The effect of nigella sativa on biomarkers of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials

Article *in* Journal of Food Biochemistry · February 2021

DOI: 10.1111/jfbc.13625

CITATIONS
2

READS
189

7 authors, including:

Somaye Fatahi
Iran University of Medical Sciences
61 PUBLICATIONS **270** CITATIONS
SEE PROFILE

Mohammadhassan Sohouli
Shahid Beheshti University of Medical Sciences
24 PUBLICATIONS **25** CITATIONS
SEE PROFILE

Ahmed Abu-Zaid
Alfaisal University
170 PUBLICATIONS **726** CITATIONS
SEE PROFILE

Heitor O. Santos
Universidade Federal de Uberlândia (UFU)
65 PUBLICATIONS **380** CITATIONS
SEE PROFILE

Received: 11 September 2020 | Revised: 6 January 2021 | Accepted: 7 January 2021

DOI: 10.1111/jfbc.13625

REVIEW

Journal of Food Biochemistry WILEY

The effect of nigella sativa on biomarkers of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials

Rahele Sadat Montazeri[1,2] | Somaye Fatahi[1,3] | Mohammad Hassan Sohouli[1,2] | Ahmed Abu-Zaid[4] | Heitor O. Santos[5] | Mihnea-Alexandru Găman[6] | Farzad Shidfar[2]

[1]Student Research Committee, Faculty of Public Health Branch, Iran University of Medical Sciences, Tehran, Iran

[2]Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

[3]Pediatric Gastroenterology, Hepatology and Nutrition Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical sciences, Tehran, Iran

[4]Department of Pharmacology, College of Graduate Health Sciences, University of Tennessee Health Science Center, Memphis, TN, USA

[5]School of Medicine, Federal University of Uberlandia (UFU), Uberlandia, Brazil

[6]"Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania & Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania

Correspondence
Farzad Shidfar, Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.
Email: shidfar.f@iums.ac.ir

Abstract

Inflammation and oxidative stress are involved in the pathogenesis of a myriad of chronic disorders. This systematic review and meta-analysis was designed to determine the effects of Nigella Sativa (NS) seed and seed oil consumption on several biomarkers of inflammation and oxidative stress. The Scopus, Web of Science, and PubMed-MEDLINE databases were systematically searched until August 2019. The quality assessment and heterogeneity of the selected randomized clinical trials (RCTs) were measured using the Jadad checklist, and *Q* and I^2 tests, respectively. Finally, a total of 10 clinical RCTs were found to be eligible for this meta-analysis. The pooled findings showed that NS consumption significantly reduced serum high-sensitivity C-reactive protein (hs-CRP; WMD: −0.67, 95% CI: −1.29, −0.05, I^2 = 95.7%), tumor necrosis factor-alpha (TNF-α; WMD: −2.29, 95% CI: −4.48, −0.11, I^2 = 93%), and malondialdehyde (MDA; WMD: −1.18, 95% CI: −2.24, −0.12, I^2 = 85.4%), and significantly increased total antioxidant capacity (TAC; WMD: 0.35, 95% CI: 0.10, 0.59, I^2 = 77.1%), and superoxide dismutase (SOD; WMD: 66.30, 95% CI: 1.03, 131.57, I^2 = 99.4%) levels. Overall, the results of this systematic review and meta-analysis imply that NS consumption may decrease inflammatory response and oxidative stress markers.

Practical applications

Overall, the evidence supports the consumption of NS to reduce hs-CRP, TNF-α, and MDA, and to increase SOD and TAC levels. In addition, the subgroup analyses findings concluded that lower dosages of NS, longer durations of the intervention, and the use of NS seed oil may result in more effective action on inflammatory markers, but because of the limited number of trials, the results must be analyzed with caution, especially for the subgroup analysis. However, further prospective studies regarding the effect of NS consumption on biomarkers of inflammation and oxidative stress, with larger sample sizes, from various countries and longer follow-up periods, are required to confirm whether NS possesses veritable anti-inflammatory and antioxidant effects.

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NS, Nigella Sativa Linn; RCT, randomized controlled trial; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; WMD, weighted mean difference.

KEYWORDS
inflammation, meta-analysis, Nigella Sativa, oxidative stress

1 | INTRODUCTION

Many chronic diseases such as obesity, diabetes mellitus, atherosclerosis, and other cardiovascular disorders are associated with a higher production of reactive oxygen species (ROS; Hussain et al., 2016). In such diseases, oxidative stress stimulates the expression of inflammatory genes and promotes the cascade of inflammatory mediators, that is prooxidant molecules, cytokines, lytic enzymes, and eicosanoids (Gholamnezhad et al., 2016). Furthermore, when oxidative stress levels are high, endogenous antioxidants, such as catalase (CAT), superoxide dismutase (SOD), and glutathione, may fail to counteract the excessive production of ROS which can damage cellular proteins, lipids, and DNA/RNA, subsequently resulting in cell death and the development of disease (Hadi et al., 2016; Hussain et al., (2016). Biomarkers of oxidative stress and inflammation, such as serum malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hs-CRP), have generally been used to assess the relationship between oxidative damage to macromolecules and disease progression (Hadi et al., 2016; Mahdavi et al., 2016).

Alternative natural treatments with lower adverse effects may help in the management of various noncommunicable diseases, as they contain a wide range of bioactive phytochemicals with diverse properties which can influence the metabolism (Mahboubi et al., 2018). One such example is *Nigella sativa Linn* (NS), which is a dicotyledonous flowering plant from the family of *Ranunculaceae* and is generally known as black cumin, black seed, or Kalonji (Namazi et al., 2015). It is traditionally used in some areas such as the Middle East, Northern Africa, and Southern Europe, for the prevention or treatment of several conditions, for example, rheumatoid arthritis, cardiovascular diseases, dyslipidemia, diabetes, gastrointestinal disorders (e.g., diarrhea), asthma, and bronchitis (Daryabeygi-Khotbehsara et al., 2017; Namazi et al., 2015).

NS contains several chemical ingredients with pharmacological properties, such as thymoquinone (TQ), nigellone (a carbonyl polymer of TQ), polyunsaturated fatty acids, tocopherols, phytosterols, flavonoids, and terpenes (Namazi et al., 2015). TQ is a major bioactive ingredient in black seed and is responsible for many of the medical benefits of NS, that is, antihistaminic, antihypertensive, hypoglycemic, antineoplastic, and immune-stimulating effects (Majdalawieh & Fayyad, 2015). Recently, it has been discovered that TQ may act as a potent antioxidant and inhibit lipid peroxidation and generation of superoxide radicals, as well as enhance the activity of antioxidant enzymes in normal tissues (Schneider-Stock et al., 2014).

The effects of NS consumption on biomarkers of inflammation and oxidative stress have already been studied in some clinical trials, although the current findings are controversial. For example, NS oil consumption at a dosage of 3 g/day for 8 weeks was associated with a significant reduction in inflammatory markers, namely hs-CRP and TNF-α in obese women (Mahdavi et al., 2016). Conversely, Hadi et al. (2018) have indicated that 1 g/day of NS oil for 8 weeks resulted in no changes in TNF-α and SOD levels in patients diagnosed with diabetes mellitus type 2. Furthermore, there is some evidence that, in therapeutical terms, NS oil is more effective than the seeds of the same plant (Ahmad et al., 2013).

To the best of our knowledge, there are two recent meta-analyses which have reported the beneficial effects of NS on hs-CRP levels (Tavakoly et al., 2019) and oxidative stress parameters (Ardiana et al., 2020). However, none of these studies comprehensively examined the effects of black seed consumption on biomarkers of inflammation and oxidative stress. Therefore, we intended to conduct a systematic review and meta-analysis to investigate the effects of NS seeds and NS seed oil consumption on serum biomarkers of inflammation and oxidative stress in humans.

