in Diabetic Rats: A Systematic Review and Meta-analysis of Animal Studies

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Abstract

Background: Diabetes, as a chronic metabolic disease, can induce oxidative stress, leading to severe damage to various tissues, including the kidneys, heart, and others. This study aimed to assess the influence of saffron and its active component on oxidative stress markers in diabetic rats.

Methods: The databases were searched until December 24, 2021. The quality of the included articles was assessed using SYRCLE's Risk of Bias tool. To estimate the effects of saffron and its active component, SMD with 95% confidence intervals (CIs) were pooled using a random-effects model. Subgroup analysis and meta-regression were used to explore heterogeneity. Publication bias was assessed using the Begg and Egger tests. The results were reported under the PRISMA guidelines.

Results: The meta-analysis comprising 42 articles revealed that prolonged hyperglycemia leads to increased oxidative markers, including malondialdehyde (MDA), nitric oxide (NO), total oxidant status (TOS), xanthine oxidase (XO), and reactive oxygen species (ROS)), and decreased antioxidant defense system, including glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), thiol groups (SH), and total antioxidant capacity (TAC). Treatment of diabetic rats with saffron, crocin, and safranal decreased the oxidant markers and increased the antioxidant markers.

Conclusion: Saffron, crocin, and safranal reduce oxidative stress by reinforcing the antioxidant defense system and reducing oxidant markers. Hence, we believe that saffron and its active ingredients can be favorable options for managing diabetes and its complications. However, further human studies are required to draw definite conclusions.

Keywords: Diabetes, Oxidative stress, Saffron, Crocin, Safranal, Meta-analysis, Diabetic rats

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Introduction

Diabetes mellitus (DM) is a globally recognized non-communicable chronic disease gaining epidemic proportions. The deficiency of insulin or the body's poor performance in effectively utilizing insulin can be due to auto-immune destruction of pancreatic cells, genetics, urbanization, and a sedentary lifestyle. The disease is affecting individuals throughout the age spectrum, and those who have it are estimated to reach 552 million by 2030 (1).

The loss of pancreatic insulin-producing cells (type 1 diabetes) or a reduction in insulin sensitivity in its target receptors in muscles and fatty tissues (type 2 diabetes) (2) elucidates its pathophysiology. Chronic and uncontrolled hyperglycemia activates and initiates signals that cause severe structural damage, resulting in functional defects of various tissues such as the kidney, liver, pancreas, brain, and eyes. In due course, it can cause nephropathy, retinopathy, neuropathy, and micro/macrovascular tissue damage (3). The normal metabolism of substrates diverges to atypical pathways in the diabetic state, releasing toxic byproducts, such as reactive oxygen species (ROS).

The ROS released in uncontrolled hyperglycemia is triggered by protein kinase C activation, glucose autoxidation, oxidative phosphorylation, methylglyoxal

generation, and sorbitol formation (4). Free radicals can overwhelm the body's natural antioxidant defense mechanism and cause oxidative stress (5). Oxidative stress is associated with protein inactivation, protein glycation, and impaired glutathione metabolism, and damages various cellular components (6,7). Superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT) are some of the free radical scavenging mechanisms that help remove superoxide, hydrogen peroxide, and hydroxyl radicals (8). Apoptosis and tissue dysfunction are eventually induced by oxidative stress produced by an imbalance between the antioxidant defense system and free radicals (3,5). As a result, rebalancing the oxidant-antioxidant defense system is a significant concern in diabetes. Diabetes is treated with various medicines, although synthetic treatments have always caused adverse reactions and other side effects (9). In this sense, using effective natural remedies instead of chemical and pharmaceutical treatments is preferable.

Saffron (*Crocus sativus* L.) is a small but prominent species from the Iridaceae family, known in Iran for its coloring, seasoning, and aromatizing properties. It is a food additive used in traditional medicine to cure various illnesses, including depression, cognitive problems, seizures, and cancer. In addition, saffron has anticancer, antioxidant, and anti-inflammatory properties and can improve memory function and learning ability. The pharmacological activities of saffron are attributed to many of its active constituents, including crocin, picrocrocin, and safranal (10). Evidence suggests that saffron might be used to treat diabetes. Although the hypoglycemic effects of saffron have been explored, the antioxidant benefits of saffron have not been well investigated. Therefore, this study aimed to see how saffron and its active components affect oxidative stress markers in a diabetic rat model.

Methods

The current systematic review and meta-analysis used the *Cochrane Handbook for Systematic Reviews of Intervention criteria* to investigate the effect of saffron and its active components on oxidative stress markers in diabetes. The results were reported under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines (11).

PECOS

- Population: Rats with induced diabetes.
- Comparison: Untreated diabetic rats (control group).
- Outcome: Oxidants (including malondialdehyde [MDA], nitric oxide [NO], total oxidant status [TOS], xanthine oxidase [XO], reactive oxygen species [ROS]) and antioxidants (including GSH, CAT, SOD, GPx, total antioxidant status [TAS], thiol groups [SH], total antioxidant capacity [TAC]).
- Study designs: experimental animal studies

Data sources and search strategy

Two researchers (Y.M., H.B.) independently conducted a comprehensive electronic search using the five following databases: PubMed ($n=89$), Scopus ($n=164$), ProQuest ($n=57$), Web of Science ($n=24$), and Cochrane ($n=80$) until December 24, 2021, with no language restrictions. Database sources were searched using relevant keywords from MeSH and non-MeSH terms. The Boolean search terms (AND and OR) were used for the search strategy. In addition, the reference lists of the selected articles were manually checked ($n=2$). An automated Google Scholar database search was also done to ensure that the most recent papers on the subject were considered (Table 1).

Eligibility criteria

Studies were deemed acceptable if they met the following criteria: (a) animal studies, (b) investigations on the effects of saffron, crocin, and safranal on oxidative stress markers, and (c) studies on DM. Exclusion criteria include (a) randomized controlled trials (RCTs), (b) studies on other diseases and other parameters, (c) conference abstracts, reviews, editorials, protocols, letters, case reports, and commentaries, (d) studies with incomplete data, and (e) interventions by combining saffron and its constituents with other compounds or supplements.

Study selection and data extraction

Endnote Reference Manager X9 was used to store all identified papers from each database, eliminating duplicates using the Endnote function "Find duplicates." Two reviewers (Y.M. and A.R.F.) evaluated the remaining publications individually with 97% agreement to verify their eligibility for inclusion after duplicate records were removed. The screening process was divided into two parts. Initially, titles and abstracts were examined, and unrelated research was eliminated. The remaining papers were reviewed for eligibility in the second step using the full-text version. Throughout the research selection process, any disagreements were settled by face-to-face conversation or consultation with a third reviewer (S.M.R.). Finally, all eligible studies were included in this study. Microsoft Excel software was used to create a special form for data mining. The information in the document included the following: first author, year of publication, country of study, quality of study, animal breed, sample size, duration of intervention, dose of intervention (mg/kg/d), mean and standard deviation (SD) of findings before and after the intervention.

