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The effect of *Nigella sativa L.* supplementation on serum C-reactive protein: A systematic review and meta-analysis of randomized controlled trials

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Abstract

Objective: Evidence on the efficacy of *Nigella sativa* supplementation is equivocal, thus the aim of this systematic review and meta-analysis of randomized clinical trials (RCTs) was to examine the effect of *Nigella sativa* (*N. sativa*) supplementation on plasma C-reactive protein (CRP) concentrations.

Methods: PubMed, Scopus, ISI Web of Science, Cochrane library, and Google Scholar databases were searched (up to April, 2019) to identify RCTs investigating the effects of *N. sativa* seed and seed oil supplementation on CRP. Weighted mean differences (WMD) was pooled using a random-effects model. Standard methods were also used for assessment of heterogeneity, sensitivity analysis, and publication bias.

Results: Eventually only five articles which reported data of interest entered for data analysis. The meta-analysis showed a significant reduction in serum CRP (WMD: -0.55 mg/L, 95% CI: -1.02, -0.08, P=0.02), with significant heterogeneity between selected studies ($I^2=77.3\%$). Between-study heterogeneity disappeared following subgroup analysis, stratified by baseline BMI (≥ 30 kg/m²: $I^2=2.8\%$). However, the effect of *N. sativa* seed and seed oil supplementation on CRP was only significant in studies that were conducted on participants with BMI ≥ 30 kg/m² (WMD: -0.50 mg/L, 95% CI: -0.85, -0.15).

Conclusions: This meta-analysis suggests that *N. sativa* seed and seed oil supplementation can significantly reduce serum CRP level. However, RCTs with a larger sample size and longer follow-up periods should be conducted for future investigations to confirm the veracity of these results.

Key-words: *N. sativa*, C-reactive protein, Meta-analysis, Randomized controlled trial

Introduction

C-reactive protein (CRP) is an acute phase protein produced by liver, and its concentration is notably increased during infections and inflammation¹. CRP is classified as first-line defence molecule against pathogenic organisms, particularly given its affinity for binding to phosphocholine of bacterial and fungal membranes^{1, 2}. It also functions to stimulate phagocytic cells that remove apoptotic and necrotic cells, further contributing to the healing of injured tissues^{2, 3}. Production of CRP from the liver is stimulated by cytokines associated with non-specific tissue injury, including; interleukin-1 β , interleukin-6, and tumor necrosis factor alpha (TNF- α)^{3, 4}. It has been shown that CRP levels in apparently healthy humans may predict future risk for cardiovascular disease (CVD), independent of established risk factors. Moreover, serum CRP has been purported to predict incidence of myocardial infarction, coronary artery disease (CAD), stroke, peripheral arterial disease, and sudden death; concomitant to acting as a mediator of these conditions⁵⁻⁷. Whilst such non-communicable diseases are commonly treated by pharmacotherapies, the concept of complementary medicine is proliferating, with a reputed 80% of the global population utilising complementary therapies^{8, 9}.

Medicinal plants may serve as an adjuvant in the treatment and prevention of various non-communicable diseases as they contain a wide range of bioactive phytochemicals with diverse metabolic effects, and, moreover, have been asserted as safe adjuvant treatments to reduce the progression, morbidity as well as the cost of treatment¹⁰⁻¹². One such example is *Nigella sativa* L. (*N. sativa*), commonly known as black seed, which belongs to the botanical family of *Ranunculaceae*^{13, 14}. *N. sativa* has been traditionally in use in many Middle and Far East countries as a natural remedy for a wide range of illnesses, attributed to its wide range of pharmacological properties¹⁴. Among the active ingredients of *N. sativa*, thymoquinone (TQ)

is one of the most important ingredients, which purportedly contains anti-oxidant, anti-inflammatory and immunoprotective properties¹⁵⁻¹⁷.

Although some evidence exists to support the use of *N. Sativa* seed and seed oil to positively influence CRP^{12, 18-20}, the current evidence base is bereft of uniformity, and given the absence of a unifying and elucidatory analysis, we sought to systematically review and meta-analyse the effect of *N. Sativa* seed and seed oil supplementation on serum CRP in humans.

Methods

The present systematic review was planned, conducted, and reported in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines²¹.

Search strategy

Five databases, including PubMed (<http://www.ncbi.nlm.nih.gov>), Scopus (<http://www.scopus.com>), Cochrane Library (<http://www.cochranelibrary.com>), ISI Web of Science (<http://www.webofscience.com>) and Google Scholar (<http://scholar.google.com>) were explored with no language or time frame restriction up to April, 2019. The search strategy was drawn up using following keywords and medical subject heading (MeSH) terms: “*Nigella sativa*” OR “black seed” OR “kalonji” OR “black cumin” OR “black caraway” combined with “C-Reactive Protein” OR “high-sensitivity CRP” OR “hs-CRP” OR “CRP”. In addition, we manually reviewed reference lists from the retrieved articles, systematic reviews, and meta-analyses to avoid missing any pertinent articles.