2 | MATERIALS AND METHODS

The current meta-analysis was conducted in agreement with the Systematic Reviews and Meta-Analyses statement guidelines for RCTs (Liberati et al., 2009).

2.1 | Search strategy

A systematic literature search was performed using several medical databases (PubMed-MEDLINE, SCOPUS, and Web of Science) up to August 2019 using the following keywords and medical subject heading (MeSH) terms: ("Nigella sativa" OR "Kalonji" OR "Kalonjus" OR "Black Cumin" OR "black seed" OR "N. sativa" OR "thymoquinone" OR "nigella sativum" OR "karayal" OR "black onion seed") AND ("oxidative stress" OR "inflammation markers" OR "reactive oxygen species" OR "nitric oxide" OR "malondialdehyde" OR "thiobarbituric acid reactive substances" OR "glutathione" OR "total antioxidant capacity" OR "superoxide dismutase" OR "interleukin 6" OR "interferon gamma" OR "tumor necrosis factor-alpha" OR "nuclear factor kappa B") AND ("clinical trials as topic" OR "cross-over studies" OR "double-blind method" OR "single-blind method"). To identify possibly omitted trials, the reference lists of the related articles were manually searched.

2.2 | Study selection

After the duplicates were removed, the remaining papers were separately screened based on their titles and abstracts. Reviews, irrelevant records and animal studies were excluded. Then, the full texts of the remaining studies were reviewed to select only the records

related to the topic of our systematic review and meta-analysis. Ultimately, all the randomized controlled trials (RCTs) that met the following criteria were included in this report: (a) original RCTs either with a parallel or a crossover design that investigated the effects of NS seed and seed oil consumption on biomarkers of inflammation and oxidative stress, (b) RCTs conducted in adults (age ≥18 years), and (c) RCTs that reported appropriate data on the mean [standard deviation (*SD*) or 95% confidence intervals (CI)] changes on biomarkers of inflammation and oxidative stress, namely hs-CRP, TNF-α, MDA, TAC, and SOD at baseline and at the endpoint of intervention (NS seed and seed oil consumption) for each group (intervention/placebo).

The studies which were not RCTs, those without an appropriate control group, those that did not report enough data for the results regarding the effects of NS seed and seed oil consumption on biomarkers of inflammation and oxidative stress and for the control groups, those which were in vitro or animal studies, reviews, unpublished trials, and trials without a published full text were excluded. In addition, only studies published in English were considered.

2.3 | Data extraction

Data extraction was performed by two independent investigators. This process was examined by another investigator. The following data from each included trial were extracted: first author's name, publication time, study location, study duration, study design, participants' characteristics, sample size, type and dose of supplements/placebo used in intervention/control group, and the main outcomes. When the results were reported for multiple periods, only the data related to the longest duration of treatment was evaluated.

2.4 | Quality assessment

The methodological quality of the eligible studies was evaluated using the quantitative 5-point scoring system developed by Jadad et al., (1996). High-quality studies were defined by scores ≥3, whereas a score of 0–2 was indicative of a low-quality report.

2.5 | Statistical analysis

The pooled weighted mean difference (WMD) and its 95% confidence interval (CI) were calculated to evaluate the effects of NS seed and seed oil on serum hs-CRP, TNF-α, MDA, SOD, and TAC levels. Data from the last time point were used for analyses in studies that presented more than one time point for outcomes throughout the intervention. To ensure that this meta-analysis was not sensitive to the selected correlation coefficient, all the analyses for each parameter were repeated using a correlation coefficient of 0.2 and 0.8. The presence of between-study heterogeneity was determined by the I^2 statistic. Low, moderate, and high heterogeneity was attributed to I^2 values less than 25%, between 25% and 50%, and over 50%, respectively (Higgins & Green, 2011). To determine the possible sources of between-study heterogeneity, we carried out a preplanned subgroup analysis based on NS dosage (>2 or ≤2 g/day), sex (male, female, or male/female), duration of the intervention (≤8 or >8 weeks), and intervention form (powder or oil). Heterogeneity between subgroups was assessed using a fixed-effect model. Sensitivity analysis was also performed to evaluate the impact of every single study on the overall effect size. Publication bias was estimated using visual assessment of funnel plots and Egger's weighted regression tests (Egger et al., 1997). All statistical analyses were conducted using the Stata 12.0 software with a two-sided *p* value significance level of <.05.

3 | RESULTS

3.1 | Flow-chart of study selection

The selection and the identification process of the eligible studies are presented in Figure 1. Initially, 735 studies were identified via the search strategy, of which 331 were excluded for being duplicates. After reviewing the titles and abstracts of the remaining articles, 386 publications that were irrelevant to the study purposes were excluded (supplementary file). Finally, 10 articles (Amin et al., 2015; Darand et al., 2019; Hadi et al., 2016, 2018; Kaatabi et al., 2015; Kheirouri et al., 2016; Mahdavi et al., 2016; Mohtashami et al., 2016; Namazi et al., 2015; Nikkhah-Bodaghi et al., 2019) were eligible to be included into this systematic review and meta-analysis.

3.2 | Study and participant characteristics

The characteristics of 10 RCTs using NS seed powder and its oil have been summarized in Table 1. This meta-analysis included 630 individuals. Nine trials were double-blinded (Amin et al., 2015; Darand et al., 2019; Hadi et al.,2016, 2018; Kheirouri et al., 2016; Mahdavi et al., 2016; Mohtashami et al., 2016; Namazi et al., 2015; Nikkhah-Bodaghi et al., 2019) and one study (Kaatabi et al., 2015) was single-blinded. Among the included trials, one (Mohtashami et al., 2016) was conducted using the crossover method. The duration of the intervention varied from 6 to 48 weeks (Kaatabi et al., 2015; Nikkhah-Bodaghi et al., 2019). The daily recommended dosage of NS seed and seed oil varied between 1 and 3 g/day across different studies (Hadi et al., 2016, 2018; Kheirouri et al., 2016; Mahdavi et al., 2016; Namazi et al., 2015). The included studies were conducted in different populations, namely patients diagnosed with diabetes mellitus type 2 (Hadi et al., 2018; Kaatabi et al., 2015), obesity (Mahdavi et al., 2016; Namazi et al., 2015), metabolic syndrome (Amin et al., 2015; Mohtashami et al., 2016), nonalcoholic fatty liver disease (NAFLD; Darand et al., 2019), rheumatoid arthritis (Hadi et al., 2016; Kheirouri et al., 2016), and ulcerative colitis (Nikkhah-Bodaghi et al., 2019). Overall, six RCTs reported changes in

