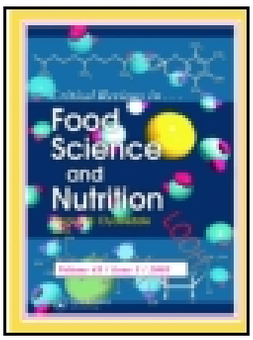

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REVIEW

Does saffron supplementation have favorable effects on liver function indicators? A systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Several pharmaceutical and non-pharmaceutical approaches have been suggested to improve liver health. There is a large discrepancy in the effects of saffron supplementation on liver function in adults. To fill this knowledge gap, this systematic review and meta-analysis of randomized controlled trials (RCTs) assess the effects of saffron supplementation on liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). A systematic search current to August 2020 was performed in PubMed/Medline, Scopus, Web of Science, and Google Scholar using relevant keywords to detect eligible articles. A random-effects model was used to estimate the weighted mean difference (WMD) and 95% confidence (95% CI). Nine eligible trials were included in the final analysis. The pooled analysis revealed that serum ALT concentrations were significantly reduced using saffron compared to placebo (WMD: −2.39 U/L; 95% CI: −4.57 to −0.22; $P = 0.03$, $I^2 = 87.9\%$, $P < 0.001$). However, saffron supplementation did not affect levels of serum AST (WMD: 1.12 U/L; 95% CI: −1.42 to 3.65; $P = 0.39$) or ALP (WMD: 4.32 U/L; 95% CI: −6.91 to 15.54; $P = 0.78$). In the dose-response analysis, we did not find a significant dose-response relationship between dosage and duration of saffron supplementation on serum levels of ALT, AST, and ALP. We found that saffron supplementation can reduce ALT serum concentrations without significant effects on other liver function indicators, including AST and ALP. Nevertheless, future large RCTs on diverse populations are needed to understand better the effects of saffron and its constituents on these enzymes.

KEYWORDS

Saffron; crocin; liver enzyme; alanine transaminase; meta-analysis

Introduction

Liver disorders make up a significant global health concern, accounting for approximately 2% of total deaths worldwide (Lozano et al. 2012). Adverse drug reactions and ischemia-reperfusion (IR) injuries are the major causes of acute liver damage. Viral infection, alcohol abuse, and obesity-associated metabolic disorder are the most common causes of chronic liver disease and often lead to end-stage liver cirrhosis and hepatocellular carcinoma (Fitzmaurice et al. 2015). There has been a rapid accumulation of knowledge in the field of hepatocyte injury leading to numerous screening tests to indicate the severity of liver damage (Dufour et al. 2000). Assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which predominantly indicate structural and cellular liver damage, are standard laboratory tests in this regard. AST and ALT have a pivotal role in catalyzing the transfer of amino groups to produce products required for gluconeogenesis and amino acid metabolism (Jiang et al. 2015). Although ALT is present in various organs, hepatocytes are the predominant sources (Ruhl and Everhart 2009), while AST can be produced in the heart, liver, skeletal muscle, and kidney (Dufour et al. 2000). Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity (Dufour et al. 2000). ALP, which appears in the placenta, ileal mucosa, kidney, bone, and liver, contributes to metabolite transport across cell membranes (Dufour et al. 2000). Some epidemiological evidence has linked these enzymes and type 2 diabetes mellitus, cardiovascular disease, and mortality from vascular and non-vascular causes (Kunutsor, Apekey, and Walley 2013; Rahmani et al. 2019). As most liver disorders are

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preventable, effective primary preventions are required to relieve liver disorders' growing burden (Asrani et al. 2019).

Regarding preventative measures, lifestyle modifications stand out as the most promising strategies to reduce the prevalence of liver disorders (Promrat et al. 2010; Mousavi et al. 2019a). Some of these strategies include complementary and herbal medicine, which have long been a central treatment strategy for liver disorders (Feher and Lengyel 2012; Mousavi et al. 2020). Indeed, due to the side effects of conventional drugs and inconclusive treatment in some patients, more concentration has been placed on herbal remedies in recent years (Liu et al. 2013; Mousavi et al. 2018). One such medicinal herb is saffron, the dried stigma of the *Crocus sativus* L. flower, with a red-gold coloration that is used for dying, flavoring, and therapeutic reasons (Jose Bagur et al. 2018). Saffron owes its pharmacologic potential to three constituents, including volatile agents (e.g., safranal), bitter principles (e.g., picrocrocin), and dye materials (e.g., crocetin and its glycoside, crocin) (Schmidt, Betti, and Hensel 2007). In addition, it has other carotenoids, carbohydrates, raw fiber, proteins, fats, anthocyanins, flavonoids, vitamins, minerals, and many other components that exhibit health benefits (Serrano-DíAZ et al. 2013). Studies have documented the traditional uses of saffron which include applications, such as antioxidant (Asbaghi et al. 2021), antitumor (Abdullaev 2002), memory-enhancing (Papandreou et al. 2006), antianxiety (Hosseinzadeh, Shamsaie, and Mehri 2009), an antidepressant (Hosseinzadeh, Shamsaie, and Mehri 2009), antiseizure (Hosseinzadeh and Sadeghnia 2007), antigenotoxic (Bhatti et al. 2009), and hypotensive (Imenshahidi, Hosseinzadeh, and Javadpour 2010) agents. Furthermore, various studies have been implemented to gain insight into the potential effect of saffron on the serum level of liver enzymes, which are predominantly markers of liver dysfunction (Parsi et al. 2020; Milajerdi et al. 2017; Sepahi et al. 2018; Ebrahimi et al. 2019; Moravej Aleali et al. 2019; Mansoori et al. 2011; Mohamadpour et al. 2013; Mousavi et al. 2015). Although some studies found significant changes in some liver enzymes' level (Parsi et al. 2020) others failed to find any significant changes (Mousavi et al. 2015; Ebrahimi et al. 2019; Pour et al. 2020). For this reason, more reliable measures are currently frequently used as markers of synthetic liver function, namely albumin levels and coagulation (Muniraman et al. 2017). Nevertheless, due to the wide applicability of assessing transaminase levels for liver function and that hepatocyte injury is highly indicative of disease severity and overall function, this study chose to focus predominantly on the effect of saffron supplementation on levels of transaminases.

Due to differences in supplementation dosages, the length of intervention, and the health status of participants in the available studies, saffron's true effect on the serum level of liver enzymes has been inconsistently reported. Nevertheless, the beneficial effect cannot be ruled out. Moreover, despite several publications on the impact of saffron on the serum level of liver enzymes, we are aware of no previous studies summarizing findings in this regard. Therefore, we conducted a systematic review and meta-analysis of published randomized controlled trials (RCTs) to investigate the quantitative impact of saffron supplementation on the serum level of liver enzymes in adults.