Study selection

EndNote software, version X6 (Thomson Reuters) was used for record management. After removing duplicates and publications without an English abstract, the remaining articles were independently screened by two authors (A.H and A.A) based on the title and abstract and

irrelevant records, animal studies and reviews were excluded. Then, the full text of remaining articles was investigated profoundly to choose only related studies. Finally, all randomized controlled trials (RCTs) (either parallel or cross-over designs) that examined the effects of *N. sativa* seed and seed oil supplementation on CRP in adults (age \geq 18-year old) were selected. Studies that prescribed *N. sativa* mixed with other substances, publications with duplicate data, and also trials without an appropriate control group were excluded. Disagreement between reviewers was resolved by discussion or, if it was necessary, by consulting with a third reviewer (E.Gh).

Data extraction

Two investigators (A.H and A.A) abstracted relevant data from the selected trials. This process was verified by another investigator (E.Gh). The following methodological and outcome variables from each included papers were extracted, using a data abstraction form: first author's last name, publication time, country in which the study was performed, research design, participant characteristics (mean age, gender and body mass index [BMI]), sample size, intervention duration (week), type and dose of supplements/placebo used in intervention/control group, and the main results. When results were presented in multiple time points, only data relating to the longest duration of treatment were considered.

Quality assessment

The methodological quality of enrolled studies was assessed using the scoring system developed by Jadad et al.²², where total score ranges 0 to 5 points based on the 5 items: 1) randomization, 2) methods of randomization, 3) blinding, 4) suitable method of double blinding, and 5) withdrawn or drop-outs explanation. The scores of 3 or more represented high quality while 0-2 indicated a low-quality study. This section was also independently

accomplished by two researchers (A.H and A.A). Final scores were discussed by the investigators to make a consensus.

Statistical analysis

The pooled weighted mean difference (WMD) and its 95% confidence interval (CI) were estimated to assess the effects of *N. sativa* on levels of serum CRP. In studies in which the net changes were not directly reported, the WMD was calculated by following formula: (measure at the end of follow-up in the treatment group - measure at baseline in the treatment group) - (measure at the end of follow-up in the control group - measure at baseline in the control group). Also, the standard deviation (SD) of WMD was calculated as follows: [SD=square root $[(SD \text{ pre-treatment})^2 + (SD \text{ post-treatment})^2 - (2R \times SD \text{ pre-treatment} \times SD \text{ post-treatment})]$, assuming a correlation coefficient of 0.5. To ensure that our meta-analysis was not sensitive to the selected correlation coefficient, all the analyses for each parameter was repeated using a correlation coefficient of 0.2 and 0.8. In the case that standard error (SE) of mean was only reported, SD was calculated using the following formula: $SD = SE \times \text{square root } (n)$, where n is the number of participants in each group. All meta-analyses were done using the random effects model which takes the between-study variability into account. The presence of between-study heterogeneity was assessed by the I^2 statistic. Low, moderate and high heterogeneity was ascribed to I^2 values less than 25%, between 25-50% and over 50%, respectively²³. To find the potential sources of between-study heterogeneity, we carried out a pre-planned subgroup analysis based on baseline BMI (≥ 30 or < 30 kg/m²), *N. sativa* dosage (≥ 2 or < 2 mg/day), and intervention form (powder or oil). Heterogeneity between subgroups was evaluated using a fixed-effect model. Sensitivity analysis was also executed in order to evaluate the influence of every single study on the overall effect size. Publication bias was evaluated using visual assessment of funnel plots and Egger's weighted regression tests²⁴. All analyses were carried out using the STATA software (version 11.2, Stata Corp,

College Station, TX, USA) and two-sided p-values < 0.05 were considered statistically significant.

Results

Flow of study selection

Study selection and identification process are depicted in **Figure 1**. A total of 95 publications were identified using the search strategy previously described in the method part, from which 31 were excluded after duplicate deletion. By reviewing the title and abstracts of the remaining articles, 54 publications which were irrelevant to the study objectives were excluded. Subsequently, 10 full-text articles were carefully reviewed for eligibility and 3 clinical trials were excluded because of the following reasons: two studies had interventions using other components in addition to *N. sativa*, and one article included a duplicate population. Finally, 7 eligible trials^{12, 18-20, 25-27} were considered eligible for the systematic review. However, two of the included articles^{25, 26} did not report the data required for meta-analysis. We contacted the corresponding author of studies, twice, but did not receive any response. These 2 studies were therefore excluded, leaving 5 studies^{12, 18-20, 27} for inclusion in the meta-analysis.