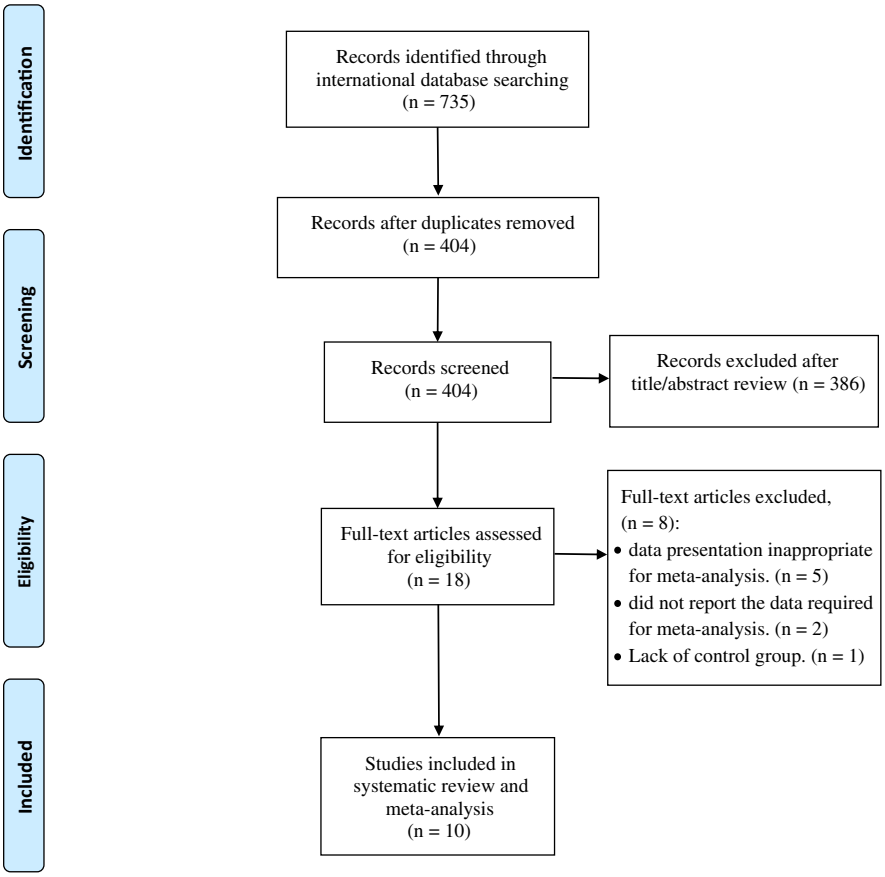

FIGURE 1 PRISMA flow diagram of study selection process

hs-CRP (Amin et al., 2015; Darand et al., 2019; Kheirouri et al., 2016; Mahdavi et al., 2016; Mohtashami et al., 2016; Nikkhah-Bodaghi et al., 2019), five in TNF-α (Darand et al., 2019; Hadi et al., 2016, 2018; Mahdavi et al., 2016; Nikkhah-Bodaghi et al., 2019), four in MDA (Hadi et al., 2016, 2018; Namazi et al., 2015; Nikkhah-Bodaghi et al., 2019), four in SOD (Hadi et al., 2016, 2018; Kaatabi et al., 2015; Namazi et al., 2015), and four in TAC (Hadi et al., 2016; Kaatabi et al., 2015; Namazi et al., 2015; Nikkhah-Bodaghi et al., 2019) levels following NS consumption. The Jadad checklist confirmed that all studies except for one (Salem et al., 2017) had a high methodological quality. The scores of the quality assessment of the included studies are presented in Table 2.

3.3 | Findings from the meta-analysis

3.3.1 | The effects of Nigella Sativa on inflammatory markers

In the pooled result of data from six studies (Amin et al., 2015; Darand et al., 2019; Kheirouri et al., 2016; Mahdavi et al., 2016; Mohtashami et al., 2016; Nikkhah-Bodaghi et al., 2019) with seven arms (n = 400), there was a significant difference at follow-up between the NS and placebo groups regarding hs-CRP concentrations, with a high heterogeneity [WMD: −0.67, 95% CI: −1.29, −0.05, I^2 = 95.7%] (Figure 2). The subgroups analyses for the intervention dose, duration, and type of intervention, and also gender, did not identify any possible sources of heterogeneity. However, doses ≤2 g were more effective in reducing hs-CRP concentrations [WMD: −1.13, 95% CI: −2.12, −0.14] compared to a NS supplementation with a dose >2 g (Figure S1). The pooled results from the random-effects model of five RCTs (n = 266), the changes in TNF-α levels in the NS group compared to the control group at follow-up were significant [WMD: −2.29, 95% CI: −4.48, −0.11, I^2 = 93%] (Figure 3). The subgroups analyses for the intervention dose, duration, and type of intervention, and also for gender, did not identify any possible sources of heterogeneity. However, these analyses showed that a duration of the intervention > 8 weeks and supplementation with NS oil as an intervention reduced TNF-α levels more effectively versus interventions lasting ≥8 weeks and supplementation with NS powder, respectively (Figure S2).

3.3.2 | The effects of Nigella Sativa on biomarkers of oxidative stress

The pooled WMD of four effect sizes displayed a significant difference in MDA (WMD: −1.18, 95% CI: −2.24, −0.12, I^2 = 85.4%), TAC (WMD: 0.35, 95% CI: 0.10, 0.59, I^2 = 77.1%), and SOD (WMD: 66.30, 95% CI: 1.03, 131.57, I^2 = 99.4%) levels in the NS groups versus the control

TABLE 1 Characteristics of included randomized controlled trials in meta-analysis

Author, year	Location	Study design	Participants	Gender (*n*)	Mean age (year)	Dose of NS	Duration (weeks)	Main outcome measures
Amin et al. (2015)	Pakistan	Randomized, Double-Blind, Placebo Controlled Trial	MetS patients	Men (125)	43.33	1.5 g/day NS powder	8	CRP
Darand et al. (2019)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	NAFLD patients	Both (50)	47.48	2 g/day NS powder	12	TNF-α, hs-CRP
Hadi et al. (2018)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	T2DM patients	Both (43)	53.7	1 g/day NS oil	8	TNF-α, MDA, SOD
Hadi et al. (2016)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	Rheumatoid arthritis patients	Women (42)	42.24	1 g/day NS oil	8	TAC, TNF-α, MDA, SOD
Kaatabi et al. (2015)	Iran	Randomized, Single-Blind, Placebo Controlled Trial	T2DM patients	Both (114)	46.47	2 g/day NS powder	48	TAC, SOD
Kheirouri et al. (2016)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	Rheumatoid arthritis patients	Women (43)	42.2	1 g/d NS oil	8	hs-CRP
Mahdavi et al. (2016)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	Obese women (BMI = 30–35 kg/m^2)	Women (84)	40.25	3 g/d NS oil + low calorie diet	8	TNF-α, hs-CRP
Mohtashami (phase 1; 2016)	Iran	Randomized, Double-Blind, Crossover Clinical Trial	MetS patients	Both (51)	47	100 g bread (3 g NS powder + 3 g wheat bran)	8	hs-CRP
Mohtashami (phase 2; 2016)	Iran	Randomized, Double-Blind, Crossover Clinical Trial	MetS patients	Both (51)	47	100 g bread (3 g NS powder + 3 g wheat bran)	8	hs-CRP
Namazi et al. (2015)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	Obese women (BMI = 30–35 kg/m^2)	Women (49)	42.95	3 g/day NS oil + low calorie diet	8	TAC, SOD, MDA
Nikkhah-Bodaghi et al. (2019)	Iran	Randomized, Double-Blind, Placebo Controlled Trial	Ulcerative colitis Patients	Both (48)	37.02	2 g/day NS powder	6	TNF-α, MDA, hs-CRP, TAC

TABLE 2 Methodological quality scores for the included studies using the Jadad scale

Study	Randomization	Methods of randomization	Blinding	Method of blinding	Description of withdrawal	Total score
Nikkhah-Bodaghi et al. (2019)	+	+	+	+	+	5
Darand et al. (2019)	+	+	+	+	+	5
Moustafa et al. (2019)	+	+	–	–	+	3
Hadi et al. (2018)	+	+	+	–	+	4
Salem et al. (2017)	+	–	–	–	+	2
Mahdavi et al. (2016)	+	+	+	+	+	5
Hadi et al. (2016)	+	–	+	+	+	4
Mahdavi et al. (2016)	+	+	+	+	+	5
Kheirouri et al. (2016)	+	–	+	–	+	3
Kaatabi et al. (2015)	+	+	+	–	+	4
Amin et al. (2015)	+	+	+	–	+	4