Methods

We performed and reported this systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) checklist (Moher et al. 2015).

Search strategy

Systematic literature searches for articles published up until August 2020 were conducted in PubMed/Medline, Scopus, Web of Science, and Google Scholar. In our search strategy, we used the combination of MeSH and non-MeSH terms, including ("Crocus sativus Linn" OR Safranal OR saffron OR crocin) AND ("liver enzymes" OR "alanine transaminase" OR "aspartate transaminase" OR "alkaline phosphatase" OR "gamma-Glutamyltransferase" OR "lactate dehydrogenase" "uric acid" OR creatinine OR "ALT" OR "AST" OR "ALP") AND ("intervention" OR "trial" OR "supplement*" OR "effect"). We applied no time and language limitation. In addition, we performed reference list checking of included articles and related reviews and meta-analyses for additional related studies to ensure a comprehensive literature search.

Inclusion criteria

Studies were selected according to the following inclusion criteria: 1) randomized controlled trials (RCTs) with either parallel or crossover design; 2) those conducted on the adult population (≥18 years); 3) those that examined the effects of saffron supplementation or crocin (the main phytochemical in saffron) on serum levels of ALT, AST, and ALP as the main outcome of interest, irrespective of the other reported measures; and 4) studies that provided sufficient information on main endpoints, including standard deviation (SD), standard error (SE), or 95% confidence interval (CI), which must have been presented at baseline and the end of study duration in both treatment and control groups.

Exclusion criteria

Studies were excluded if they: 1) were carried out on children, pregnant women, or animals; 2) had no control group; 3) contained inadequate data on the selected outcomes in saffron or control groups, and 4) examined the impact of saffron in combination with other ingredients where the independent effect of saffron could not be determined. Moreover, we did not include unpublished documents and gray literature, such as conference abstracts, theses, and patents.

Table 1. Summary of the risk of bias assessment according to the Cochrane Collaboration's tool.

Study. year	Random sequence generation	Allocation concealment	Selective reporting	Other sources of bias	Blinding (participants and personnel)	Blinding (outcome assessment)	Incomplete outcome data
Mansoori et al. 2011	L	U	L	H	L	U	L
Mohamadpour et al. 2013	L	U	L	H	L	U	L
Mousavi et al. 2015	L	U	L	H	L	U	L
Milajerdi et al. 2017	L	U	L	L	L	L	L
Sepahi et al. 2018	L	U	H	H	L	U	L
Ebrahimi et al. 2019	L	U	L	L	L	U	L
Moravej Aleali et al. 2019	L	U	L	L	L	U	L
Kaviani Pour et al. 2020	L	L	L	L	L	L	U
Parsi et al. 2020	L	L	H	H	L	U	L

U; unclear risk of bias, L; low risk of bias, H; high risk of bias.

Screening and data extraction

Titles and abstracts were screened independently by two reviewers (OS and OA). After that, full texts of eligible studies were also screened separately. Data extraction was performed by two reviewers (OS and OA) using standard data extraction forms. All conflicts were solved through consulting a principal investigator (SMM). In studies where detailed data were not reported, we contacted the corresponding author by email to obtain further information. Moreover, there was more than one article for a study, we only used the references with complete information. The following information was extracted from each eligible trial: first author's name, year of publication, study location, intervention duration, study design, characteristics of enrolled participants (numbers, mean age, sex, and health status of them), saffron intervention (type, dosage, frequency), and outcomes measured as mean ± SD of ALT, AST, and ALP at study baseline, post-intervention, and/or changes between baseline and post-intervention.

Risk of bias assessment

The likelihood of bias in the included RCTs was explored through the Cochrane Risk of Bias Tool for clinical trials (Higgins et al. 2011). Two independent authors (OS and OA) assessed each publication's quality based on the following seven domains: 1) random sequence generation. 2) allocation concealment, 3) selective outcome reporting, 4) blinding of participants and personnel, 5) detection bias (blinding of evaluators), 6) incomplete outcome data, and 7) other probable sources of biases. Every article was assigned a label of bias (low risk, high risk, or unclear risk of bias) (Table 1).

Data synthesis and statistical analysis

The mean differences and standard deviation (SD) of serum ALT, AST, and ALP levels were used to obtain the overall effect sizes. In cases where SD was not reported, we calculated it as follows: SD_{change}=square root $[(SD_{baseline})^2 + (SD_{final})^2 - (2 \times R \times SD_{baseline} \times SD_{final})]$ (Borenstein et al. 2009). The best correlation coefficient (R) for outcomes was calculated from articles in which mean (SD) changes were reported (Borenstein et al. 2011). When SD was not directly reported, and a standard error of the mean (SEM) was presented rather than SD, we estimated SD for analyses by considering SEM$\times \sqrt{n}$, in which "n" was the number of subjects in each group. Where the outcome measures were only reported in medians and ranges or 95% confidence intervals (CIs), SD values were calculated based on the method from Hozo et al. (Hozo, Djulbegovic, and Hozo 2005).

The pooled results were calculated using the random-effects model with the procedure suggested by DerSimonian and Laird (Dersimonian and Kacker 2007). We used the Q-test and the I-squared statistic for heterogeneity between studies estimates, with a significance level set at $p < 0.10$. Potential clinical sources of observed heterogeneity were explored using subgroup analyses by type and dosage of saffron, study duration, gender, mean age, and participants' health status. The potential non-linear dose-response association between dosage and duration of saffron supplementation was examined by fractional polynomial modeling (Fan and Gijbels 1996). To explore the impact of each study on the pooled effect size, sensitivity analyses were conducted. The risk of publication bias was assessed using visual inspection of the funnel plot and Egger's test. All statistical tests were performed using Stata software (Version 14.0, Stata Corp, College Station, TX), and a P-value less than 0.05 was considered as statistically significant.