Study and participant characteristics

Details characteristics of the included trials are outlined in **Table 1**. In total, 439 participants were enrolled in selected articles, of which 222 individuals allocated to *N. sativa* supplementation group and 217 subjects to the control group. These studies were published between 2010 and 2019 and were carried out in the Iran^{18-20, 26, 27}, Pakistan¹², and Indonesia²⁵. All studies except one²⁶ adopted a parallel study design. The mean age of the participants ranged from 37.02 to 47.48 years old and mean baseline BMI varied from 24.60 to 32.05 kg/m². Only, one out of included studies²⁵ was conducted exclusively in men, two in women

^{18, 19}, and the other trials^{12, 20, 26, 27} were performed in both genders. The follow-up period ranged from 6 to 12 weeks. Daily recommended dosage of *N. sativa*-based natural products varied between 1 and 3 g/day in these studies, including both crushed seeds and seed oil. Included studies were carried out in various populations, including metabolic syndrome^{12, 26}, obese individuals^{19, 25}, rheumatoid arthritis¹⁸, ulcerative colitis²⁷, and non-alcoholic fatty liver disease (NAFLD)²⁰.

Quality assessment

The Jaded checklist showed that all studies except one²⁵ had high methodological quality. The details of quality assessment in individual studies are provided in **Table 2**.

Findings from the systematic review

Based on the present systematic review, 4 trials reported reduced CRP level with *N. sativa* seed and seed oil supplementation^{12, 18-20}, while 2 studies did not find such an effect^{25, 26}. Moreover, a significant increase in the levels of serum CRP was observed following *N. sativa* seed and seed oil supplementation in one of the trials²⁷.

Findings from the meta-analysis

The forest plot displaying the effect of *N. sativa* seed and seed oil supplementation on circulating CRP is demonstrated in **Figure 2**. The pooled estimate from the random-effect model that performed on 5 studies including 175 cases and 173 controls, showed *N. sativa* seed and seed oil supplementation significantly reduced CRP concentrations (WMD: -0.55 mg/L, 95% CI: -1.02, -0.08, P=0.02). As there was a significant heterogeneity (P<0.001, $I^2=77.3\%$), we performed subgroup analysis and found that baseline BMI (≥ 30 kg/m²: P=0.31, $I^2=2.8\%$) could explain between-study heterogeneity. However, the effect of *N. sativa* seed and seed oil supplementation on CRP was only significant in studies that were conducted on participants with BMI ≥ 30 kg/m² (WMD: -0.50 mg/L, 95% CI: -0.85,

-0.15, $I^2=2.8\%$). In another subgroup analysis by intervention forms, *N. sativa* powder subset showed non-significant differences in the mean change of CRP which is in contrast with the overall results. The subgroup analysis by dose of intervention did also not show any significant difference in the mean change of circulating CRP (**Table 3**).

Moreover, the sensitivity analysis demonstrated that with the removal of the study by Mahdavi et al.¹⁹ and Amin et al.¹² the effect of *N. sativa* on CRP became non-significant (WMD: -0.53 mg/L, 95% CI: -1.17, 0.10, P=0.41 and WMD: -0.56 mg/L, 95% CI: -1.23, 0.10, P=0.39, respectively).

Publication bias

Assessment of publication bias by visual inspection of funnel plot indicated no evidence of publication bias in the meta-analysis of *N. sativa* supplementation on circulating CRP concentrations (**Figure 3**). Egger's linear regression test also showed the same result (P=0.77).

Discussion

The present meta-analysis included a total of 439 adults presenting with NAFLD, metabolic syndrome, obesity, ulcerative colitis and rheumatoid arthritis from 7 RCTs. Despite considerable heterogeneity among the studies, our findings indicate improvement in the levels of serum CRP following *N. sativa* seed and seed oil supplementation. To our knowledge, this is the first systematic review that has assessed the effects of *N. sativa* on serum CRP. Significant reductions in serum CRP levels by -0.55 mg/L were observed following *N. sativa* seed and seed oil supplementation with no detectable changes in the control group.

Reductions in the levels of serum CRP, as observed in the present study, are important in the clinical setting, because levels ≥ 3 mg/dL are related to increases in the risk of coronary heart

disease by up to 58%^{28, 29}. CRP, and particularly hs-CRP, is one of the most frequently used biomarkers for assessing the inflammatory status, with predictive values for various chronic diseases including CVD. Since metabolic syndrome is considered a chronic mild inflammatory state, levels of acute-phase reactants are usually elevated among patients with it³⁰⁻³². In this meta-analysis, patients with metabolic syndrome, or the hepatic component of it, i.e. NAFLD, and obesity were mainly included. Therefore, the chronic mild inflammatory state of the above-mentioned metabolic disorders could account for the association between the observed increases in CRP among those patients. On the contrary, when *N. sativa* seed and seed oil was administered, the observed significant decreases in CRP levels could be due to the anti-inflammatory properties of *N. sativa*.

When we categorized studies based on dose, the result was non-significant in both subsets, which may be attributable to the lower number of trials included in each subgroup. In addition, the findings showed that CRP reduction was more pronounced when *N. sativa* was administered as oil, suggesting that form of *N. sativa* is one of the important factors which affect CRP responses. Previous meta-analysis which investigating effect of *N. sativa* seed showed that *N. sativa* seed oil were more effective in lowering low density lipoprotein (LDL) and cholesterol³³ and glycaemic indexes³⁴. Such differences have previously been attributed to the chemical composition and preparation process of *N. sativa* seed oil, as compared with seed powder that is prepared by crushing^{35, 36}. Some potential anti-inflammatory mechanisms of *N. sativa* seed in the most studies have been attributed to antioxidant activity³⁷. TQ and dithymoquinone are the major active antioxidant components of *N. sativa*³⁷. Actually controlled thermal processing of the *N. sativa* seeds, responsible for TQ accumulation at temperatures between 50 and 150°C; on the other hand TQ is fat soluble which can lead to higher biological activity of the TQ in the oil from^{38, 39}.