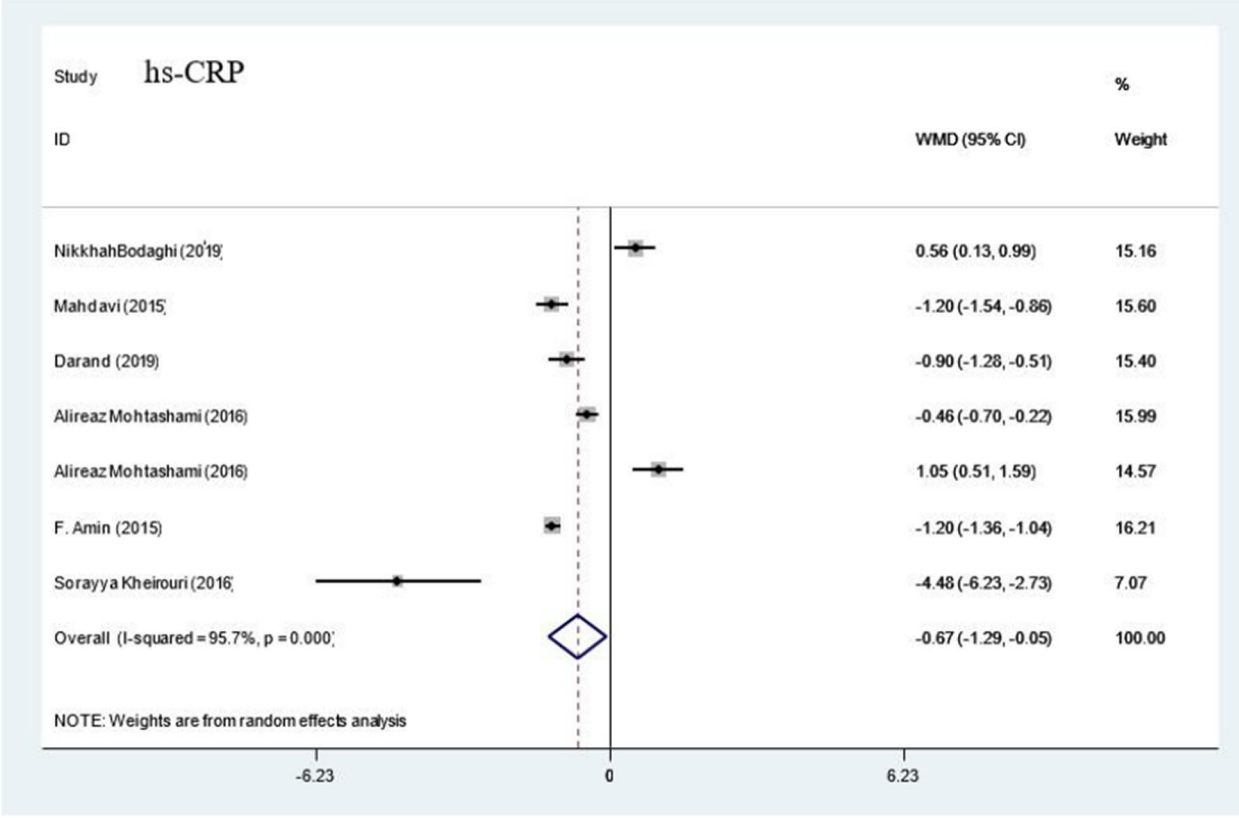

FIGURE 2 Forest plot of the mean difference for the effect of NS consumption on serum hs-CRP levels

groups (Figures 4–6). Because of the limited number of studies for each group, the subgroup analyses did not lead to reliable results. However, the gender of the participants and the type of intervention were possible sources of heterogeneity for MDA concentrations (Figures S3–S5).

3.4 | Publication bias

No publication bias was identified based on the assessment of the funnel plots (Figure 6) or via the Egger's test (p =.51 for hs-CRP, p =.24 for MDA, p =.12 for SOD, and p =.96 for TNF-α). Based on the Egger's regression test, there was a publication bias for TAC concentrations (p =.01). However, the Meta Trim and fill analyses found no unpublished article.

4 | DISCUSSION

To the best of our knowledge, this is the first systematic review or meta-analysis to comprehensively report on the effects of NS on

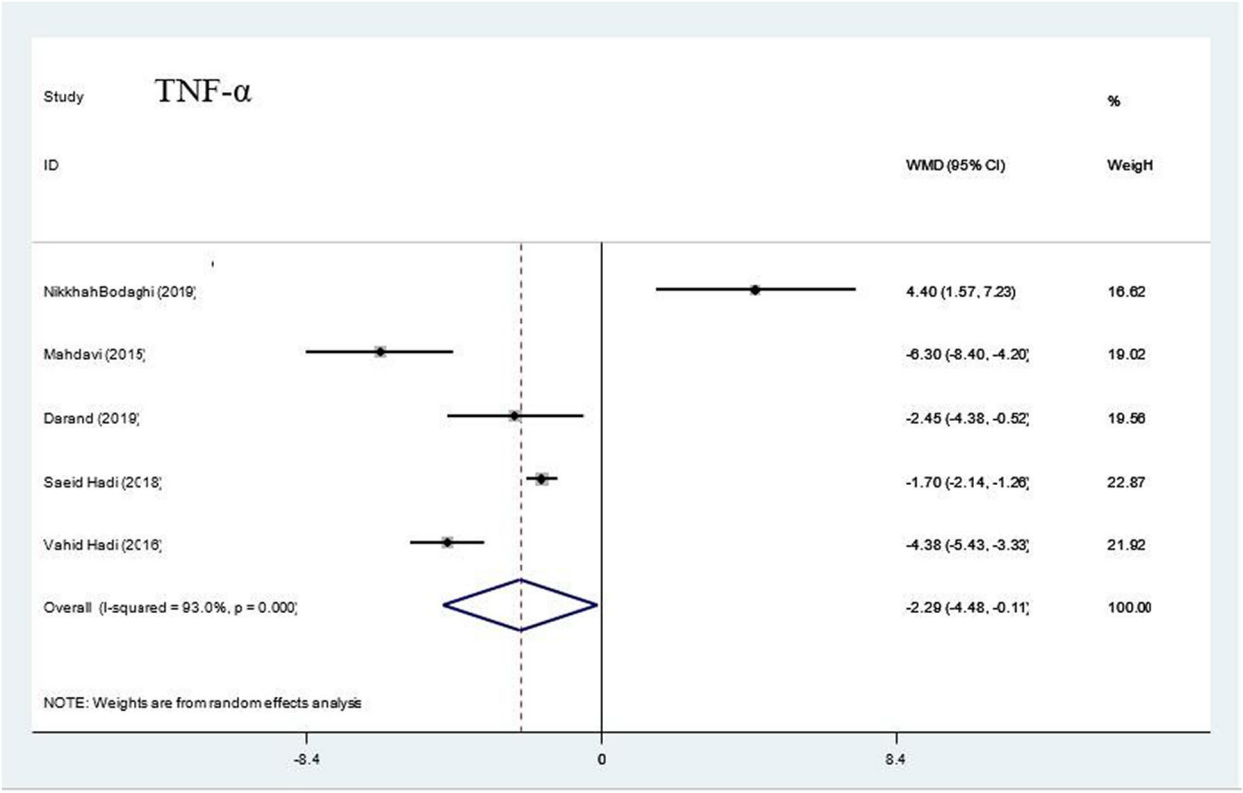

FIGURE 3 Forest plot of the mean difference for the effect of NS consumption on serum TNF-α levels

biomarkers of inflammation and oxidative stress. The current meta-analysis, conducted on 10 RCTs, showed that the administration of NS seed and seed oil significantly reduced serum hs-CRP, TNF-α, and MDA levels, and significantly increased SOD and TAC levels. All of these molecules are widely used to assess the redox balance in different conditions (Smukowska-Gorynia et al., 2019). Our findings provide more underpinnings for the results of other studies that encompassed the impact of NS supplementation on traditional cardiometabolic markers (Daryabeygi-Khotbehsara et al., 2017; Sahebkar et al., 2016).