Quality assessment

Two independent authors (Y.M., A.R.F.) assessed the articles using SYRCLE's Risk of Bias (RoB) tool, a tool specially designed for animal studies (12). This tool can be used to detect selection, performance, detection, erosion, and report bias in the included studies. The

data in the articles were labeled as high category risk of bias (H), unclear risk of bias (U), or low risk of bias (L). A "No" decision indicates a high risk of bias. When the text description was insufficient to determine the danger of bias, the term "Unclear" was used; a "Yes" decision indicates a low risk of bias. Studies with quality scores below 60% were considered high-risk in terms of bias and were excluded. Two reviewers (Y.M. and A.R.F.) independently performed quality assessment, with 97% agreement. Differences in scoring were resolved through discussion between the authors and consultation with a third author (S.M.R.) if necessary.

Statistical analysis

Stata software Version 14.0 was used to analyze the data. We assessed the metabolic effects of saffron and its active components using standardized mean difference (SMD) with a 95% confidence interval (CI). A random-effects model was used to estimate SMD (13). Q and I^2 statistics were used to determine heterogeneity. I^2 values above 0.6 were considered heterogeneous. Subgroup analysis and meta-regression approaches were used to address heterogeneity (14,15). The Egger regression test, a parametric method, was used to determine publication bias. Sensitivity analysis was also conducted to detect the influence of a single study on the overall estimate by eliminating one study and repeating the analysis. A P value of 0.1 was considered significant in the analyses, with less than ten studies.

Results

Search results

Figure 1 depicts the steps of the article selection process. In total, 414 studies were found in various databases,

Table 1. Search strategy

Databases	Search Strategy	Result
Scopus	(TITLE-ABS-KEY (saffron*) OR TITLE-ABS-KEY (safron*) OR TITLE-ABS-KEY (crocus*) OR TITLE-ABS-KEY (safrana*) OR TITLE-ABS-KEY (crocetin*) OR TITLE-ABS-KEY (picrocrocin*) OR TITLE-ABS-KEY (crocin*)) AND (TITLE-ABS-KEY (diabet*) OR TITLE-ABS-KEY ("Diabetic Nephropathies")) AND (TITLE-ABS-KEY (antioxidan*) OR TITLE-ABS-KEY (oxidativ*) OR TITLE-ABS-KEY ("oxidative stress*") OR TITLE-ABS-KEY ("Oxidative marker*") OR TITLE-ABS-KEY ("Enzymatic oxidativ*") OR TITLE-ABS-KEY ("anti-oxidative marker*") OR TITLE-ABS-KEY (oxidant*) OR TITLE-ABS-KEY ("Free radical*") OR TITLE-ABS-KEY (freeradical*) OR TITLE-ABS-KEY (malondialdehyd*) OR TITLE-ABS-KEY ("Superoxide dismutas*") OR TITLE-ABS-KEY (glutathion*) OR TITLE-ABS-KEY ("Non-enzymatic oxidative*") OR TITLE-ABS-KEY ("anti-oxidative marker*") OR TITLE-ABS-KEY (catalas*) OR TITLE-ABS-KEY ("Selenoglutathione peroxidase*"))	164
PubMed	((Antioxidan*[Text Word] OR "oxidativ*"[Text Word] OR "oxidative stress*"[Text Word] OR "Oxidative marker*"[Text Word] OR "Enzymatic oxidativ*"[Text Word] OR "anti-oxidative marker*"[Text Word] OR Oxidant*[Text Word] OR "Free radical*"[Text Word] OR Freeradical*[Text Word] OR Malondialdehyd*[Text Word] OR "Superoxide dismutas* "[Text Word] OR Glutathion*[Text Word] OR "Non-enzymatic oxidative*"[Text Word] OR "anti-oxidative marker*"[Text Word] OR "Catalas*"[Text Word] OR "Selenoglutathione peroxidase*"[Text Word] OR "oxidative stress"[MeSH Terms] OR "Antioxidants"[Mesh] OR "Malondialdehyde"[Mesh] OR "Superoxide Dismutase"[Mesh] OR "Glutathione"[Mesh] OR "Catalase"[MeSH]) AND (Saffron*[Text Word] OR safron*[Text Word] OR "Crocus*"[Text Word] OR Safrana*[Text Word] OR crocetin*[Text Word] OR picrocrocin*[Text Word] OR crocin*[Text Word] OR "Crocus"[Mesh] OR "safranal" [Supplementary Concept] OR "picrocrocin" [Supplementary Concept] OR "crocin" [Supplementary Concept])) AND (Diabet*[Text Word] OR "Diabetic Nephropathies"[Mesh] OR "Diabetes Mellitus"[Mesh] OR "Diabetes Mellitus, Type 2"[Mesh])	89
Proquest	((ti(Saffron*) OR ab(Saffron*) OR su(Saffron*) OR ti(safron*) OR ab(safron*) OR su(safron*) OR ti(Crocus*) OR ab(Crocus*) OR su(Crocus*) OR ti(Safrana*) OR ab(Safrana*) OR su(Safrana*) OR ti(crocetin*) OR ab(crocetin*) OR su(crocetin*) OR ti(picrocrocin*) OR ab(picrocrocin*) OR su(picrocrocin*) OR ti(crocin*) OR ab(crocin*) OR su(crocin*)) AND PEER(yes)) AND ((ti(Diabet*) OR ab(Diabet*) OR su(Diabet*) OR ti("Diabetic Nephropathies") OR ab("Diabetic Nephropathies") OR su("Diabetic Nephropathies")) AND PEER(yes)) AND ((ti(Antioxidan*) OR ab(Antioxidan*) OR su(Antioxidan*) OR ti(oxidativ*) OR ab(oxidativ*) OR su(oxidativ*) OR ti(("oxidative stress")) OR ab(("oxidative stress")) OR su(("oxidative stress")) OR ti("Oxidative marker*") OR ab("Oxidative marker*") OR su("Oxidative marker*") OR ti("Enzymatic oxidativ*") OR ab("Enzymatic oxidativ*") OR su("Enzymatic oxidativ*") OR ti("anti-oxidative marker*") OR ab("anti-oxidative marker*") OR su("anti-oxidative marker*") OR ti(Oxidant*) OR ab(Oxidant*) OR su(Oxidant*) OR ti(("free radical" OR "free radicals")) OR ab(("free radical" OR "free radicals")) OR su(("free radical" OR "free radicals")) OR ti(Freeradical*) OR ab(Freeradical*) OR su(Freeradical*) OR ti(Malondialdehyd*) OR ab(Malondialdehyd*) OR su(Malondialdehyd*) OR ti(("superoxide dismutase")) OR ab(("superoxide dismutase")) OR su(("superoxide dismutase")) OR ti(Glutathion*) OR ab(Glutathion*) OR su(Glutathion*) OR ti("Non-enzymatic oxidative*") OR ab("Non-enzymatic oxidative*") OR su("Non-enzymatic oxidative*") OR ti("anti-oxidative marker*") OR ab("anti-oxidative marker*") OR su("anti-oxidative marker*") OR ti(Catalas*) OR ab(Catalas*) OR su(Catalas*) OR ti("Selenoglutathione peroxidase*") OR ab("Selenoglutathione peroxidase*") OR su("Selenoglutathione peroxidase*")) AND PEER(yes))	57
Web of Science	TI=(Saffron*) OR AB=(Saffron*) OR AK=(Saffron*) OR TI=(safron*) OR AB=(safron*) OR AK=(safron*) OR TI=(Crocus*) OR AB=(Crocus*) OR AK=(Crocus*) OR TI=(Safrana*) OR AB=(Safrana*) OR AK=(Safrana*) OR TI=(crocetin*) OR AB=(crocetin*) OR AK=(crocetin*) OR TI=(picrocrocin*) OR AB=(picrocrocin*) OR AK=(picrocrocin*) OR TI=(crocin*) OR AB=(crocin*) OR AK=(crocin*) AND TI=(Diabet*) OR AB=(Diabet*) OR AK=(Diabet*) OR TI=("Diabetic Nephropathies") OR AB=("Diabetic Nephropathies") OR AK=("Diabetic Nephropathies") AND TI=(Antioxidan*) OR AB=(Antioxidan*) OR AK=(Antioxidan*) OR TI=(oxidativ*) OR AB=(oxidativ*) OR AK=(oxidativ*) OR TI=("oxidative stress*") OR AB=("oxidative stress*") OR AK=("oxidative stress*") OR TI=("Oxidative marker*") OR AB=("Oxidative marker*") OR AK=("Oxidative marker*") OR TI=("Enzymatic oxidativ*") OR AB=("Enzymatic oxidativ*") OR AK=("Enzymatic oxidativ*") OR TI=("anti-oxidative marker*") OR AB=("anti-oxidative marker*") OR AK=("anti-oxidative marker*") OR TI=(Oxidant*) OR AB=(Oxidant*) OR AK=(Oxidant*) OR TI=("Free radical*") OR AB=("Free radical*") OR AK=("Free radical*") OR TI=(Freeradical*) OR AB=(Freeradical*) OR AK=(Freeradical*) OR TI=(Malondialdehyd*) OR AB=(Malondialdehyd*) OR AK=(Malondialdehyd*) OR TI=("Superoxide dismutas*") OR AB=("Superoxide dismutas*") OR AK=("Superoxide dismutas*") OR TI=(Glutathion*) OR AB=(Glutathion*) OR AK=(Glutathion*) OR TI=("Non-enzymatic oxidative*") OR AB=("Non-enzymatic oxidative*") OR AK=("Non-enzymatic oxidative*") OR TI=("anti-oxidative marker*") OR AB=("anti-oxidative marker*") OR AK=("anti-oxidative marker*") OR TI=(Catalas*) OR AB=(Catalas*) OR AK=(Catalas*) OR TI=("Selenoglutathione peroxidase*") OR AB=("Selenoglutathione peroxidase*") OR AK=("Selenoglutathione peroxidase*")	80