Results

Study selection

A total of 1136 articles were initially retrieved after searching databases. Of those, 316 duplicate publications were removed. The detailed steps of the literature search process

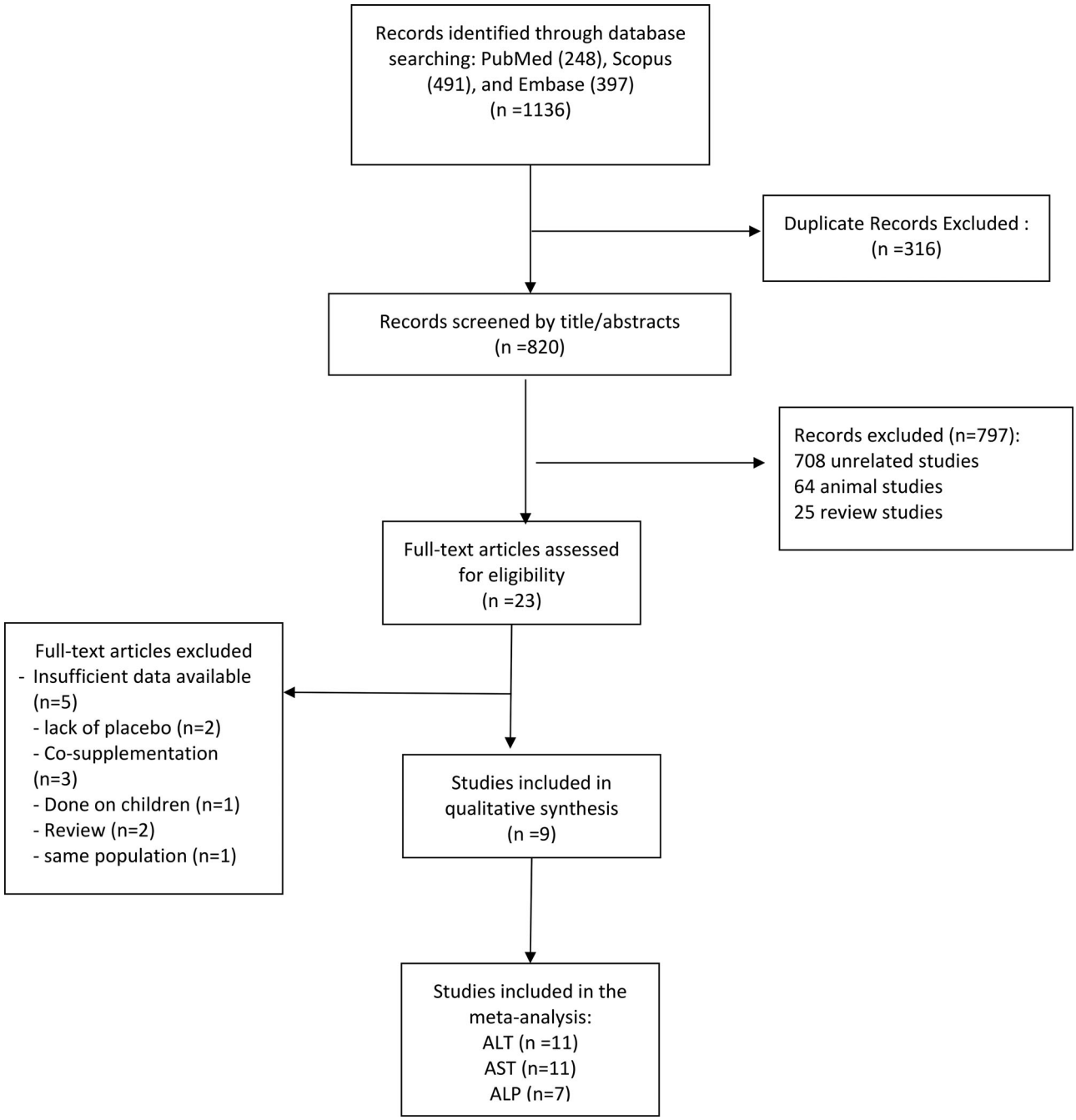

Figure 1. Flowchart of the study selection for inclusion in the meta-analysis.

are shown in the flow chart in Figure 1. After reviewing the remaining 820 titles and abstracts, we included 23 publications for further examination of full-texts. Of these, 14 records were excluded for the following reasons: duplicate dataset (n = 1), review studies (n = 2), trials were conducted on children (n = 1), saffron supplementation was done in combination with other components (n = 3), lack of placebo group (n = 2), and did not report sufficient data (n = 5). After the final assessment, nine eligible trials (with 11 treatment arms) were included in the final quantitative analysis (Parsi et al. 2020; Moravej Aleali et al. 2019; Ebrahimi et al. 2019; Sepahi et al. 2018; Mousavi et al. 2015; Mohamadpour et al. 2013; Mansoori et al. 2011; Milajerdi et al. 2017; Pour et al. 2020).

Study characteristics

The characteristics of the nine studies included in the current study are shown in Table 2. The studies were published between 2011 and 2020. Sample sizes ranged between 20 and 80 individuals, with a total sample size of 562 individuals. All studies were performed in Iran, and all were randomized, double-blinded, placebo-controlled clinical trials. Eight studies included both genders, and only one study was done exclusively on males (Mousavi et al. 2015). Out of nine studies: five assessed the effects of saffron supplementation (Mansoori et al. 2011; Milajerdi et al. 2017; Ebrahimi et al. 2019; Moravej Aleali et al. 2019; Pour et al. 2020), three assessed the effects of crocin supplementation

Table 2. Characteristics of included studies investigating the effects of saffron supplementation on liver function.