Systemic inflammation and oxidative stress partake in the development of a myriad of acute and chronic diseases (Gholamnezhad et al., 2016). The present meta-analysis reinforced the aforementioned hypothesis and demonstrated that NS seed and seed oil intake results in a significant reduction in serum hs-CRP and TNF-α levels. Our findings were similar to those reported in a meta-analysis conducted by Tavakoly et al. (2019) which included five trials and confirmed the beneficial effect of NS on hs-CRP levels. However, the current study included additionally one crossover study (Mohtashami et al., 2016) and contains subgroup analyses performed by the gender of the participants and the intervention duration. Moreover, a RCT concluded that two different doses of NS seed powder capsules (1 and 2 g/day) for 3 months improved serum inflammatory cytokine levels in adults with asthma (Salem et al., 2017).

Furthermore, animal studies have also confirmed the anti-inflammatory effects of black seed. For instance, Umar et al. (2012) have reported that the oral administration of 5 mg kg^{-1} day^{-1} of TQ for 21 days significantly reduced the levels of pro-inflammatory molecules, namely interleukin-6 (IL-6), TNF-α, and prostaglandin E (PGE), and suppressed nitric oxide (NO) production in Wistar rats with arthritis. However, Datau et al. (2010) revealed that men with central obesity who consumed NS seed powder capsules (3 g/day) for 3 months did not benefit from a decrease in hs-CRP levels. The disagreement between the results of these studies may be related to the differences in the baseline levels of inflammatory markers in patients with asthma versus men diagnosed with central obesity. In our meta-analysis, one study showed a direct relationship between NS consumption and inflammatory markers, unlike other studies (Amin et al., 2015; Darand et al., 2019; Kheirouri et al., 2016; Mahdavi et al., 2016). In this study, the clinical status of the patients with ulcerative colitis (UC) was different, since colonoscopy and tissue biopsy were not used to assess UC severity. Thus, patients with different phases of UC and different baseline inflammation levels may have been enrolled in the study (Higgins & Green, 2011). In addition, in the crossover study (phase 1) which we included in our meta-analysis, hs-CRP increased following the consumption of bread containing black seed. This finding might be related to the reduction of the anti-inflammatory properties of NS by blending it with wheat bran and by cooking it, which could have impacted on the quality and characteristics of NS (Mohtashami et al., 2016). In fact, heating NS seeds at ≥200°C leads to a significant reduction in the TQ content and could alter the properties of NS to inhibit the NF-κB activation pathway (Smukowska-Gorynia et al., 2019).

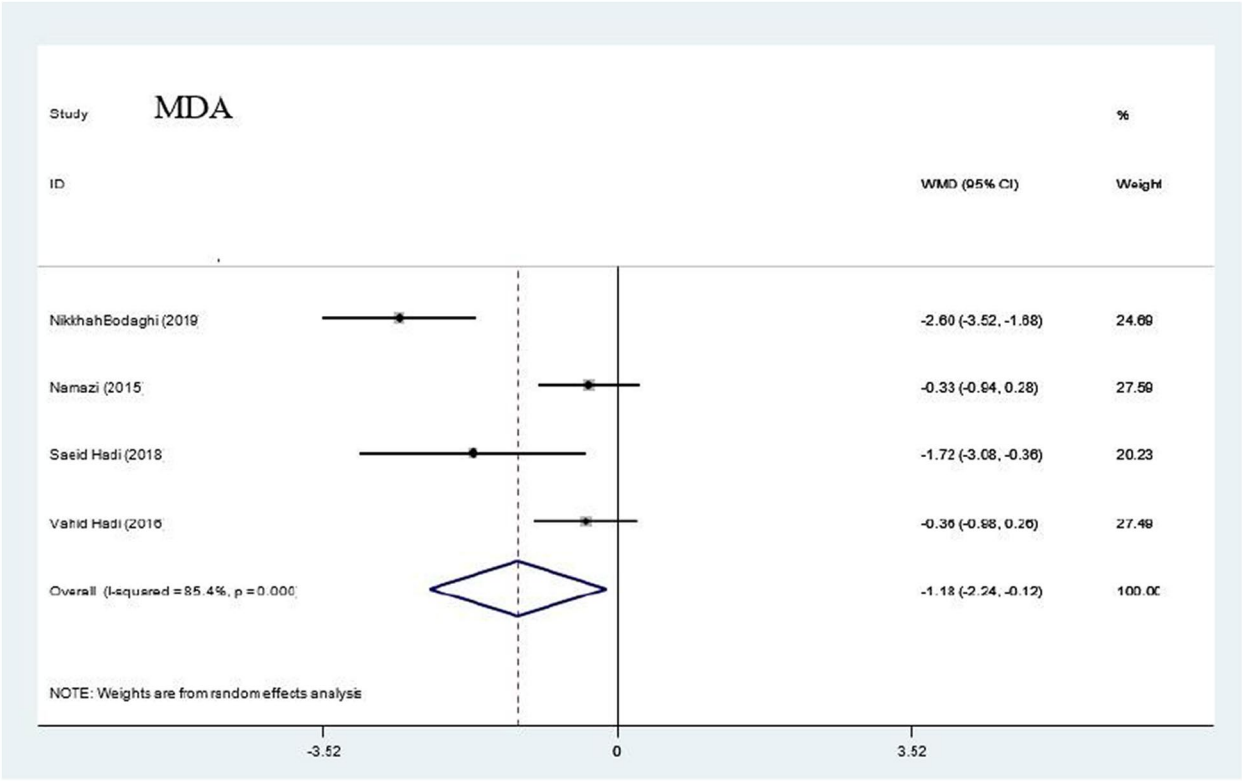

FIGURE 4 Forest plot of the mean difference for the effect of NS consumption on serum MDA levels

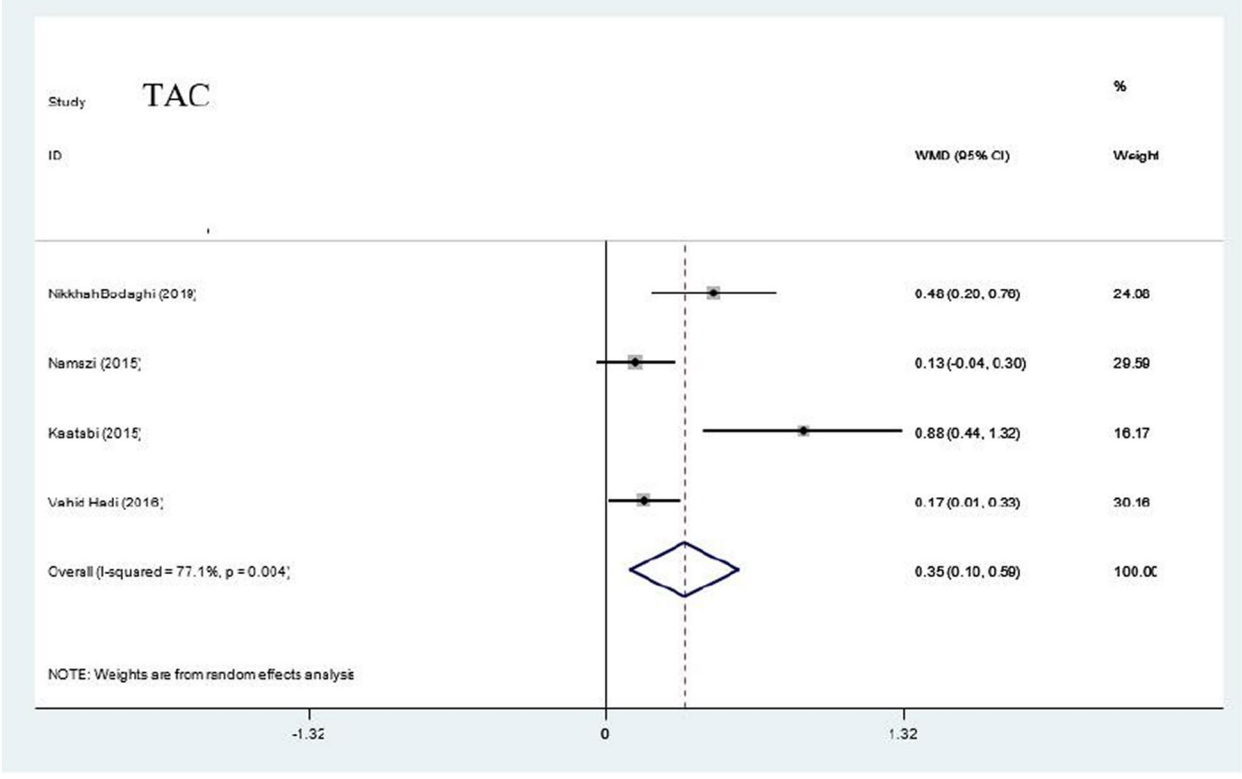

FIGURE 5 Forest plot of the mean difference for the effect of NS consumption on serum TAC levels