In subgroup analyses, we also found that *N. sativa* supplementation could reduce CRP levels in participants with baseline BMI \geq 30 kg/m², a result which is not only interesting, but also important to emphasize. As we know, obese subjects have higher CRP levels than other people due to the accumulation of free fatty acid intermediates, which subsequently activate pro-inflammatory serine kinase cascades⁴⁰. Thus, it is logical that bigger changes in CRP concentration were manifest in subjects with baseline BMI \geq 30 kg/m²

The anti-inflammatory properties of *N. sativa* have already been reviewed, and several mechanisms of action have been described. TQ, the major constituent of *N. sativa* or black seed or black cumin, possesses anti-inflammatory properties that prevent the biosynthesis of important mediators in inflammatory processes, such as cyclooxygenase (COX), prostaglandin-2 and leukotrienes^{41, 42}. TQ has been also shown to reduce LPS-induced pro-inflammatory cytokines, such as interleukins and TNF- α ^{43, 44}.

Numerous studies have been reported that *N. sativa* could improve body antioxidant defence through enhancement of antioxidant enzymes activity and reduction of reactive oxygen species^{45, 46}. Furthermore, it has been suggested that *N. sativa* supplementation, by scavenging various free radicals, including hydroxyl radicals, hypochlorous acid and singlet oxygen, suppresses inflammation and oxidative stress^{15, 17}. Besides, considering the positive role of weight loss in improving the CRP level, the beneficial effects of *N. sativa* on the CRP can be attributed to its anti-obesity activity^{47, 48}.

Generally, *N. sativa* is known as a safe herbal medicine and no serious adverse effect was reported among included studies. However, there is antecedent reports that suggest *N. sativa* consumption can result in some unwanted side effects, such as; dyspepsia, nausea, allergic reaction, dermatitis, diarrhoea, abdominal cramping and slight anorexia in high dosages^{33, 49}. Furthermore, *N. sativa* is also known to adversely interact with both chemotherapy

medications and radiation therapy⁵⁰. Since it may act like an antioxidant in the body, this herbal supplement can decrease the efficacy of standard cancer treatments^{50, 51}.

With respect to potential limitations in our study; first, a number of eligible studies in the present meta-analysis were relatively small which may have biased the results to some extent. Second, pathological status of participants was varied, including participants with with NAFLD, metabolic syndrome, obesity, ulcerative colitis and rheumatoid arthritis. Third, a high degree of heterogeneity was detected in both outcomes, and although we have explored the source of heterogeneity, the interpretation of findings may be influenced by the level of heterogeneity observed. Fourth, most of the included studies have been conducted in Iran, which makes it difficult to generalize the results to the rest of the world. Fifth, given the limited studies on each gender, duration of intervention and CRP type, we could not examine the effects of the aforementioned parameters. And finally, trials used various durations and doses with two different forms, although subgroup analyses were assessed the effects of seed oil and powder.

Conclusion

This meta-analysis suggests that *N. sativa* seed and seed oil supplementation in doses of 1g to 3g daily can significantly reduce serum CRP level. This result may be attributable to the widely reputed anti-inflammatory properties of *N. sativa* seed and seed oil. However, RCTs with a larger sample size and a longer follow-up period should be considered in the near future in order to support the veracity these findings.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

None.

Author Contribution

A.H and E.Gh carried out the concept, design, and drafting of this study. A.H, E.Gh and A.A searched databases, screened articles and extracted data. A.H and R.T performed the acquisition, analysis, and interpretation of data. C.C critically revised the manuscript. All authors approved the final version of the manuscript. A.H and E.Gh are the guarantor of this study.