Author, year	Design	Participants, n	Health condition	Age mean or range, year	Intervention: Treatment group	Intervention: Control group	Duration (week)	Outcomes (changes, U/L)[1]: Treatment group	Outcomes (changes, U/L)[1]: Control group	Adjust/ matching
Mansoori et al. 2011	RA/DB/ Parallel	F/M: 20, Int: 10, Con: 10	Depression	Int: 35.3, Con: 42.4	30 mg/ d saffron	Placebo: unclear	4	ALT: 4.30 ± 6.86 AST: 2.00 ± 2.88 ALP: 8.10 ± 20.49	ALT: 4.60 ± 3.58 AST: −1.00 ± 3.78 ALP: −7.60 ± 24.57	No
Mohamadpour et al. 2013	RA/DB/ Parallel	F/M: 44, Int: 22, Con: 22	Healthy	Int: 31.1, Con: 31.1	20 mg/d crocin	Placebo: unclear	4	ALT: −7.89 ± 19.08 AST: 5.96 ± 10.74 ALP: −12.50 ± 41.22	ALT: −1.5 ± 2.86 AST: −1.68 ± 6.00 ALP: 5.95 ± 44.32	No
Mousavi et al. 2015 (A)	RA/DB/ Parallel	M: 44, Int: 22, Con: 22	Schizophrenia	Int: 49.3, Con: 48.1	30 mg/ d saffron	Placebo: unclear	12	ALT: −0.7 ± 4.53 AST: 2.90 ± 4.21 ALP: 9.50 ± 29.87	ALT: 1.00 ± 3.31 AST: −10.00 ± 4.56 ALP: −8.10 ± 26.63	No
Mousavi et al. 2015 (B)	RA/DB/ Parallel	M: 44, Int: 22, Con: 22	Schizophrenia	Int: 48.1, Con: 48.1	30 mg/d crocin	Placebo: unclear	12	ALT: −4.00 ± 4.73 AST: −13.60 ± 4.08 ALP: 15.00 ± 21.45	ALT: 1.00 ± 3.31 AST: −10.00 ± 4.56 ALP: 2.89 ± 16.09	No
Milajerdi et al. 2017	RA/DB/ Parallel	F/M: 54, Int: 27, Con: 27	Diabetes	>50 years	30 mg/ d saffron	Placebo: starch, lactose, magnesium stearate, and gelatin	8	ALT: −1.00 ± 4.70 AST: 0.95 ± 3.33 ALP: 15.00 ± 21.45	ALT: 0.7 ± 4.71 AST: −0.80 ± 4.31 ALP: 2.89 ± 16.09	Baseline values
Sepahi et al. 2018 (A)	RA/DB/ Parallel	F/M: 40, Int: 20, Con: 20	Diabetes	Int: 54.3, Con: 57.1	5 mg/d crocin	Placebo: unclear	13	ALT: 0.95 ± 4.70 AST: −0.90 ± 3.59	ALT: 0.25 ± 1.05 AST: 0.10 ± 1.04	No
Sepahi et al. 2018 (B)	RA/DB/ Parallel	F/M: 40, Int: 20, Con: 20	Diabetes	Int: 56.0, Con: 57.1	15 mg/d crocin	Placebo: unclear	13	ALT: 0.30 ± 1.41 AST: −0.85 ± 1.37	ALT: 0.25 ± 1.05 AST: 0.10 ± 1.04	No
Ebrahimi et al. 2019	RA/DB/ Parallel	F/M: 80, Int: 20, Con: 20	Diabetes	Int: 55.2, Con: 53.0	100 mg/ d saffron	Placebo: maltodextrin	12	ALT: −1.35 ± 4.67 AST: 1.20 ± 4.51 ALP: −4.80 ± 21.65	ALT: −2.8 ± 4.42 AST: −2.3 ± 3.70 ALP: −0.70 ± 20.57	No
Moravej Aleali et al. 2019	RA/DB/ Parallel	F/M: 64, Int: 32, Con: 32	Diabetes	Int: 53.5, Con: 52.4	30 mg/ d saffron	Placebo: lactose, magnesium stearate, and starch	13	ALT: 1.93 ± 9.69 AST: −5.03 ± 43.9 ALP: −7.90 ± 57.8	ALT: −0.62 ± 10.21 AST: −1.5 ± 13.5 ALP: 34.3 ± 93.60	No
Kaviani Pour et al. 2020	RA/DB/ Parallel	F/M: 72, Int: 36, Con: 36	NAFLD	Int: 43.4, Con: 43.4	100 mg/ d saffron	Placebo: maltodextrin	12	ALT: −8.15 ± 10.97 AST: −4.34 ± 7.16	ALT: −5.19 ± 5.57 AST: −4.41 ± 4.06	Baseline values
Parsi et al. 2020	RA/DB/ Parallel	F/M: 60, Int: 30, Con: 30	NAFLD	Int: 33.0, Con: 36.1	15 mg/d crocin	Placebo: starch	8	ALT: −17.06 ± 9.45 AST: −6.56 ± 7.83	ALT: 0.53 ± 8.19 AST: 2.87 ± 12.41	No

Abbreviations: ALT: alanine transaminase, AST: aspartate transaminase, ALP: alkaline phosphatase, M: male, F: female, RA: randomized, DB: double-blinded; SB: single-blinded; Con: control; NAFLD: nonalcoholic fatty liver disease.

[1]Values are mean ± SD

(Mohamadpour et al. 2013; Sepahi et al. 2018; Parsi et al. 2020), and one evaluated both (Mousavi et al. 2015). Since crocin is the main phytochemical in saffron, we included studies that assessed the effects of crocin supplementation on liver enzymes. Four studies were done on diabetic patients (Milajerdi et al. 2017; Sepahi et al. 2018; Ebrahimi et al. 2019; Moravej Aleali et al. 2019), two on patients with nonalcoholic fatty liver disease (Parsi et al. 2020; Pour et al. 2020), one on healthy individuals (Mohamadpour et al. 2013), and one on patients with schizophrenia (Mousavi et al. 2015). Of nine studies, only two studies controlled their analyses for baseline measurements (Milajerdi et al. 2017; Pour et al. 2020).

Findings of the systematic review

All studies evaluated the serum concentrations of ALT, of which two revealed a significant reduction through saffron supplementation, and others showed no significant impact. Serum concentrations of AST were significantly reduced following saffron supplementation in three studies, increased dramatically in three studies, and did not change in the remaining three studies. In terms of ALP levels, three studies indicated an increasing effect of saffron supplementation, one showed a reducing effect, and others did not find any significant effect.

Effect of saffron on ALT levels

A total of nine trials (with 11 treatment arms) involving 562 subjects (case = 281, control = 281) investigated the effect of saffron supplementation on serum ALT levels. The pooled analysis indicated that serum ALT concentrations were significantly reduced with the use of saffron supplementation compared to placebo (WMD: −2.39 U/L; 95% CI: −4.57 to −0.22; $P = 0.03$), with a significant degree of heterogeneity (I^2= 87.9%, $P < 0.001$) (Figure 2A). The sensitivity analysis suggested that the overall effect size was heavily influenced by the study by Mohamadpour et al. (Mohamadpour et al. 2013), as the removal of this study resulted in no significant association between saffron intake and ALT concentrations (WMD: −2.20 U/L; 95% CI: −4.42 to 0.21). The heterogeneity disappeared when a subgroup analysis was performed by type of supplementation (I^2=41.1%, P = 0.14), participant's mean age (I^2=19.3%, P = 0.30), and population's characteristic (I^2=19.3%, P = 0.30). These results also showed that serum ALT levels were significantly reduced in trials using crocin as an intervention (WMD: −5.15 U/L; 95% CI: −9.73 to −0.60; $P = 0.03$), but not in trials using saffron (WMD: −0.52 U/L; 95% CI: −2.15 to 1.11; $P = 0.53$). In addition, a significant reduction in serum ALT was observed following saffron supplementation in participants <50 years' old (WMD: −5.52 U/L; 95% CI: −9.90 to −1.13; $P = 0.01$), non-diabetic subjects (WMD: −5.52 U/L; 95% CI: −9.90 to −1.13; $P = 0.01$), and males (WMD: −3.33 U/L; 95% CI: −6.57 to −0.10; $P = 0.04$) (Table 3).