Table 1. Continued.

Databases	Search Strategy		Result
Cochrane	#1	(Saffron*):ti,ab,kw = 276	24
	#2	(safron*):ti,ab,kw = 4	
	#3	(Crocus*):ti,ab,kw = 145	
	#4	(Safrana*):ti,ab,kw = 4	
	#5	(crocetin*):ti,ab,kw = 28	
	#6	(picrocrocin*):ti,ab,kw = 0	
	#7	(crocin*):ti,ab,kw = 75	
	#8	MeSH descriptor: [Crocus] explode all trees = 55	
	#9	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 = 349	
	#10	(Diabet*):ti,ab,kw = 90985	
	#11	MeSH descriptor: [Diabetic Nephropathies] explode all trees = 1412	
	#12	MeSH descriptor: [Diabetes Mellitus] explode all trees = 30783	
	#13	MeSH descriptor: [Diabetes Mellitus, Type 2] explode all trees = 17348	
	#14	#10 OR #11 OR #12 OR #13 = 91206	
	#15	(Antioxidan*):ti,ab,kw = 12679	
	#16	(oxidativ*):ti,ab,kw = 12436	
	#17	(oxidative stress*):ti,ab,kw = 10239	
	#18	(Oxidative marker*):ti,ab,kw = 3891	
	#19	(Enzymatic oxidativ*):ti,ab,kw = 214	
	#20	(anti-oxidative marker*):ti,ab,kw = 69	
	#21	(Oxidant*):ti,ab,kw = 2091	
	#22	(Free radical*):ti,ab,kw = 4533	
	#23	(Freeradical*):ti,ab,kw = 145	
	#24	(Malondialdehyd*):ti,ab,kw = 2998	
	#25	(Superoxide dismutas*):ti,ab,kw = 2198	
	#26	(Glutathion*):ti,ab,kw = 3559	
	#27	(Non-enzymatic oxidative*):ti,ab,kw = 52	
	#28	(anti-oxidative marker*):ti,ab,kw = 69	
	#29	(Catalas*):ti,ab,kw = 926	
	#30	(Selenoglutathione peroxidase*):ti,ab,kw = 1	
	#31	MeSH descriptor: [Oxidative Stress] explode all trees = 2978	
	#32	MeSH descriptor: [Antioxidants] explode all trees = 4812	
	#33	MeSH descriptor: [Malondialdehyde] explode all trees = 1252	
	#34	MeSH descriptor: [Superoxide Dismutase] explode all trees = 774	
	#35	MeSH descriptor: [Glutathione] explode all trees = 671	
	#36	MeSH descriptor: [Catalase] explode all trees = 260	
	#37	#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 = 25778	
	#38	#9 AND #14 AND #37 = 24	

and two were found in other sources. After removing duplicates ($n = 228$), the titles and abstracts of 188 articles were assessed, 78 of which were excluded from the study. A total of 110 full-text articles were reviewed, and 68 studies were omitted, leaving 42 papers to be included and evaluated for quality in the present study.