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References

1. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *The Journal of clinical investigation*. 2003;111(12): 1805-1812.
2. Black S, Kushner I, Samols D. C-reactive protein. *Journal of Biological Chemistry*. 2004;279(47): 48487-48490.
3. Volanakis JE. Human C-reactive protein: expression, structure, and function. *Molecular immunology*. 2001;38(2-3): 189-197.
4. Du Clos TW, Mold C. C-reactive protein. *Immunologic research*. 2004;30(3): 261-277.
5. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107(3): 363-369.
6. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? *Hypertension*. 2004;44(1): 6-11.
7. Guijarro C. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*. 2001;104(22): E127.
8. Power M, Pratley R. Alternative and complementary treatments for metabolic syndrome. *Current diabetes reports*. 2011;11(3): 173-178.
9. Debas HT, Laxminarayan R, Straus SE. Complementary and alternative medicine. *Disease control priorities in developing countries*. 2006;2.
10. Anderson JG, Taylor AG. Use of complementary therapies by individuals with or at risk for cardiovascular disease: results of the 2007 National Health Interview Survey. *The Journal of cardiovascular nursing*. 2012;27(2): 96.
11. Mehta D. Integrative medicine and cardiovascular disorders. *Primary Care: Clinics in Office Practice*. 2017;44(2): 351-367.
12. Amin F, Islam N, Anila N, Gilani A. 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*. 2015;23(2): 165-174.
13. Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. *International immunopharmacology*. 2005;5(13-14): 1749-1770.
14. Gali-Muhtasib H, El-Najjar N, Schneider-Stock R. The medicinal potential of black seed (Nigella sativa) and its components. *Advances in Phytomedicine*. 2006;2: 133-153.
15. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytotherapy research*. 2000;14(5): 323-328.
16. Ali B, Blunden G. Pharmacological and toxicological properties of Nigella sativa. *Phytotherapy Research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives*. 2003;17(4): 299-305.
17. Ahmad A, Husain A, Mujeeb M, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pacific journal of tropical biomedicine*. 2013;3(5): 337-352.
18. Kheirouri S, Hadi V, Alizadeh M. Immunomodulatory effect of Nigella sativa oil on T lymphocytes in patients with rheumatoid arthritis. *Immunological investigations*. 2016;45(4): 271-283.
19. Mahdavi R, Namazi N, Alizadeh M, Farajnia S. 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*. 2016;10(5): 1203-1211.
20. Darand M, Darabi Z, Yari Z, et al. 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*. 2019.

21. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine*. 2009;151(4): 264-269.
22. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Controlled clinical trials*. 1996;17(1): 1-12.
23. Higgins JP. Cochrane handbook for systematic reviews of interventions version 5.0. 1. The Cochrane Collaboration. <http://www.cochrane-handbook.org>. 2008.
24. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj*. 1997;315(7109): 629-634.
25. Datau E, Surachmanto E, Pandelaki K, Langi J. Efficacy of *Nigella sativa* on serum free testosterone and metabolic disturbances in central obese male. *Acta Medica Indonesiana*. 2010;42(3): 130-134.
26. Mohtashami A, Mahaki B, Azadbakht L, Entezari MH. Effects of bread with *Nigella sativa* on lipid profiles, apolipoproteins and inflammatory factor in metabolic syndrome patients. *Clinical nutrition research*. 2016;5(2): 89-95.
27. Nikkhah-Bodaghi M, Darabi Z, Agah S, Hekmatdoost A. 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*. 2019.
28. Eisenhardt SU, Habersberger J, Murphy A, et al. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. *Circulation research*. 2009;105(2): 128-137.
29. Vallianou N, Tsang C, Taghizadeh M, Davoodvandi A, Jafarnejad S. Effect of cinnamon (*Cinnamomum Zeylanicum*) supplementation on serum C-reactive protein concentrations: A meta-analysis and systematic review. *Complementary therapies in medicine*. 2018.
30. Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. *Current opinion in immunology*. 2014;29: 23-28.
31. Shrivastava AK, Singh HV, Raizada A, Singh SK. C-reactive protein, inflammation and coronary heart disease. *The Egyptian Heart Journal*. 2015;67(2): 89-97.
32. Seven E, Husemoen LL, Sehested TS, et al. Adipocytokines, C-reactive protein, and cardiovascular disease: a population-based prospective study. *PloS one*. 2015;10(6): e0128987.
33. Sahebkar A, Beccuti G, Simental-Mendía LE, Nobili V, Bo S. *Nigella sativa* (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials. *Pharmacological research*. 2016;106: 37-50.
34. Askari G, Rouhani MH, Ghaedi E, Ghavami A, Nouri M, Mohammadi H. Effect of *Nigella sativa* (black seed) supplementation on glycemic control: A systematic review and meta-analysis of clinical trials. *Phytotherapy Research*. 2019.
35. Paarakh PM. *Nigella sativa* Linn.—A comprehensive review. 2010.
36. Khan MA. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology*. 1999;7(1): 15-35.
37. Fararh KM, Ibrahim AK, Elsonosy YA. Thymoquinone enhances the activities of enzymes related to energy metabolism in peripheral leukocytes of diabetic rats. *Research in veterinary science*. 2010;88(3): 400-404.
38. Agbaria R, Gabarin A, Dahan A, Ben-Shabat S. Anticancer activity of *Nigella sativa* (black seed) and its relationship with the thermal processing and quinone composition of the seed. *Drug design, development and therapy*. 2015;9: 3119.
39. Mahdavi R, Namazi N, Alizadeh M, Farajnia S. Effects of *Nigella sativa* oil with a low-calorie diet on cardiometabolic risk factors in obese women: a randomized controlled clinical trial. *Food & function*. 2015;6(6): 2041-2048.
40. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obesity reviews*. 2013;14(3): 232-244.

41. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. *Journal of pharmacopuncture*. 2017;20(3): 179.
42. Shaterzadeh-Yazdi H, Noorbakhsh M-F, Hayati F, Samarghandian S, Farkhondeh T. Immunomodulatory and Anti-inflammatory Effects of Thymoquinone. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders)*. 2018;18(1): 52-60.
43. Alkharfy KM, Ahmad A, Jan BL, Raish M. Thymoquinone reduces mortality and suppresses early acute inflammatory markers of sepsis in a mouse model. *Biomedicine & Pharmacotherapy*. 2018;98: 801-805.
44. Hadi S, Mirmiran P, Daryabeygi-Khotbesara R, Hadi V. 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*. 2018;20(1-S): 127-133.
45. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ. Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β -cells of streptozotocin-induced diabetic rats. *Journal of diabetes*. 2010;2(4): 256-266.
46. Sultan MT, Butt MS, Karim R, et al. *Nigella sativa* fixed and essential oil supplementation modulates hyperglycemia and allied complications in streptozotocin-induced diabetes mellitus. *Evidence-Based Complementary and Alternative Medicine*. 2014;2014.
47. Namazi N, Larijani B, Ayati MH, Abdollahi M. The effects of *Nigella sativa* L. on obesity: A systematic review and meta-analysis. *Journal of ethnopharmacology*. 2018;219: 173-181.
48. Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-reactive protein: a systematic review. *Archives of internal medicine*. 2007;167(1): 31-39.
49. Sharma N, Ahirwar D, Jhade D, Gupta S. Medicinal and pharmacological potential of *nigella sativa*: a review. *Ethnobotanical Leaflets*. 2009;2009(7): 11.
50. Lamson DW, Brignall M. Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. *Alternative Medicine Review*. 1999;4: 304-329.
51. Bang S-h, Han S-s, Cho J-h, Lee Y-w, Cho C-k, Yoo H-s. Study about herb-drug interaction for cancer patients. *The Journal of Internal Korean Medicine*. 2008;29(4): 887-903.

Legends of figures:

Figure 1. PRISMA flow diagram of study selection process

Figure 2. Forest plot of the effect of *Nigella sativa* supplementation on C-reactive protein

Figure 3. Funnel plots detailing publication bias in the studies selected for analysis

Table 1. Characteristics of included studies

First author (publication year)	Country	Sample size	Gender	Mean age (year)	Mean BMI (kg/m ²)	RCT design (blinding)	Duration (week)	Target population	Intervention (name and daily dose)	Control	Results
Datau et al (2010)	Indonesia	40	Male	38	NR	Parallel (Yes)	12	Obese	1500 mg/day of <i>N. sativa</i> powder	Placebo	hs-CRP ↔
Amin et al (2015)	Pakistan	124	Both	43.33	27.45	Parallel (Yes)	8	Metabolic syndrome	1500 mg/day of <i>N. sativa</i> powder	Placebo	CRP ↓
Mahdavi et al (2016)	Iran	90	Female	40.25	32.05	Parallel (Yes)	8	Obese	3000 mg/day of <i>N. sativa</i> oil	Placebo	hs-CRP ↓
Kheirouri et al (2016)	Iran	43	Female	42.19	25.22	Parallel (Yes)	8	Rheumatoid arthritis	1000 mg/day of <i>N. sativa</i> oil	Placebo	hs-CRP ↓
Mohtashami et al (2016)	Iran	51	Both	47	29.90	Cross-over (Yes)	8	Metabolic syndrome	Wheat bread + 3000 mg/day of <i>N. sativa</i> powder	Wheat bread	hs-CRP ↔
Darand et al (2019)	Iran	43	Both	47.48	31.78	Parallel (Yes)	8	NAFLD	2000 mg/day of <i>N. sativa</i> powder	Placebo	hs-CRP ↓
Nikkhah-Bodaghi et al (2019)	Iran	48	Both	37.02	24.60	Parallel (Yes)	6	Ulcerative colitis	2000 mg/day of <i>N. sativa</i> powder	Placebo	hs-CRP ↑

RCT, randomized controlled trial; BMI, body mass index; NR, not reported; NAFLD, nonalcoholic fatty liver disease; CRP, C-reactive-protein; hs-CRP, high-sensitivity CRP

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
Datau et al (2010)	-	-	+	-	+	2
Amin et al (2015)	+	+	+	+	+	5
Mahdavi et al (2016)	+	+	+	+	+	5
Kheirouri et al (2016)	+	-	+	+	+	4
Mohtashami et al (2016)	+	+	+	+	+	5
Darand et al (2019)	+	+	+	+	+	5
Nikkhah-Bodaghi et al (2019)	+	+	+	+	+	5

Table 3. Result of subgroup analysis of included studies in meta-analysis.