Effect of saffron on AST levels

Nine trials (with 11 treatment arms), including a total of 562 participants (281 cases and 281 controls) assessed the effect of saffron supplementation on circulating AST levels. The overall meta-analysis reported that saffron supplementation does not significantly change serum AST levels (WMD: 1.12 U/L; 95% CI: −1.42 to 3.65; $P = 0.39$). A significant between-studies degree of heterogeneity was observed (I^2= 92.8%, $P < 0.001$) (Figure 2B). Based on sensitivity analyses, the step-by-step omission of each study individually minimally changed the overall effect size (WMD altered between −0.59 to 5.05). Performing subgroup analyses, we did not find any potential sources of heterogeneity or a significant effect of saffron on AST levels among these subgroups (Table 3).

Effect of saffron on ALP levels

The meta-analysis of six trials (with seven treatment arms) involving 353 individuals revealed no significant change in ALP levels after saffron intervention (WMD: 4.32 U/L; 95% CI: −6.91 to 15.54; $P = 0.78$) with a high heterogeneity between studies (I^2= 70.3%, P = 0.003) (Figure 2C). This association was not altered significantly with the sequential elimination of each study one at a time (WMD altered between −12.03 to 18.96). The potential source of heterogeneity, identified by subgroup analysis, was the participants' gender (I^2=0.0%, P = 0.95) (Table 3).

Dose-response analysis

In the dose-response analysis, we failed to find a significant non-linear relationship between the duration of saffron intake and serum ALT levels ($P_{non\text{-}linearity} = 0.18$) (Figure 3A). Similarly, for AST and ALP levels, no significant non-linear association was observed ($P_{non\text{-}linearity} = 0.22$, $P_{non\text{-}linearity} = 0.26$, respectively) (Figure 3). In addition, significant associations were not found between the saffron dosage and outcomes of interest ($P_{non\text{-}linearity} = 0.47$, $P_{non\text{-}linearity} = 0.93$, $P_{non\text{-}linearity} = 0.20$) (Figure 4).

Publication bias

The evaluation of publication bias by visual inspection of the funnel plot illustrated a slight asymmetry in ALT and ALP plots (Figure 5). However, Egger's test revealed no evidence of publication bias for studies examining the effect of saffron supplementation on serum concentrations of ALT (P = 0.14), AST (P = 0.42), and ALP (P = 0.60).

Discussion

Nutraceuticals, such as saffron, have been shown to exert therapeutic and protective effects on chronic diseases, such as inflammatory bowel diseases, Alzheimer's, rheumatoid arthritis, colon, stomach, lung, breast, skin, and liver diseases (Ashktorab et al. 2019; Mousavi et al. 2019b). The

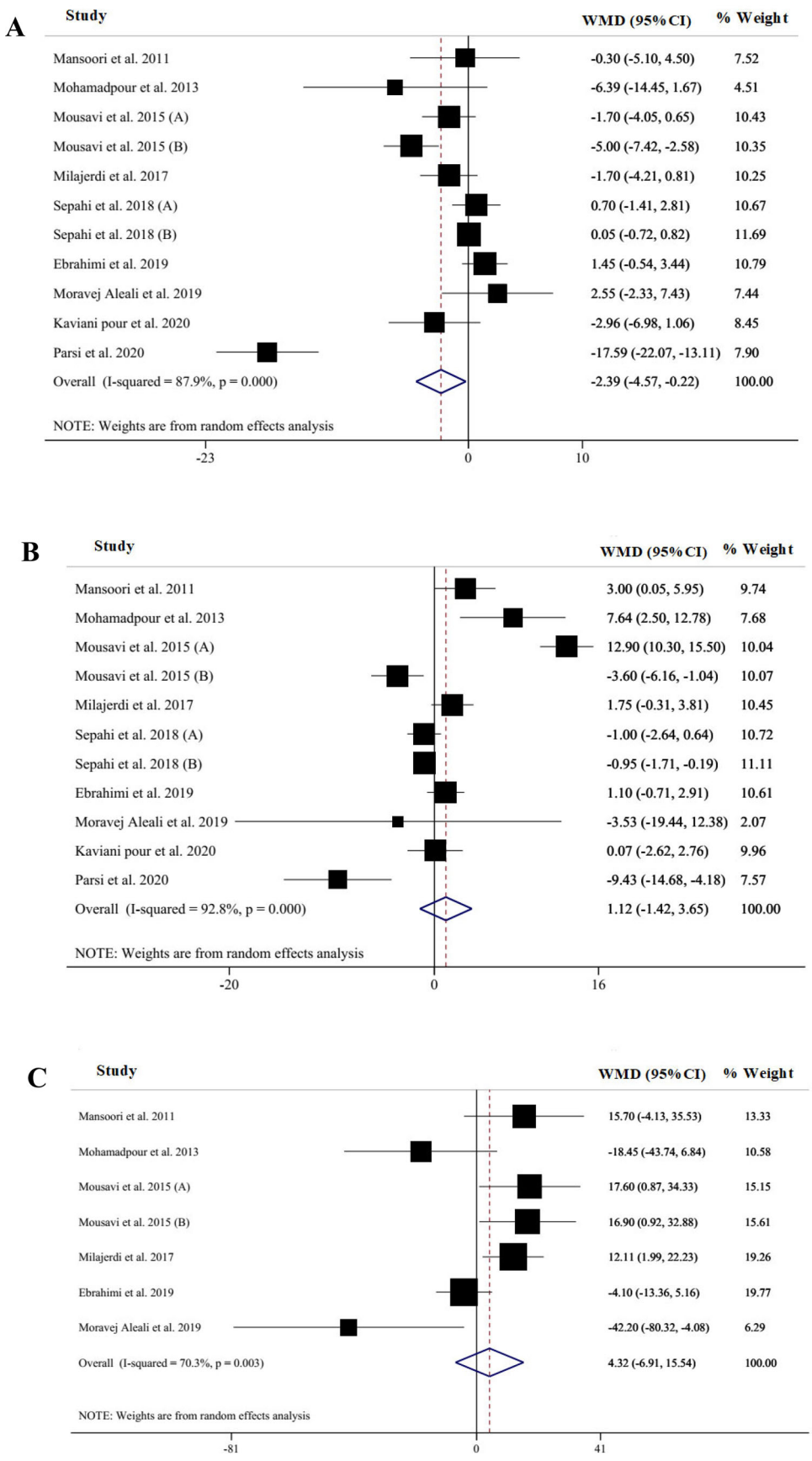

Figure 2. Forest plot representing weighted mean difference and 95% confidence intervals (CIs) for the effect of saffron supplementation on A) ALT, B) AST, and C) ALP.

Table 3. Meta-analysis presenting the effects of saffron supplementation on liver enzymes (U/L) based on several subgroups (all analyses were conducted using random-effects model).