Study characteristics

The specifications of 42 eligible articles are shown in Table 2. These studies were done in Iran ($n = 26$), China ($n = 2$), Egypt ($n = 3$), Greece ($n = 2$), Turkey ($n = 8$), and Poland ($n = 1$) and were published between 2009 and 2021. They included 41 studies on rats and one study on mice. In the trials, 37 publications used streptozotocin (STZ) (dose range 30 to 75 mg/kg), two papers used a high-fat diet, and three articles used a combination of STZ and a high-fat diet to induce diabetes. The duration of the intervention using saffron, crocin, and safranal was from 14 to 70 days. The chemical used was crocin in 28 articles (dose range 2.5 to 150 mg/kg), saffron in 10 articles (dose range 10 to 200 mg/kg), and safranal in 6 articles (dose range 0.25 to 5 mg/kg).

Quality assessment

Table 2 shows the results, and Figure S1 (Supplementary file 1) shows the details of the quality assessment of the included articles. The quality evaluation results showed that 15 studies scored 88.89, 21 articles scored 77.78, and 6 papers scored 66.67.

Meta-analysis results

In these studies, diabetic groups treated with saffron, crocin, and safranal were compared to diabetic control groups. The meta-analysis results are presented in Table 3 (subgroup analysis in different specimens) and Table 4 (subgroup analysis by type of intervention).

The subgroup analysis for the effect of saffron and its active compounds on antioxidant markers in different specimens

The results of our meta-analysis showed that in the treated diabetic group compared with the diabetic control group, antioxidant markers, including GSH, CAT, SOD, GPx, TAS, TAC, and SH, increased with high effectiveness in the different specimens. However, the heterogeneity of the studies was high. Most studies had been performed on

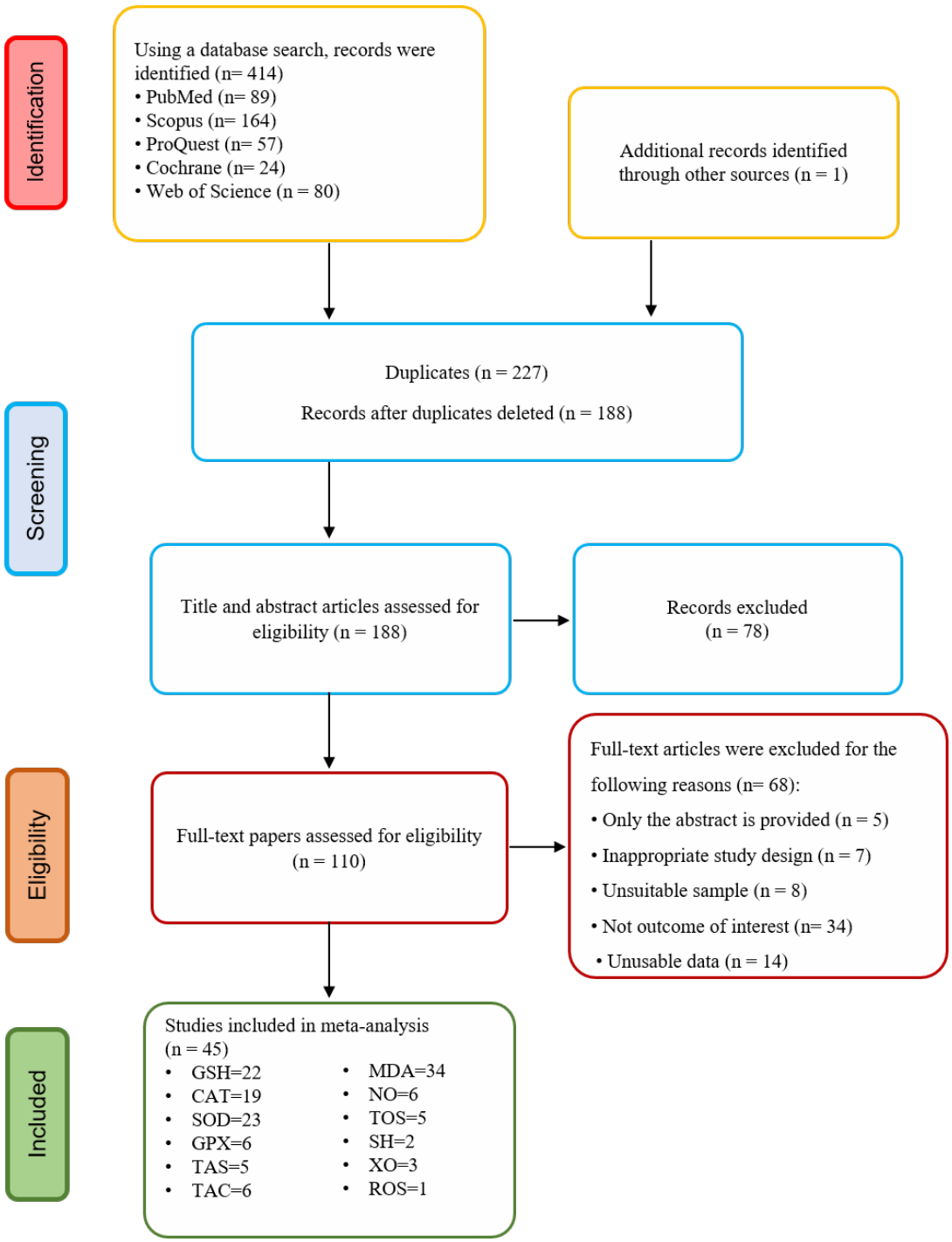

Figure 1. Flowchart of the study selection process for the systematic review and meta-analysis

GSH ($n=22$), and the results showed that the effectiveness of saffron and its active compounds on GSH was more in the lung tissue and less in the brain tissue (for lung: SMD, 5.31 (µmol/L) [95% CI, 3.03 to 7.59]; $P=0.001$, $I^2=67.5\%$; for the brain: SMD, 0.79 (µmol/L) [95% CI, 0.32 to 1.25]; $P=0.001$, $I^2=0.9\%$).

The subgroup analysis for the effect of saffron and its active compounds on oxidant markers in different specimens

The results of this study highlighted the effectiveness of saffron in decreasing the oxidant markers, including MDA, NO, TOS, XO, and ROS, in different specimens in the diabetic group compared to the control group.