Sub-grouped by	No. of trials	Effect size ¹	95% CI	I ² (%)	P for heterogeneity	P for between subgroup heterogeneity
Baseline BMI						0.88
≥30 kg/m ²	2	-0.50	-0.85, -0.15	2.8	0.31	
<30 kg/m ²	3	-0.64	-1.50, 0.23	87.9	<0.001	
<i>N. sativa</i> dose						0.04
≥2 mg/day	3	-0.26	-0.74, 0.22	61.1	0.07	
<2 mg/day	2	-1.04	-2.16, 0.07	88.0	<0.001	
Intervention type						<0.001
Powder	3	-0.24	-0.65, 0.18	53.0	0.11	
Oil	2	-1.10	-2.11, -0.10	84.1	0.01	

¹Calculated by Random-effects model
 BMI, body mass index

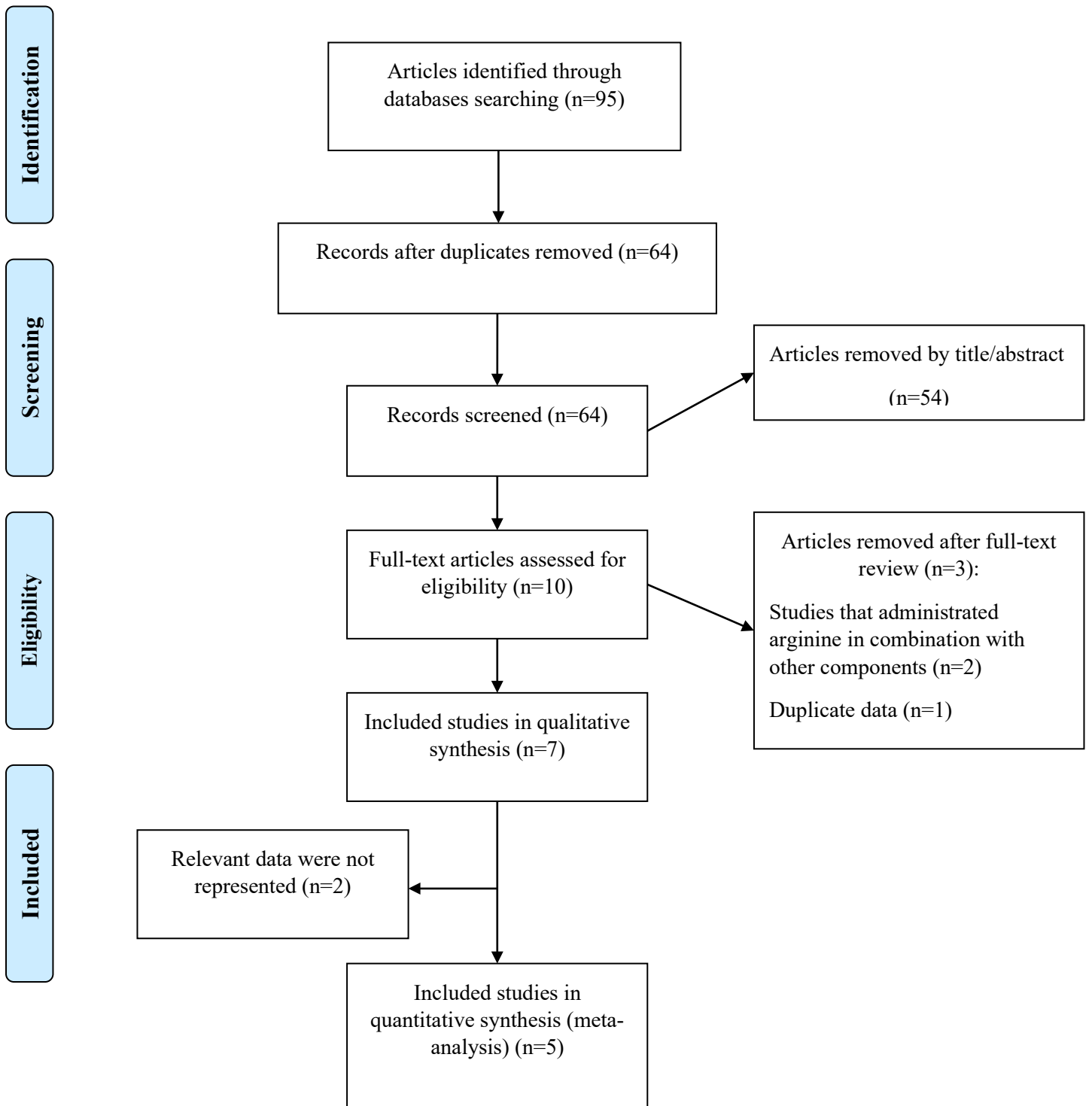


Figure 1

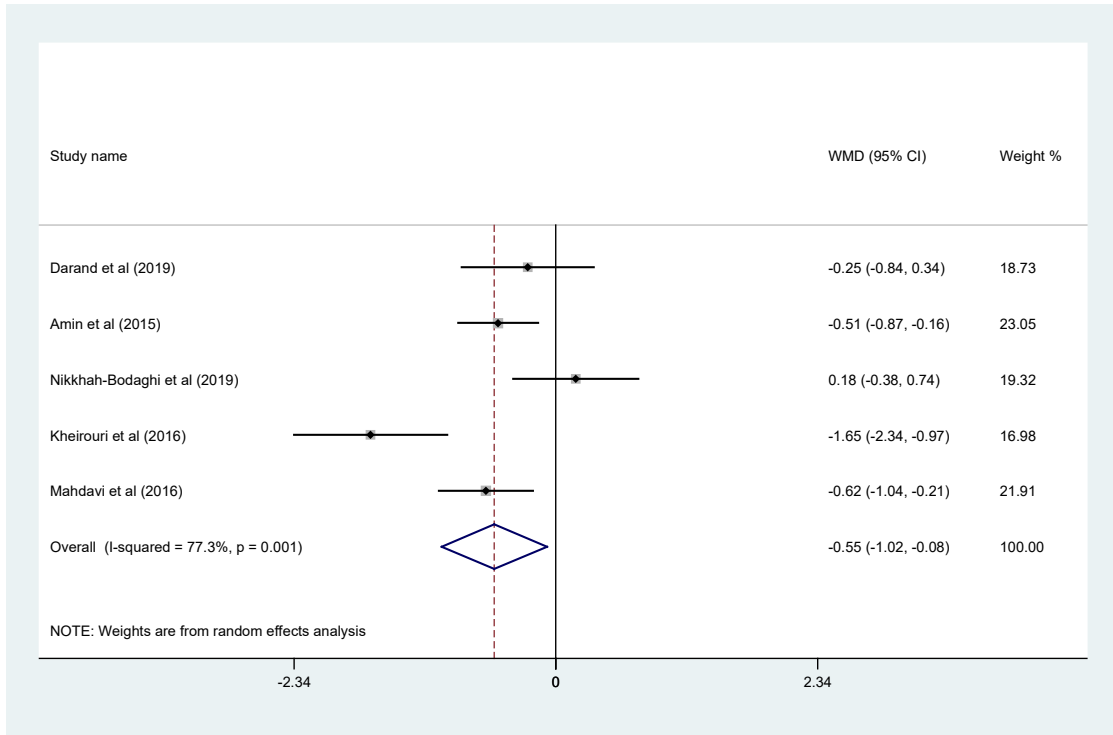


Figure 2

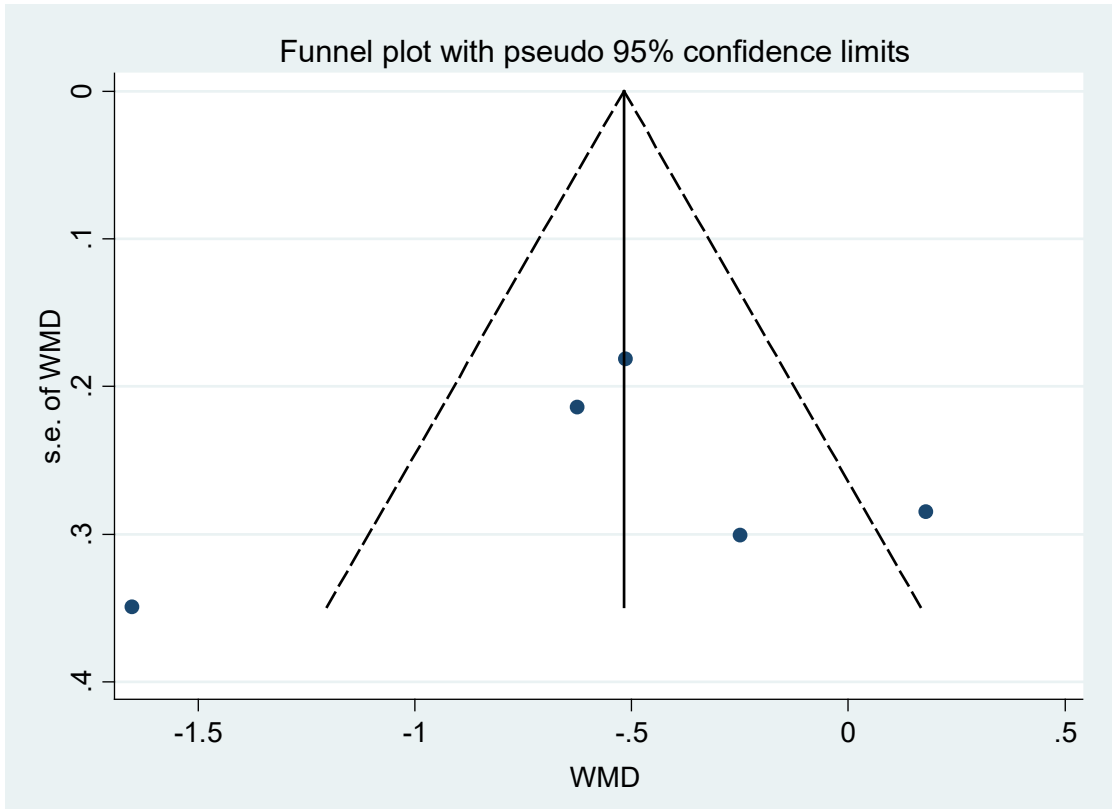


Figure 3