		Meta-analysis		Heterogeneity		
Study group	Number of trials	WMD (95% CI), U/L	P-effect	I^2 (%)	P-within group	P-between group
ALT						
Type of intervention						0.62
Saffron	6	−0.52 (-2.15, 1.11)	0.53	41.0	0.13	
Crocin	5	−5.15 (-9.73 , −0.60)	0.03	94.6	<0.001	
Saffron dosage (mg/d)						0.16
<30	4	−5.31 (-11.07, 0.44)	0.07	95.11	<0.001	
≥30	7	−1.28 (-3.10, 0.75)	0.21	69.6	0.003	
Study duration (week)						<0.001
<12	4	−6.44 (-14.68, 1.75)	0.12	92.6	<0.001	
≥12	7	−0.72 (-2.34, 0.90)	0.38	74.4	0.001	
Participant's mean age						<0.001
<50 years	6	−5.52 (-9.90, −1.13)	0.01	88.1	<0.001	
≥50 years	5	0.26 (-0.62, 1.15)	0.56	19.3	0.30	
Population's characteristic						<0.001
Diabetic	5	0.26 (-0.62, 1.15)	0.56	19.3	0.30	
Non-diabetic	6	−5.52 (-9.90, −1.13)	0.01	88.1	<0.001	
Gender						0.001
Male	2	−3.33 (-6.57, −0.10)	0.04	72.8	0.05	
Both	9	−2.18 (-4.74, 0.37)	0.09	88.3	<0.001	
AST						
Type of intervention						<0.001
Saffron	6	3.37 (-0.76, 7.50)	0.11	92.5	<0.001	
Crocin	5	−1.50 (-3.95, 0.95)	0.23	83.7	<0.001	
Saffron dosage (mg/d)						<0.001
<30	4	−0.91 (-3.83, 2.01)	0.54	85.5	<0.001	
≥30	7	2.24 (-1.73, 6.23)	0.27	93.3	<0.001	
Study duration (week)						0.17
<12	4	0.93 (-3.91, 5.77)	0.70	87	<0.001	
≥12	7	1.18 (-2.08, 4.56)	0.47	94.6	<0.001	
Participant's mean age						<0.001
<50 years	6	1.84 (-4.26, 7.95)	0.55	95.5	<0.001	
≥50 years	5	−0.01 (-1.26, 1.23)	0.98	57.7	0.05	
Population's characteristic						<0.001
Diabetic	5	−0.01 (-1.26, 1.23)	0.98	57.7	0.05	
Non-diabetic	6	1.84 (-4.26, 7.95)	0.55	95.5	<0.001	
Gender						<0.001
Male	2	4.64 (-11.52, 20.81)	0.57	98.7	<0.001	
Both	9	0.31 (-1.33, 1.95)	0.71	77.4	<0.001	
ALP						
Type of intervention						0.49
Saffron	5	4.90 (-8.21, 18.02)	0.46	73	0.005	
Crocin	2	0.64 (-33.89, 35.17)	0.97	81.4	0.02	
Saffron dosage (mg/d)						0.09
<30	1	−18.45 (-43.74, 6.84)	0.15	–	–	
≥30	6	7.12 (-4.24, 18.49)	0.21	69.9	0.005	
Study duration (week)						0.99
<12	3	5.81 (-10.96, 22.59)	0.49	62.4	0.07	
≥12	4	2.34 (-15.52, 20.21)	0.79	77.8	0.004	
Participant's mean age						0.006
<50 years	4	10.25 (-3.73, 24.24)	0.15	53.8	0.09	
≥50 years	3	−3.46 (-22.23, 15.29)	0.71	81.3	0.005	
Population's characteristic						0.006
Diabetic	3	−3.46 (-22.23, 15.29)	0.71	81.3	0.005	
Non-diabetic	4	10.25 (-3.73, 24.24)	0.15	53.8	0.09	
Gender						0.003
Male	2	17.23 (5.68, 28.78)	0.003	0.0	0.95	
Both	4	−1.78 (-16.04, 12.47)	0.80	73.4	0.005	

Abbreviations: ALT; Alanine aminotransferase, AST ; Aspartate aminotransferase, ALP; Alkaline phosphatase, WMD; weighted mean difference.

liver, the largest internal digestive organ, plays an indispensable role in our body's physiological mechanisms (Shamsi-Baghbanan et al. 2014). The liver is susceptible to impairment by a broad array of factors, such as metabolic products, circulatory materials, toxins, and microorganisms (Xiong and Guan 2017; Shamsi-Baghbanan et al. 2014). Saffron (*Crocus sativus* L.) is a dietary spice which belongs to the Iridaceae family (Dehghan et al. 2016). This plant contains three main phytochemical compounds, including carotenoids (crocin and crocetin), glycoside (picrocrocin), and a volatile oil component (safranal) (Bhattacharjee et al. 2012; Tung and Shoyama 2013). Crocin and crocetin are the essential carotenoids and the main bioactive components of saffron with a large spectrum of biological activities (Ashktorab et al. 2019; Dehghan et al. 2016). Their anti-inflammatory and antioxidant characteristics have been evaluated in many in vitro and in vivo studies of various organs, such as the kidney, liver, intestine, and stomach (Chen et al.