MDA, an indicator of lipid peroxidation, is often used as a marker, and its level has been explored in several studies. The results showed that the highest effect of saffron and its active compounds on MDA was in the kidney tissue and its lowest effect was seen in the gastric mucosa (for kidney: SMD, -3.69 (µmol/L) [95% CI, -5.17 to -2.75]; $P=0.001$, $I^2=78.6\%$; for gastric mucosa: SMD, -1.41

Table 2. Characteristics of articles included in the meta-analysis

Reference	Year	Country	Animal	Sample size in each group	Type of Diabetes induction/dosage (mg/kg)	Treatment period (day)	Type of intervention/ dosage (mg/kg)	Measurement*	Quality score
Yaribeygi et al (16)	2019	Iran	Rats	6	STZ/45	56	Crocin/40	MDA, GSH, SOD, CAT	88.89
Yaribeygi et al (17)	2019	Iran	Rats	6	STZ/40	56	Crocin/40	MDA, GSH, SOD, CAT	88.89
Yaribeygi et al (18)	2017	Iran	Rats	6	STZ/40	56	Crocin/40	MDA	88.89
Wu et al (19)	2018	China	Rats	8	STZ/60	14	Crocin/60	MDA, SOD, NO	88.89
Talebanzadeh et al (20)	2018	Iran	Rats	8	STZ/60	35	Saffron/200	MDA, GPx, SOD, CAT	77.78
Sefidgar et al (21)	2019	Iran	Rats	6	STZ/60	28	Crocin/60	TAS, TOS	88.89
Samarghandian et al (22)	2013	Iran	Rats	8	STZ/60	28	Safranal/0.25, 0.5, 0.75	MDA, GSH, SOD, CAT, NO	77.78
Samarghandian et al (23)	2014	Iran	Rats	10	STZ/60	28	Saffron/20, 40, 80	GSH, SOD, CAT	88.89
Samarghandian et al (24)	2016	Iran	Rats	9	STZ/60	28	Saffron/10, 20, 40	MDA, GSH, SOD, CAT, NO	88.89
Samarghandian et al (25)	2016	Iran	Rats	9	STZ/60	28	Crocin/10, 20, 30	MDA, GSH, SOD, CAT, NO	77.78
Samarghandian et al (26)	2013	Iran	Rats	8	STZ/60	28	Safranal/0.25, 0.5, 0.75	MDA, GSH, SOD, CAT, NO	88.89
Samaha et al (27)	2019	Egypt	Rats	10	STZ/50	28	Crocin/10	MDA, GSH, SOD, TAC, CAT	66.67
Rajaei et al (28)	2012	Iran	Rats	7	STZ/55	42	Crocin/15, 30, 60	MDA, SH	77.78
Rahbani et al (29)	2012	Iran	Rats	10	STZ/75	56	Saffron/40	MDA, GSH, GPx, SOD, CAT	66.67
Qiuet al (30)	2020	China	Mice	12	High- fat diet	56	Crocin/50	MDA, GSH, SOD, CAT, ROS	77.78
Motamedrad et al (31)	2019	Iran	Rats	7	STZ/60	21	Saffron/25, 100	MDA, TAC	66.67
Rahbani et al (32)	2011	Iran	Rats	10	STZ/65	56	Saffron/40	MDA, GSH, SOD, CAT	88.89
Margaritis et al (33)	2020	Greece	Rats	6	STZ/65	28	Crocin/20, 50	GSH, SOD	88.89
Kianbakht et al (34)	2009	Iran	Rats	10	STZ/50	35	Saffron (25, 100, 250) /Crocin (2.5, 5, 10)/ Safranal (0.25, 2, 5)	MDA, GSH	77.78
Hazman et al (35)	2014	Turkey	Rats	8	STZ (30)/High -fat diet	28	Safranal	TAS, TOS	77.78
Hazman et al (36)	2015	Turkey	Rats	8	STZ (30)/High -fat diet	28	Safranal	GSH, TAS, TOS	77.78
Hazman et al (37)	2016	Turkey	Rats	8	STZ (30)/High-fat diet	42	Crocin/150	TAS, TOS	77.78
Hasanpour et al (38)	2018	Iran	Rats	8	STZ/65	35	Saffron/200	MDA, GPx, SOD, CAT	88.89
Yaribeygi et al (39)	2018	Iran	Rats	6	STZ/45	56	Crocin/40	MDA, GSH, SOD, CAT	77.78
Ghorbanzadeh et al (40)	2016	Iran	Rats	7	STZ/35	56	Crocin/50	MDA, GPx, SOD, CAT	88.89
Farshid et al (41)	2016	Iran	Rats	8	STZ/50	56	Crocin/5, 10, 20	MDA, SOD	77.78
Farshid et al (42)	2015	Iran	Rats	6	STZ/60	56	Safranal/1	MDA	66.67
El-Fawal et al (43)	2018	Egypt	Rats	8	High-fat diet	70	Crocin/50	MDA	77.78
Bajerska et al (44)	2013	Poland	Rats	6	STZ/40	35	Saffron	MDA, TAC	88.89
Bahmani et al (45)	2016	Iran	Rats	10	STZ/65	56	Crocin/100	GSH, SOD, TAC, CAT	66.67
Asri_Rezaei et al (46)	2014	Iran	Rats	8	STZ/50	42	Crocin/12.5, 25, 50	MDA, TAC	77.78
Ashrafi et al (47)	2016	Iran	Rats	7	STZ/60	35	Crocin/200	MDA, GPx, SOD, CAT	88.89
Altinoz et al (48)	2015	Turkey	Rats	10	STZ/50	21	Crocin/20	MDA, GSH	77.78
Altinoz et al (49)	2014	Turkey	Rats	10	STZ/50	21	Crocin/20	MDA, GSH, XO	77.78
Altinoz et al (50)	2015	Turkey	Rats	10	STZ/50	21	Crocin/20	MDA, GSH, XO	77.78
Ahmadi et al (51)	2017	Iran	Rats	7	STZ/55	42	Crocin/15	MDA, SH	66.67
AbouHany et al (52)	2018	Egypt	Rats	10	STZ/50	56	Crocin/20	MDA, GSH, SOD, NO	77.78
Tamaddonfard, E et al (53)	2012	Iran	Rats	6	STZ/60	30	Crocin/7.5, 15, 30	MDA, TAC	77.78
Altinoz et al (54)	2014	Turkey	Rats	10	STZ/50	21	Crocin/20	MDA, GSH, XO	77.78
Ayşegül et al (55)	2020	Turkey	Rats	7	STZ/65	21	Crocin/50	MDA, SOD, TAS, TOS	88.89
Kapucu et al (56)	2020	Greece	Rats	5	STZ/55	70	Saffron/60	GPx, CAT	77.78
Yaribeygi et al (57)	2021	Iran	Rats	6	STZ/45	56	Crocin/40	MDA, GSH, SOD, CAT	77.78

Abbreviations: glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), total antioxidant capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), total oxidant status (TOS), thiol groups (SH), xanthine oxidase (XO), reactive oxygen species (ROS).