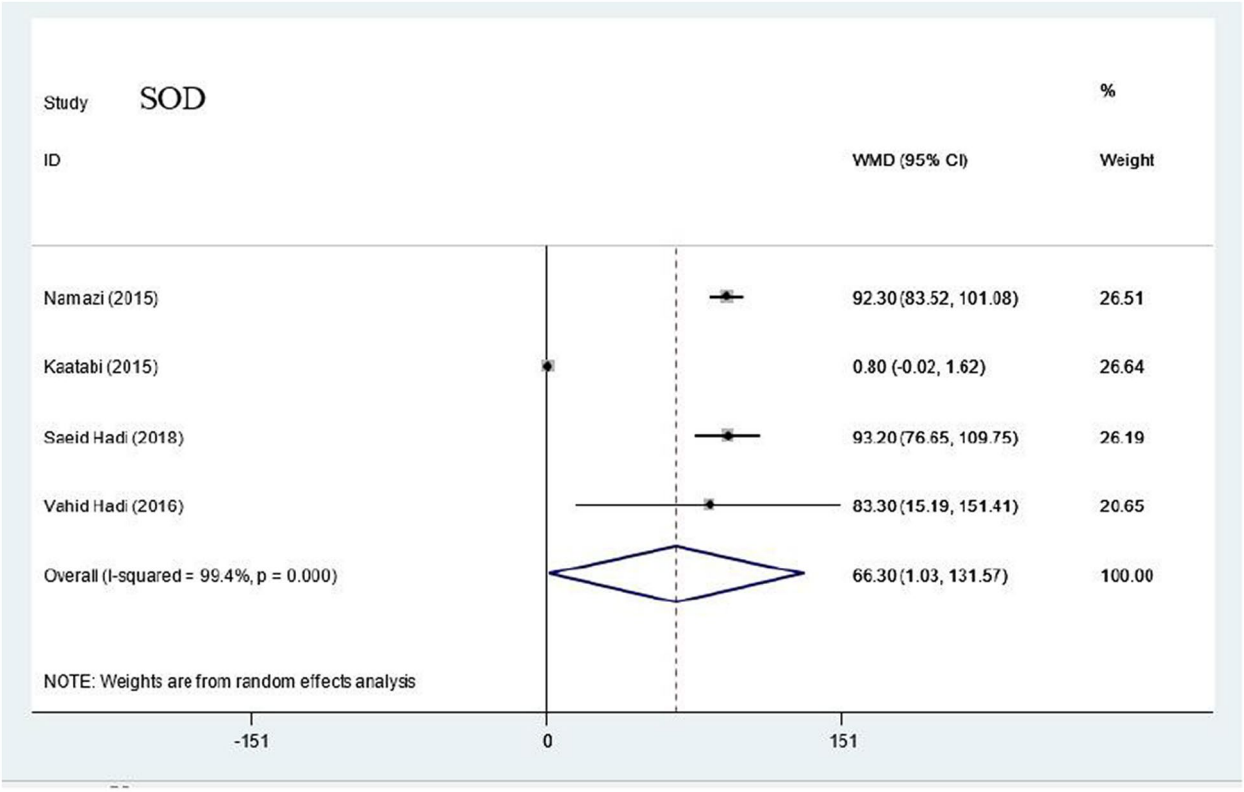

FIGURE 6 Forest plot of the mean difference for the effect of NS consumption on serum SOD levels

Because of the high heterogeneity of the studies, subgroup analyses were performed, but due to the low number of studies in each group, the heterogeneity source could not be well established. However, the subgroup analyses showed that the greatest impact of NS in decreasing TNF-α levels was when the duration of the intervention lasted more than 8 weeks, and when NS oil was employed as an intervention form. Since the main phytochemical component of the volatile NS oil is TQ, which accounts for 28%–45% of the NS oil, the intake of NS oil in longer durations may cause an increased intake of total TQ versus NS powder. The beneficial effects of NS seed oil consumption was also pointed out by prior meta-analyses, which observed that it can reduce triglycerides (TG; Daryabeygi-Khotbehsara et al., 2017), body weight (Namazi et al., 2018), and fasting plasma glucose (FPG) more efficiently than NS seed powder (Askari et al., 2019). The subgroup analyses also revealed that an intervention dose ≤2 g was more effective in decreasing hs-CRP levels. Therefore, this finding should be interpreted with caution because the between-study heterogeneity was considerable and the number of studies was limited.

Several mechanisms explaining the anti-inflammatory effects of NS have been reported. Black seed and TQ can inhibit both pathways (lipoxygenase and cyclooxygenase) of the arachidonate metabolism, which are involved in the production of inflammatory mediators. Moreover, decreasing NO via the inhibition of inducible nitric oxide synthase (iNOS) can reduce the inflammation process (Mahboubi et al., 2018). TQ mediates its inhibitory influence on NO production via a reduction of the iNOS mRNA and protein expression and nitrite production in lipopolysaccharide (LPS)-activated macrophages (El-Mahmoudy et al., 2002). The inhibition of the overproduction of NO, as a free oxygen radical (NO.), may have therapeutic benefits in patients with inflammatory disorders by stopping its harmful effects, including the inhibition of mitochondrial respiration and damaging a variety of mitochondrial components, inhibition of the activity of antioxidant enzymes such as glutathione peroxidase, reducing the levels of some cellular antioxidants such as ascorbic acid, uric acid, and plasma thiols and changing the structure and function of proteins, lipids, carbohydrates, and DNA, that could lead to oxidative damage to tissues (Aktan, 2004). Other possible anti-inflammatory effects are due to the inhibition of the TNF-α-mediated activation of NF-κB via suppressing the activity of the inhibitor of NF-κB kinase (IKK), as well as reducing the transportation of NF-κB (transcription factor aggravating inflammation) from the cytosol to the nucleus (Woo et al., 2012). Thus, these molecular events can suppress the expression of pro-inflammatory mediators, namely including interleukin-1β (IL-1β), TNF-α, matrix metalloproteinase 13, cyclooxygenase-2, and PGE2 (Majdalawieh & Fayyad, 2015).

Our meta-analysis showed that the intake of NS seeds resulted in a significant increase in TAC and SOD levels, and also in a significant decrease of MDA levels (which is as an indicator of lipid peroxidation). Consistent with our results, a recent meta-analysis by Ardiana et al. (2020) showed that NS supplementation was associated with improved SOD levels but had no significant effect on MDA and TAC levels. However, unlike in our paper, that study did not report data on subgroup analyses. Moreover, in a randomized

preliminary study, the twice-daily administration for 3 months of two capsules containing 500 mg of NS in patients with oral submucous fibrosis was accompanied by a significantly increase in SOD levels (Pipalia et al., 2016). On the contrary, Moustafa et al. (2019) reported no significant reduction in serum TAC levels after 3 g/day of NS administration for 3 months in type 2 diabetes mellitus patients. Furthermore, in another study recruiting epileptic children, the administration of 40–80 mg kg^{-1} day^{-1} of black seed did not change TAC and MDA levels significantly as compared to placebo (Shawki et al., 2013). In general, it seems that the differences in study design, study populations, baseline inflammatory markers, NS dosage, duration of intervention, dietary intakes, and physical activity may lead to different outcomes among the current evidence.