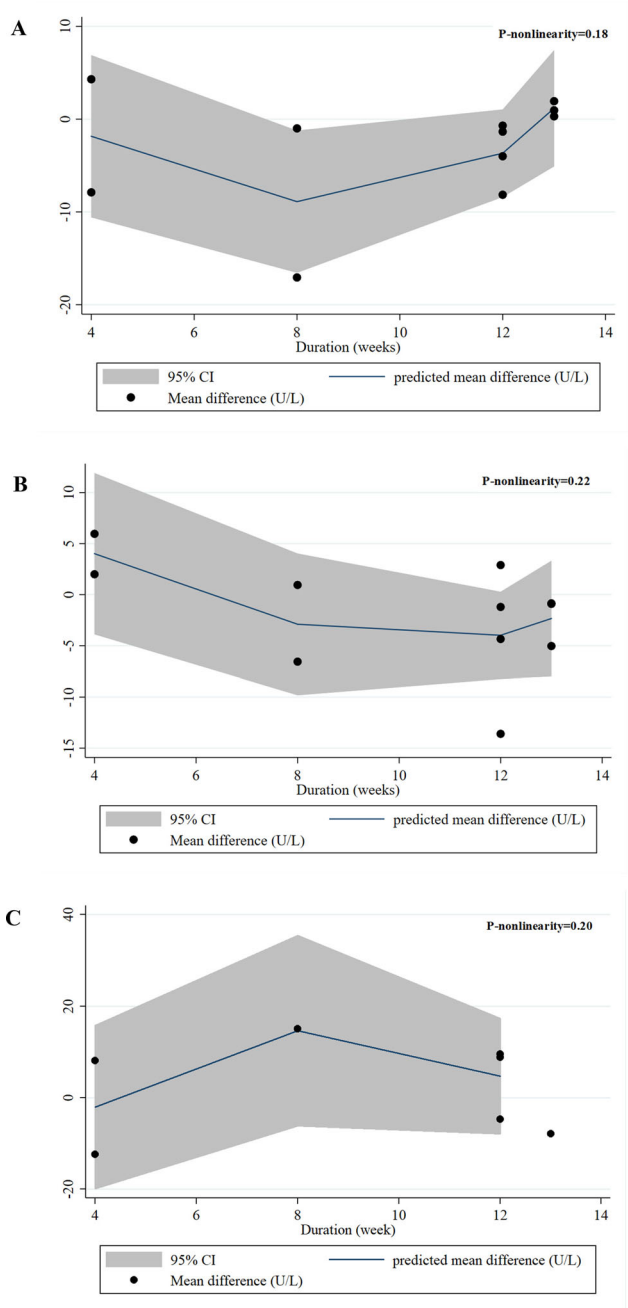

Figure 3. Dose-response relations between duration of saffron supplementation (week) and mean difference (U/L) in A) ALT, B) AST, and C) ALP. The 95% CI is demonstrated in the shaded regions.

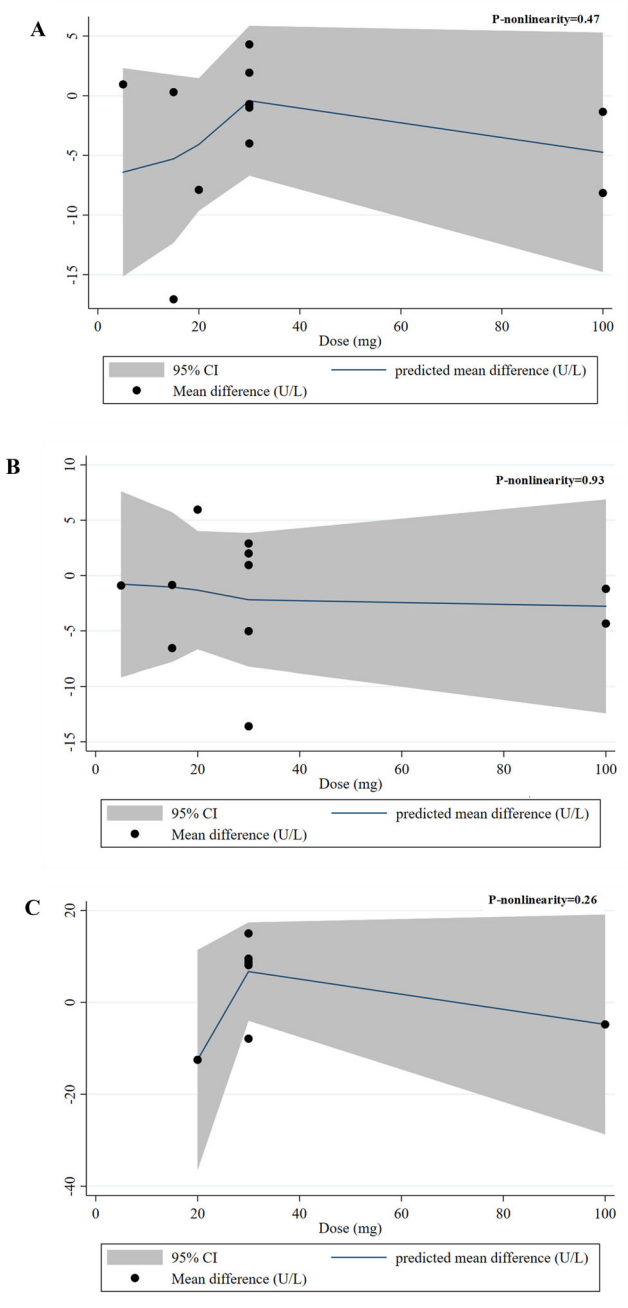

Figure 4. Dose-response relations between saffron dosage (mg/d) and mean difference (U/L) in A) ALT, B) AST, and C) ALP. The 95% CI is demonstrated in the shaded regions.

2010; Farahmand et al. 2013; Khorasany and Hosseinzadeh 2016).

In this systematic review and meta-analysis, the effects of saffron supplementation on serum levels of liver enzymes, including ALT, AST, and ALP, were assessed. In the examined RCTs, the intake of saffron significantly decreased serum ALT concentrations, however, the effect was seen to a greater extent in a subgroup analysis when crocin supplements were used compared to saffron supplements alone. This may be due to the fact that crocin has been reported to have a stronger hypolipidemic effect, faster absorption, and better bioavailability than saffron, thus rendering it a more effective hepatoprotective agent and resulting in a more significant drop in ALT levels (Mashmoul et al. 2014; ALMODóVAR et al. 2020). Overall, the reduction in ALT exhibited by both saffron and crocin supplementation is of clinical significance and could be applied to patients with liver diseases and dysfunction or those who are overweight or have metabolic disorders and at risk of liver damage. In addition, there was a significantly increased reduction in this enzyme's concentration in the subgroup of patients with mean age <50 years, non-diabetic subjects, and males. The circulating levels of AST and ALP were not significantly changed following taking saffron supplements. Moreover, the dose-response analysis indicated that higher supplementation has no effect on serum ALT, AST, and ALP levels. The individually reported findings of the included studies