Table 3. Effect of saffron and its effective compounds on oxidative stress markers (oxidants and antioxidants) according to the type of specimens

Variable	*N*	SMD (95% CI)	*P* value for SMD	*Q*	I^2	Tau
Antioxidant markers						
GSH						
Gastric mucosa	9	4.94 (4, 5.88)	<0.001	18.56	0.0	0.00
Liver	6	0.87 (0.44, 1.29)	<0.001	1.98	0.0	0.00
BALF	3	3.87 (2.71, 5.03)	<0.001	2.58	22.6	0.24
Lung	3	5.31 (3.03, 7.59)	<0.001	6.14	67.5	2.72
Serum	11	1.06 (0.75, 1.36)	<0.001	8.98	0.0	0.00
Testis	1	2.96 (1.24, 4.68)	<0.001	0.00	0.0	0.00
Brain	4	0.79 (0.32, 1.25)	<0.001	3.03	0.9	0.0021
Heart	1	4.99 (3.14, 6.85)	<0.001	0.00	0.0	0.00
Kidney	7	1.09 (-0.37, 2.55)	0.145	57.30	89.5	3.33
Lens	1	4.43 (2.74, 6.12)	<0.001	0.00	0.0	0.00
Pancreas	2	3.82 (-2.99, 10.63)	0.272	13.87	92.8	22.46
CAT						
Liver	3	1.49 (-1.15, 4.14)	0.26	26.37	92.4	4.84
BALF	3	3.65 (1.71, 5.59)	<0.001	7.64	73.8	2.13
Lung	3	2.67 (1.6, 3.74)	<0.001	3.39	40.9	0.36
Serum	10	0.93 (0.62, 1.24)	<0.001	5.32	0.0	0.00
Brain	3	0.74 (0.16, 1.31)	0.012	2.35	14.8	0.038
Lens	2	2.76 (1.82, 3.71)	<0.001	0.04	0.0	0.00
Kidney	2	4.59 (1.28, 7.9)	0.007	3.87	74.1	4.35
Heart	1	4.66 (2.52, 6.80)	<0.001	0.00	0.0	0.00
Testis	2	5.37 (-3.11, 13.86)	0.215	13.33	92.5	34.81
Pancreas	2	1.39 (0.47, 2.30)	0.003	0.34	0.0	0.00
Retina	1	4.83 (2.16, 7.50)	<0.001	0.00	0.0	0.00
SOD						
Liver	5	1.05 (-0.24, 2.35)	0.11	22.68	82.4	1.74
BALF	3	3.06 (1.82, 4.31)	<0.001	3.95	49.4	0.59
Lung	3	3.69 (1.55, 5.84)	<0.001	9.32	78.5	2.71
Serum	11	1.88 (1.21, 2.55)	<0.001	35.89	72.1	0.88
Brain	3	0.90 (0.32, 1.49)	<0.003	2.36	15.1	0.04
Lens	2	1.77 (-2.28, 5.82)	0.392	17.14	94.2	8.04
Heart	4	4.90 (3.81, 6)	<0.001	3.14	4.5	0.05
Kidney	6	1.07 (-1.86, 4)	0.473	91.94	94.6	12.51
Testis	2	4.12 (-4.79, 13.02)	0.365	18.25	94.5	39.12
Pancreatic	2	2.66 (-0.65, 5.97)	0.116	6.62	84.9	4.87
GPx						
Liver	1	0.80 (-0.11, 1.72)	0.085	0.00	0	0.00
Lens	1	2.42 (1.09, 3.75)	<0.001	0.00	0.0	0.00
Testis	1	2.40 (1.08, 3.73)	<0.001	0.00	0.0	0.00
Heart	1	4.30 (2.29, 6.32)	<0.001	0.00	0.0	0.00
Kidney	1	4.81 (2.90, 6.32)	<0.001	0.00	0.0	0.00
TAS						
Serum	6	0.84 (-0.32, 2.01)	0.15	29.24	82.9	1.74
Pancreas	4	0.14 (-1.38, 1.10)	0.82	16.13	81.4	1.29
Kidney	3	2.40 (0.12, 4.68)	0.03	15.52	87.1	3.37
TAC						
Serum	10	2.29 (1.36, 3.21)	<0.001	32.63	72.4	1.53
SH						
Liver	3	0.78 (0.14, 1.41)	0.016	0.24	0.0	0.00
Kidney	3	1.68 (0.95-2.40)	<0.001	1.37	0.0	0.00
Brain	3	0.17 (-0.65, 0.98)	0.68	3.41	41.4	0.21
Oxidant markers						
MDA						
Liver	7	-1.61 (-2.17, -1.06)	<0.001	9.27	35.3	0.19
Serum	22	-1.87 (-2.36, -1.38)	<0.001	69.41	69.7	0.90
Kidney	10	-3.69 (-5.17, -2.75)	<0.001	42	78.6	2.78
Heart	5	-3.44 (-4.94, -1.94)	<0.001	17.76	77.5	2.18
Brain	3	-1.47 (-2.18, -0.75)	<0.001	4.54	33.9	0.17
Pancreas	2	-1.71 (-3.06, -0.36)	0.013	1.85	46.1	0.44
BALF	3	-1.95 (-3.12, -0.78)	<0.001	5.20	61.6	0.65
Lung	3	-2.41 (-3.57, -1.25)	<0.001	4.35	54	1.8
Gastric mucosa	9	-1.41 (-2.23, -0.59)	<0.001	44.73	82.1	0.00
Testis	2	-3.38 (-10.91, 4.15)	0.379	17.76	94.4	27.92
Sciatic nerve	2	-2.69 (-3.85, -1.53)	<0.001	0.39	0	0.00
NO						
BALF	3	-2.47 (-4.07, -0.88)	0.002	7.93	74.8	1.46
Lung	3	-4.67 (-7.63, -1.71)	0.002	12.84	84.4	5.65
Serum	9	-2.13 (-2.95, -1.31)	<0.001	31.54	74.6	1.12
Kidney	1	-4.67 (-6.43, -2.91)	<0.001	0.00	0	0.00
TOS						
Serum	6	-1.50 (-3.38, 0.38)	0.11	56.90	91.2	4.82
Pancreas	4	-1.49 (-3.29, -0.32)	0.10	26.18	88.5	2.96
Kidney	3	-1.26 (-3.59, -1.06)	0.287	20.51	90.2	3.76
XO						
Liver	1	-1.40 (-2.39, -0.41)	<0.001	0.00	0	0.00
Brain	1	-1.60 (-2.62, -0.58)	<0.001	0.00	0	0.00
Kidney	1	-1.44 (-2.44, -0.45)	<0.001	0.00	0	0.00
ROS						
Serum	1	-0.80 (-1.63, 0.04)	<0.001	0.00	0	0.00

Abbreviations: glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), total antioxidant capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), total oxidant status (TOS), thiol groups (SH), xanthine oxidase (XO), reactive oxygen species (ROS).