We conducted a subgroup analysis for oxidative stress biomarkers, but we were unable to reach a conclusion based on these results since the number of studies evaluating each marker was limited. The antioxidant mechanism of NS can be due in part to its properties to regulate and enhance the expression of antioxidant enzyme genes (SOD, catalase, glutathione peroxidase, and glutathione S-transferase) and restore the activities of nonenzymatic antioxidants, that is, glutathione (GSH) and vitamin C (Kanter et al., 2005; Sheikh & Ahmed, 2012). In addition, some NS compounds can inhibit the production of reactive oxygen species (ROS), including thymol which acts as a singlet oxygen quencher, TQ and dithymoquinone which showed superoxide dismutase (SOD)-like activity (Leong et al., 2013). Moreover, TQ can antagonize the excessive lipid peroxidation and stabilize the integrity of cellular membranes (Abdel-Wahab, 2013). Previous studies did not report any adverse effects of NS consumption in humans in commonly used doses, with NS being regarded as a safe herbal medicine (Amin & Hosseinzadeh, 2016; Mahboubi et al., 2018).

The current meta-analysis has multiple limitations. First, the number of studies assessing other biomarkers of inflammation and oxidative stress, that is IL-6, NF-κB, glutathione peroxidase, and catalase was low. Therefore, we did not analyze other biomarkers of inflammation and oxidative stress in this study. Second, most of the included studies were characterized by short treatment periods (≤8 weeks). Third, the current study included trials with multiple disease conditions, risk factors, and confounding factors. Finally, the subjects enrolled suffered from various disorders: NAFLD, type 2 diabetes mellitus, metabolic syndrome, obesity, UC, and rheumatoid arthritis. Because of the limited number of studies, it is difficult to judge the effects of NS in different diseases. Each of these disorders is characterized by its own pathogenic mechanisms and pathophysiological processes that can ultimately affect inflammation and oxidative stress levels. Thus, this can be regarded as another limitation of the study. However, since inflammation and oxidative stress are key factors in the development and evolution of all of these illnesses, NS decreased inflammatory response and oxidative stress markers, regardless of the type of disease.

5 | CONCLUSION

Overall, the evidence supports the consumption of NS to reduce hs-CRP, TNF-α, and MDA, and increase SOD and TAC levels. Subgroup analysis findings also proposed that lower dosages of NS, longer durations of the intervention, and the use of NS seed oil may result in more effective action on inflammatory markers, but due to the limited number of trials, the results must be analyzed with caution, especially for the subgroup analysis. However, further prospective studies regarding the effect of NS consumption on biomarkers of inflammation and oxidative stress, with larger sample sizes, from various countries and longer follow-up periods, are required to confirm whether NS possesses veritable anti-inflammatory and antioxidant effects.

ACKNOWLEDGMENTS

This study was supported by the Student Research Committee, Faculty of Public Health Branch, Iran University of Medical Sciences, Tehran, Iran (code: IR.IUMS.REC.1398.1257).

CONFLICT OF INTEREST

On behalf of all the authors, the corresponding author states that there are no conflicts of interest associated with this paper.

AUTHOR CONTRIBUTION

Rahele Sadat Montazeri: Resources; Writing-original draft; Writing-review & editing. **Somaye Fatahi:** Formal analysis; Methodology; Supervision; Validation; Writing-original draft; Writing-review & editing. **Mohammadhassan Sohouli:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Writing-original draft; Writing-review & editing. **Ahmed Abu-Zaid:** Supervision; Writing-review & editing. **HO Santos:** Supervision; Writing-review & editing. **Mihnea-Alexandru Găman:** Writing-original draft; Writing-review & editing. **Farzad Shidfar:** Conceptualization; Supervision; Writing-original draft; Writing-review & editing.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

ORCID

Farzad Shidfar https://orcid.org/0000-0002-6531-9253

REFERENCES

Abdel-Wahab, W. M. (2013). Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. *The Journal of Basic & Applied Zoology*, *66*(5), 263–270. https://doi.org/10.1016/j.jobaz.2013.04.002

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A., & Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, *3*(5), 337–352.

Aktan, F. (2004). iNOS-mediated nitric oxide production and its regulation. *Life Sciences*, *75*(6), 639–653. https://doi.org/10.1016/j.lfs.2003.10.042

Amin, B., & Hosseinzadeh, H. (2016). Black cumin (Nigella sativa) and its active constituent, thymoquinone: An overview on the analgesic and anti-inflammatory effects. *Planta Medica*, *82*(01/02), 8–16. https://doi.org/10.1055/s-0035-1557838

Amin, F., Islam, N., Anila, N., & Gilani, A. (2015). Clinical efficacy of the co-administration of Turmeric and Black seeds (Kalongi) in metabolic syndrome–A double blind randomized controlled trial–TAK-MetS trial. *Complementary Therapies in Medicine*, *23*(2), 165–174.

Ardiana, M., Pikir, B., Santoso, A., Hermawan, H., & Al-Farabi, M. J. (2020). Effect of nigella sativa supplementation on oxidative stress and antioxidant parameters: A meta-analysis of randomized controlled trials. *The Scientific World Journal*, 2390706. https://doi.org/10.1155/2020/2390706.

Askari, G., Rouhani, M. H., Ghaedi, E., Ghavami, A., Nouri, M., & Mohammadi, H. (2019). Effect of Nigella sativa (black seed) supplementation on glycemic control: A systematic review and meta-analysis of clinical trials. *Phytotherapy Research*, *33*(5), 1341–1352.

Darand, M., Darabi, Z., Yari, Z., Saadati, S., Hedayati, M., Khoncheh, A. Hosseini-Ahangar, B., Moayyed Alavian, S., & Hekmatdoost, A. (2019). Nigella sativa and inflammatory biomarkers in patients with non-alcoholic fatty liver disease: Results from a randomized, double-blind, placebo-controlled, clinical trial. *Complementary Therapies in Medicine*, *44*, 204–209.

Daryabeygi-Khotbehsara, R., Golzarand, M., Ghaffari, M. P., & Djafarian, K. (2017). Nigella sativa improves glucose homeostasis and serum lipids in type 2 diabetes: A systematic review and meta-analysis. *Complementary Therapies in Medicine*, *35*, 6–13. https://doi.org/10.1016/j.ctim.2017.08.016

Datau, E., Surachmanto, E., Pandelaki, K., & Langi, J. (2010). Efficacy of Nigella sativa on serum free testosterone and metabolic disturbances in central obese male. *Acta Medica Indonesiana*, *42*(3), 130–134.

Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, *315*(7109), 629–634.

El-Mahmoudy, A., Matsuyama, H., Borgan, M., Shimizu, Y., El-Sayed, M., Minamoto, N., & Takewaki, T. (2002). Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. *International Immunopharmacology*, *2*(11), 1603–1611. https://doi.org/10.1016/S1567-5769(02)00139-X

Gholamnezhad, Z., Havakhah, S., & Boskabady, M. H. (2016). Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review. *Journal of Ethnopharmacology*, *190*, 372–386. https://doi.org/10.1016/j.jep.2016.06.061

Hadi, S., Mirmiran, P., Daryabeygi-Khotbesara, R., & Hadi, V. (2018). Effect of Nigella sativa oil extract on inflammatory cytokine response and oxidative stress among people with type 2 diabetes mellitus: A randomized, double-blind, placebo controlled trial. *Progress in Nutrition*. *20*(1-S):127–133.