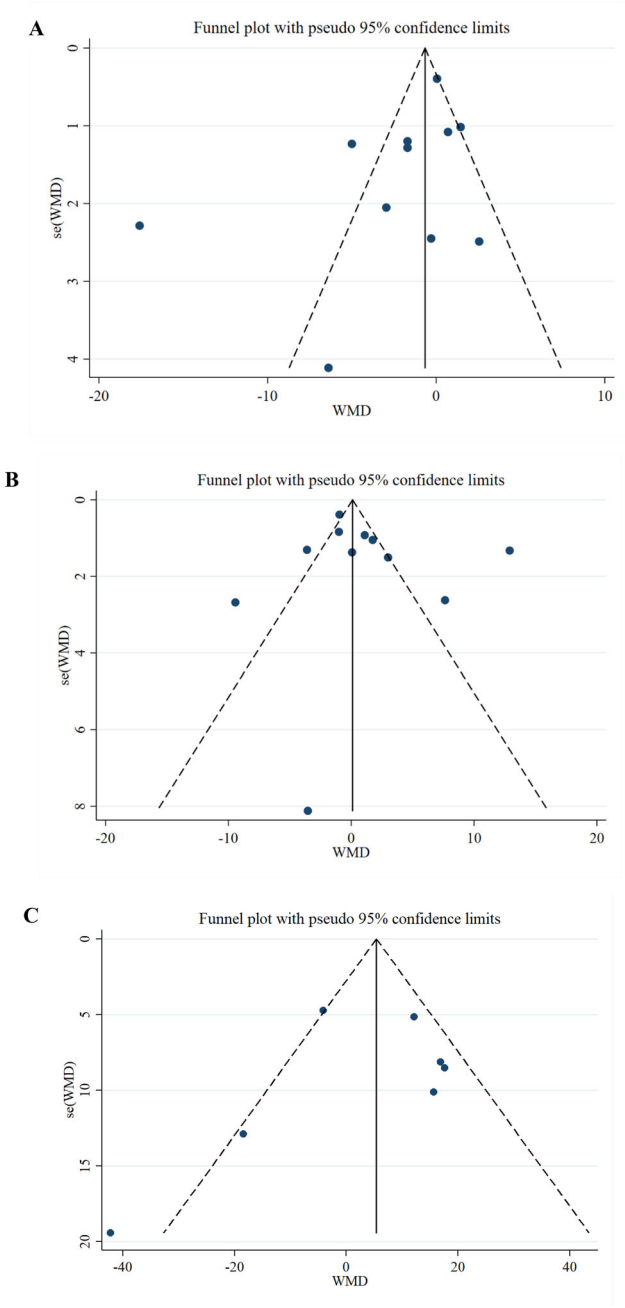

Figure 5. Funnel plot demonstrating publication bias in the trials reporting the effect of saffron supplementation on A) ALT, B) AST, and C) ALP.

mirrored our results. Although saffron and crocin were safely administered in the patient population, the overall effect on various laboratory and enzymatic parameters was generally not significant apart from ALT and reflected the need for larger RCTs.

Our findings are consistent with previous animal and mechanistic studies. The results of animal studies have shown that the AST and ALT levels in rats with fatty liver decreased following the administration of saffron and crocin for eight weeks (Mashmoul et al. 2016; Hemmati et al. 2015). It is suggested that the impact of saffron can be explained by liver enzyme modulation in parallel with normalization of liver structure and size along with decreased fatty infiltration in hepatocytes (Mashmoul et al. 2016). The hepatoprotective effects of saffron are predominantly through their antioxidant properties (Ashktorab et al. 2019). In this regard, Chen et al. reported a remarkable antioxidant capacity of crocetin and crocin on the liver in an *in-vivo* evaluation (Chen et al. 2010). Crocin has been shown to play a significant role in the inhibition of stress-induced oxidative damage in the liver (Bandegi et al. 2014). The results of one study revealed the antioxidant effects of saffron accompanied by a reduction in the markers of liver damage, such as serum ALT and gamma-glutamyl transpeptidase (GGT) levels. These findings confirmed concomitant protection against hepatic toxicity and oxidative stress credited to saffron's robust antioxidant capacity (Amin et al. 2011). Further, saffron's antioxidant property has been reported to be attributed to the phenolic content of saffron and its active ingredients, such as safranal, crocin, crocetin, and carotene (Karimi et al. 2010).

In another study, the therapeutic effect of saffron extract and crocin was investigated on an induced-obesity rat model following treatment with saffron extract and crocin (Ramli et al. 2020). It was demonstrated that in obese rats treated with saffron and crocin supplementation, the glucose level was decreased, which could be related to the activity of two transcriptional regulators, including sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP), that are responsible for lipogenesis and carbohydrate metabolism in the liver (Konstantopoulos et al. 2017). Interestingly, ALT activity in obese rats treated with 80 mg saffron was significantly decreased, and obese rats treated with saffron and lean rats managed to revert to the same lactate and alanine levels (Ramli et al. 2020). In addition, higher serum lactate and alanine accumulation imply lactate dehydrogenase (LDH) and ALT impairment (Abedimanesh et al. 2017). LDH and ALT are enzymes involved in the pyruvate-lactate and pyruvate-alanine interconversions (Xia et al. 2015).

Some potential mechanisms may explain the hepatoprotective effects of saffron; however, there is still much to clarify to understand the mechanisms more precisely. One proposed mechanism could be explained by the role of saffron in lowering carbon tetrachloride activity (CCl-4), as it was shown that CCl-4 induced hepatotoxicity and increased ALT and AST levels in plasma (Khorasany and Hosseinzadeh 2016). Saffron significantly decreases AST and ALT levels in plasma and lowers the incidence of liver lesions induced by CCl-4. It was proposed that saffron consumption may protect liver damage caused by CCl-4 by fixating the hepatic cell membrane, antioxidant effects, radical scavenging, and reducing of CCl-4 metabolic activation by cytochrome P450 inhibition (Khorasany and Hosseinzadeh 2016; Amin et al. 2011). Moreover, saffron may prevent liver damage by significantly reducing the incidence and the number of hepatic nodules. Saffron may decrease the GGT, ALT, and alpha-fetoprotein elevation, all of which are proteins that indicate liver damage (Amin et al. 2011). Given the contradictory results, further RCTs using various saffron dosages with large, diverse sample sizes should be performed to find the exact mechanisms underlying such effects.

To the best of our knowledge, this is the first meta-analysis to examine the effects of saffron supplementation on the levels of liver enzymes ALT, AST, and ALP in serum. We also assessed the dose-response analysis for the intervention duration of saffron and liver enzyme levels. This study has several limitations. The number of included RTCs was limited, and most of the existing data was from Iran, which likely contributed to the heterogeneity exhibited and rendered the generalizability of the results to the global population limited. Thus, the results should be interpreted with caution. Due to the lack of information on other liver function indicators, we were unable to evaluate the effects of saffron on other biomarkers relevant to liver damage. This should be considered in future studies. Moreover, the differences in saffron supplement dosage, participants' health conditions, and the lack of high-quality trials resulted in conflicting results, highlighting the need for future large RCTs.

Conclusion

This study found that saffron supplementation significantly reduced serum ALT levels, while it has no significant effect on serum AST and ALP levels. For future research, long-term clinical trials with different saffron dosages and larger populations are recommended to draw a more precise and comprehensive conclusion on the effects of saffron on liver function.

Conflicts of interest

The authors declared no personal or financial conflicts of interest.

Authors' contributions

SMM and OS conceived the study. OA and OS contributed to the literature search, screening articles, data extraction, and quality assessment. PM and SR contributed to the literature search and manuscript drafting. SMM, AJ, and HR analyzed and interpreted data. EP and YMS revised the statistical analysis and final version of the manuscript. OS supervised the study. The final form of the manuscript has been read and accepted by all authors.

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