Table 4. Comparison of oxidative stress markers (oxidants and antioxidants) by type of intervention

Variable	*N*	SMD (95%CI)	*P* value for SMD	*Q*	I^2	Tau
Antioxidant markers						
GSH						
Saffron	11	1.58 (0.92, 2.24)	<0.001	42.38	76.4	0.92
Crocin	23	2.16 (1.45, 2.86)	<0.001	157.64	86	2.4
Safranal	14	3.28 (1.97, 4.6)	<0.001	140.14	90.7	5.53
CAT						
Saffron	12	1.15 (0.52, 1.79)	<0.001	45.80	76	0.91
Crocin	11	2.35 (1.38, 3.31)	<0.001	54.40	81.6	1.9
Safranal	9	2.48 (1.73, 3.23)	<0.001	21.51	62.8	0.79
SOD						
Saffron	12	1.57 (0.90, 2.24)	<0.001	45.06	75.6	0.98
Crocin	21	2.01 (1.02, 3.01)	<0.001	203.59	90.2	4.57
Safranal	9	3.33 (2.58, 4.08)	<0.001	15.62	48.8	0.62
GPx						
Saffron	5	3.14 (1.24, 5.04)	<0.001	27.77	85.6	3.43
Crocin	1	4.30 (2.29, 6.32)	<0.001	0.00	0	0.00
TAS						
Crocin	7	1.06 (-0.18, 2.30)	0.094	42.20	85.8	2.31
Safranal	6	0.60 (-0.55, 1.75)	0.30	31.92	84.3	1.72
TAC						
Saffron	3	3.90 (0.78, 7.03)	0.01	15.26	86.9	6.41
Crocin	7	1.82 (0.98, 2.67)	<0.001	14.5	58.6	0.74
SH						
Crocin	9	0.84 (0.32, 1.35)	0.001	14.63	45.3	0.27
Oxidant markers						
MDA						
Saffron	15	-2.55 (-3.58, -1.53)	<0.001	120.76	88.4	3.13
Crocin	43	-2.20 (-2.58, -1.82)	<0.001	146.73	71.4	1.09
Safranal	13	-2.14 (-2.75, -.153)	<0.001	36.11	66.8	0.81
NO						
Saffron	3	-1.58 (-2.72, -0.43)	0.007	6.44	68.9	0.7
Crocin	5	-2.18 (-3.35, -1.02)	<0.001	18.13	77.9	1.34
Safranal	8	-3.70 (-4.97, -2.43)	<0.001	32.60	78.5	2.5
TOS						
Crocin	7	-1.44 (-2.70, -0.18)	0.025	40.42	85.2	2.42
Safranal	6	-1.40 (-3.19, 0.39)	0.125	58.36	91.4	4.33
XO						
Crocin	3	-1.48 (-2.06, -0.90)	0.001	0.08	0.0	0.00
ROS						
Crocin	1	-0.80 (-1.63, 0.04)	0.001	0.00	0	0.00

Abbreviations: glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), total antioxidant capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), total oxidant status (TOS), thiol groups (SH), xanthine oxidase (XO), reactive oxygen species (ROS).

(μmol/L) [95% CI, -2.23 to -0.59]; $P=0.001$, $I^2=82.1\%$).

The subgroup analysis for comparison of oxidative stress markers (oxidants and antioxidants) by type of intervention

The results of our meta-analysis showed that saffron, crocin, and safranal increase the levels of antioxidants markers (GSH, CAT, SOD, GPx, TAS, TAC, and SH) and decrease the levels of oxidant markers (MDA, NO, TOS, XO, and ROS). The results of our meta-analysis suggest that safranal was more effective than saffron and crocin on antioxidant and oxidant markers. In addition, saffron showed the least effectiveness compared to crocin and safranal.

Meta-regression

Meta-regression was performed to evaluate the effect of quantitative covariates on each of the markers of oxidative stress (oxidant and antioxidant), intervention dose, duration of intervention, publication year, quality score, and sample size. Meta-regression for MDA showed that there was no relationship between intervention dose ($b=-0.0061$; [95% CI = -0.01 to 0.003]; $P=0.2$), duration of the intervention ($b=-0.0057$; [95% CI = -0.03 to 0.02]; $P=0.6$), publication year ($b=-0.16$; [95% CI = -0.30 to -0.03]; $P=0.01$), quality score ($b=-0.011$; [95% CI = -0.078 to 0.05]; $P=0.7$), and sample size ($b=0.22$; [95% CI = -0.059 to 0.51]; $P=0.1$). Figure 2 shows the meta-regression for MDA. This analysis was performed for other oxidative stress markers (oxidant and antioxidant), but no significant relationship was observed (data are not shown).

Publication bias

In this study, publication bias was assessed by funnel plot and Egger's test, and it was observed that there was no evidence of publication bias ($P\text{-value}_{\text{Egger's test}}>0.05$) (data are not shown).

Sensitivity analysis

In this study, sensitivity analysis was performed by excluding studies with a quality score below 70%, and no change was observed in the results (data are not shown).

Discussion

Main findings

This is the first systematic review and meta-analysis to comprehensively investigate the effect of saffron and its active compounds on oxidative stress markers (oxidants and antioxidants).

DM is a complex metabolic disease caused by a defect in the amount or function of insulin (52). Various studies show that oxidative stress begins at the onset of diabetes and gradually increases following long-term hyperglycemia (44,53). Free radicals facilitate tissue damage through lipid peroxidation, changes in antioxidant enzyme activity,