Hadi, V., Kheirouri, S., Alizadeh, M., Khabbazi, A., & Hosseini, H. (2016). Effects of Nigella sativa oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: A randomized, double-blind, placebo-controlled clinical trial. *Avicenna Journal of Phytomedicine*, *6*(1), 34.

Higgins, J. P., & Green, S. (2011). *Cochrane handbook for systematic reviews of interventions*. John Wiley & Sons.

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative stress and inflammation: What polyphenols can do for us? *Oxidative Medicine and Cellular Longevity*, *2016*. https://doi.org/10.1155/2016/7432797

Jadad, A. R., Moore, R. A., Carroll, D., Jenkinson, C., Reynolds, D. J. M., Gavaghan, D. J., McQuay, H. J. (1996). Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Controlled Clinical Trials*, *17*(1), 1–12.

Kaatabi, H., Bamosa, A. O., Badar, A., Al-Elq, A., Abou-Hozaifa, B., Lebda, F., Al-Khadra, A., & Al-Almaie, S. (2015). Nigella sativa improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: Placebo controlled participant blinded clinical trial. *PLoS One*, *10*(2), e0113486.

Kanter, M., Coskun, O., & Budancamanak, M. (2005). Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World Journal of Gastroenterology*, *11*(42), 6684.

Kheirouri, S., Hadi, V., & Alizadeh, M. (2016). Immunomodulatory effect of Nigella sativa oil on T lymphocytes in patients with rheumatoid arthritis. *Immunological Investigations*, *45*(4), 271–283. https://doi.org/10.3109/08820139.2016.1153649

Leong, X.-F., Rais Mustafa, M., & Jaarin, K. (2013). Nigella sativa and its protective role in oxidative stress and hypertension. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 120732. https://doi.org/10.1155/2013/120732.

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*, *6*(7), e1000100.

Mahboubi, M., Kashani, L. M. T., & Mahboubi, M. (2018). Nigella sativa fixed oil as alternative treatment in management of pain in arthritis rheumatoid. *Phytomedicine*, *46*, 69–77. https://doi.org/10.1016/j.phymed.2018.04.018

Mahdavi, R., Namazi, N., Alizadeh, M., & Farajnia, S. (2016). Nigella sativa oil with a calorie-restricted diet can improve biomarkers of systemic inflammation in obese women: A randomized double-blind, placebo-controlled clinical trial. *Journal of Clinical Lipidology*, *10*(5), 1203–1211. https://doi.org/10.1016/j.jacl.2015.11.019

Majdalawieh, A. F., & Fayyad, M. W. (2015). Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. *International Immunopharmacology*, *28*(1), 295–304. https://doi.org/10.1016/j.intimp.2015.06.023

Mohtashami, A., Mahaki, B., Azadbakht, L., & Entezari, M. H. (2016). Effects of bread with Nigella sativa on lipid profiles, apolipoproteins and inflammatory factor in metabolic syndrome patients. *Clinical Nutrition Research*, *5*(2), 89–95.

Moustafa, H. A. M., El Wakeel, L. M., Halawa, M. R., Sabri, N. A., El-Bahy, A. Z., & Singab, A. N. (2019). Effect of Nigella Sativa oil versus metformin on glycemic control and biochemical parameters of newly diagnosed type 2 diabetes mellitus patients. *Endocrine*, , *65*(2), 286–294. https://doi.org/10.1007/s12020-019-01963-4

Namazi, N., Larijani, B., Ayati, M. H., & Abdollahi, M. (2018). The effects of Nigella sativa L. on obesity: A systematic review and meta-analysis. *Journal of Ethnopharmacology*, *219*, 173–181. https://doi.org/10.1016/j.jep.2018.03.001

Namazi, N., Mahdavi, R., Alizadeh, M., & Farajnia, S. (2015). Oxidative stress responses to Nigella sativa oil concurrent with a low-calorie diet in obese women: A randomized, double-blind controlled clinical trial. *Phytotherapy Research*, *29*(11), 1722–1728.

Nikkhah-Bodaghi, M., Darabi, Z., Agah, S., & Hekmatdoost, A. (2019). The effects of Nigella sativa on quality of life, disease activity index, and some of inflammatory and oxidative stress factors in patients with ulcerative colitis. *Phytotherapy Research*, *33*(4), 1027–1032.

Pipalia, P. R., Annigeri, R. G., & Mehta, R. (2016). Clinicobiochemical evaluation of turmeric with black pepper and Nigella sativa in management of oral submucous fibrosis—a double-blind, randomized preliminary study. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *122*(6), 705–712. https://doi.org/10.1016/j.oooo.2016.07.023

Sahebkar, A., Soranna, D., Liu, X., Thomopoulos, C., Simental-Mendia, L. E., Derosa, G., Maffioli, P., & Parati, G. (2016). A systematic review

and meta-analysis of randomized controlled trials investigating the effects of supplementation with Nigella sativa (black seed) on blood pressure. *Journal of Hypertension*, *34*(11), 2127–2135. https://doi.org/10.1097/HJH.0000000000001049

Salem, A. M., Bamosa, A. O., Qutub, H. O., Gupta, R. K., Badar, A., Elnour, A., Afzal, M. N. (2017). Effect of Nigella sativa supplementation on lung function and inflammatory mediators in partly controlled asthma: A randomized controlled trial. *Annals of Saudi Medicine*, *37*(1), 64–71.

Schneider-Stock, R., Fakhoury, I. H., Zaki, A. M., El-Baba, C. O., & Gali-Muhtasib, H. U. (2014). Thymoquinone: Fifty years of success in the battle against cancer models. *Drug Discovery Today*, *19*(1), 18–30. https://doi.org/10.1016/j.drudis.2013.08.021

Shawki, M., El Wakeel, L., Shatla, R., Gamila, E.-S., Ibrahim, S., & Badary, O. (2013). The clinical outcome of adjuvant therapy with black seed oil on intractable paediatric seizures: A pilot study. *Epileptic Disorders*, *15*(3), 295–301. https://doi.org/10.1684/epd.2013.0602

Sheikh, B. Y., & Ahmed, M. M. (2012). Thymoquinone a potential therapy for cerebral oxidative stress. *Asian J Nat Appl Sci*, *1*, 76–92.

Smukowska-Gorynia, A., Rzymski, P., Marcinkowska, J., Poniedziałek, B., Komosa, A., Cieslewicz, A., Slawek-Szmyt, S., Janus, M., Araszkiewicz, A., Jankiewicz, S., Tomaszewska-Krajniak, I., & Mularek-Kubzdela, T. (2019). Prognostic value of oxidative stress markers in patients with pulmonary arterial or chronic thromboembolic pulmonary hypertension. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–10. https://doi.org/10.1155/2019/3795320

Tavakoly, R., Arab, A., Vallianou, N., Clark, C. C., Hadi, A., Ghaedi, E. et al (2019). The effect of Nigella sativa L. supplementation on serum C-reactive protein: A systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Medicine*, *45*, 149–155.

Umar, S., Zargan, J., Umar, K., Ahmad, S., Katiyar, C. K., & Khan, H. A. (2012). Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *Chemico-Biological Interactions*, *197*(1), 40–46. https://doi.org/10.1016/j.cbi.2012.03.003

Woo, C. C., Kumar, A. P., Sethi, G., & Tan, K. H. B. (2012). Thymoquinone: Potential cure for inflammatory disorders and cancer. *Biochemical Pharmacology*, *83*(4), 443–451. https://doi.org/10.1016/j.bcp.2011.09.029

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Montazeri RS, Fatahi S, Sohouli MH, et al. The effect of nigella sativa on biomarkers of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials. *J Food Biochem*. 2021;00:e13625. https://doi.org/10.1111/jfbc.13625