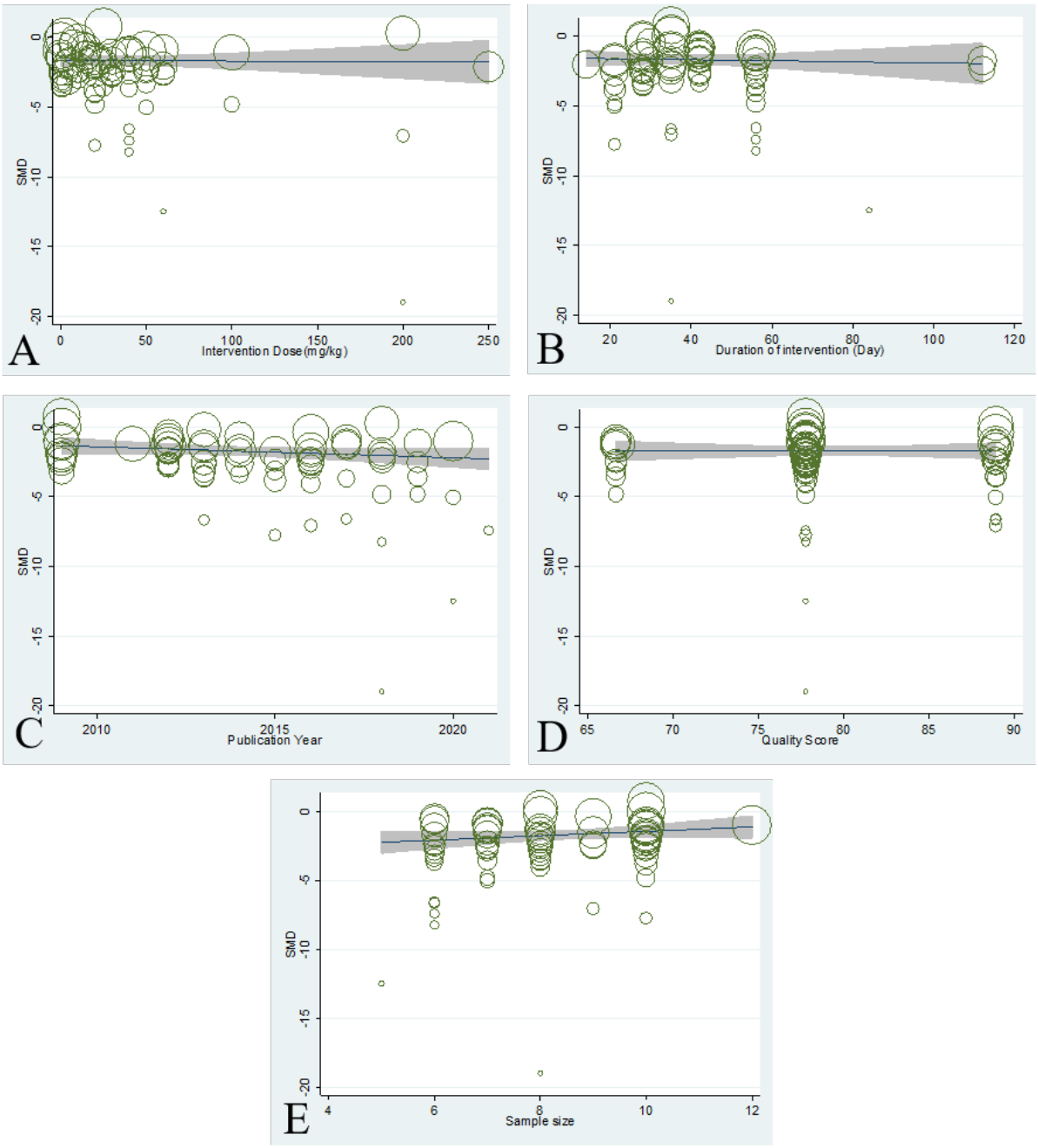

Figure 2. Meta-regression for malondialdehyde (MDA) based on **a:** Intervention dose, **b:** Duration of intervention, **c:** Publication year, **d:** Quality score, and **f:** Sample size

nuclear factor kappa B (NF-κβ) activation, and induction of apoptosis (47). MDA disrupts membrane function by reducing membrane fluidity and altering the activity of membrane-bound enzymes and receptors, eventually leading to damage to cellular components (22).

A mounting body of evidence in the literature has highlighted the hypoglycemic properties of saffron, making it a potential nutraceutical for diabetes. The enhancement in insulin sensitivity, initiation of insulin signaling pathways, enhancement of β-cell actions, advancement of glucose transporter type 4 (GLUT-4) expression, reduction of oxidative stress, and suppression of the expression of inflammatory mediators explains the hypoglycemic action of saffron (23, 58).

Our meta-analysis showed that saffron and its active compounds significantly decrease oxidant markers, including MDA, NO, TOS, XO, and ROS, in different tissues, with a large effect size. Although there is heterogeneity in some studies, numerous studies have shown that saffron, crocin, and safranal have a hypoglycemic effect (22,38,51). Thus, according to our results and previous studies, saffron and its active compounds have protective effects on different tissues by reducing oxidant markers and hyperglycemia.

The results of our meta-analysis showed that saffron and its active compounds increased antioxidant markers,

including GSH, CAT, SOD, GPx, TAS, TAC, and SH, in different tissues, with a large effect size. SOD, CAT, and GSH are defense factors against free radicals (30). However, the heterogeneity of the studies was substantial. Long-term hyperglycemia decreases antioxidant enzyme function due to non-enzymatic glycation or structural damage caused by excessive ROS generation (59). SOD is a metalloprotein and the first enzyme in the antioxidant defense chain that converts O^{-2} to H_2O_2 (24), followed by CAT and GPx. CAT is localized in peroxisomes or microperoxysomes, which catalyze the breakdown of H_2O_2 into water and oxygen (23,25). GPx uses GSH as a proton donor to convert H_2O_2 to H_2O and O_2 (56). Taking a cue from this metanalysis, saffron, crocin, and safranal reinforce the antioxidant defense system and protect different tissues in hyperglycemic conditions.

The results show that saffron and its active compounds (crocin and safranal) have an overall protective effect against oxidative stress. In our study, safranal showed a larger effect on oxidative stress markers than saffron and crocin. Among the studies included in the meta-analysis, the study of Kianbakht et al examined the concurrent effect of saffron, crocin, and safranal. It concluded that safranal is more effective than saffron and crocin in minimizing oxidative stress (34). Safranal is a monoterpene aldehyde and a principal constituent of the essential oil of saffron, but it should be noted that safranal is usually present in less than 1% of saffron (60). saffron contains more than 150 chemicals (61) and may be less effective than safranal due to its diverse chemicals. Therefore, further research on different saffron, crocin, and safranal doses is necessary to confirm the hypothesis.

Strengths and limitations

Strengths: An exploratory search was conducted to identify articles on the role of saffron and its active compounds in diabetes and oxidative stress markers, and the results were included in the meta-analysis. A subgroup analysis was done based on the type of specimen and intervention. Lastly, we tried to identify the causes of heterogeneity using meta-regression and subgroup analysis techniques.

Limitations: First, in a few of the analyses, the total number of included articles was low, especially papers on XO and ROS. Second, some studies showed high heterogeneity in the analyses.

Conclusion

The radical scavenging trait of saffron is well known to reinforce the antioxidant defense system. Saffron can play a role in offsetting the multifactor metabolic disorder seen in DM by modulating the glucose levels and the expression of inflammatory mediators. Also, further in vivo human studies are essential to confirm the affirmative effects of saffron as a complementary therapy for DM.

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Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

This study was approved by Birjand University of Medical Sciences Ethics Committee (IR.BUMS.REC.1400.138).

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None.

Supplementary Files

Supplementary file 1 contains Figure S1.

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