

The Effect of *Nigella sativa* Supplementation on Immune-Inflammatory Parameters: A Systematic Review and Meta-Analysis



Zahrah Hikmah¹, Anang Endaryanto², Syaiful Arifin³, Thoha Muhajir Albaar³, Albert Christianto³

¹Medical Doctoral Program Student, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Dr Soetomo Academic Hospital, Surabaya, Indonesia

³Postgraduate School of Biomedicine, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Abstract—*Nigella sativa* (NS) is a commonly used traditional medicine which has been shown to have anti-inflammatory and immunomodulatory properties especially thymoquinone. However, its supplementation in patients of clinical trials showed conflicting results. **Objectives:** To perform a meta-analysis regarding the effects of NS supplementation on immune-inflammatory parameters such as Th1, Th2, Th17, Treg, CD4, CD8, CD4/CD8 ratio. **Methods:** Relevant articles were systematically searched through PubMed/Medline, SCOPUS, and Google Scholar databases from inception until June 6, 2020. Information related to: author's name and year, study location, target population, sample size, age of participants, number of participants in each group, type and dose of NS supplements, study duration, and the main results were extracted from each study. To determine the effect estimates, data were assessed using fixed or random effect model. **Results:** 15 studies using 509 subjects were enrolled for meta-analysis. Our results found a significant reduction in Th17 cells (SMD -0.58 [-1.08, -0.34], $p=0.02$) following supplementation with NS. There was a significant increase in Treg cells (SMD 0.32 [0.01, 0.63], $p=0.05$) and CD4 (SMD 2.29 [0.27, 4.32], $p=0.03$) following NS supplementation. In addition, there was a significantly higher ratio of CD4/CD8 (SMD 0.65 [0.30, 1.00], $p=0.0003$) following NS supplementation. Effects of NS on Th1 cells (SMD -0.04 [-0.56, 0.47], $p=0.87$), Th2 cells (SMD -0.08 [-0.49, 0.34], $p=0.72$) and CD8 (SMD 1.58 [-0.44, 3.61], $p=0.12$) were insignificant. **Conclusion:** This meta-analysis suggests that NS supplementation in humans may have a beneficial effect on immune-inflammatory parameters especially CD4+ T cells, CD4/CD8 ratio, Treg, and Th17.

Keywords—*Nigella sativa*, Immunomodulator, meta-analysis

1. Introduction

Nigella sativa (NS), commonly known as black seed or black caraway, is a medicinal plant that has been used in Asian, Middle Eastern, Far Eastern, Eastern European and Northern African countries as a traditional medicine (1). NS belongs to the family of *Ranunculaceae* (buttercup), which have been explored with many pharmacological actions such as gastroprotective, renal protective, hepatoprotective, bronchodilator, spasmolytic, anticancer, antidiabetic, analgesic, immunomodulator, antimicrobial, and antioxidant properties (2,3). The active and important constituents of NS are thymoquinone (30%–48%), thymohydroquinone, dithymoquinone (nigellone), p-cymene (7%–15%), carvacrol (6%–12%), 4-terpineol (2%–7%), α -anethole (1%–4%), sesquiterpenelimonene (1%–8%), α -pinene, thymol, and some alkaloids, which have been shown to have a therapeutic effect for a wide range of diseases (4–6).

The severity of several diseases such as autoimmune is mediated by high inflammatory parameters. T cells play many essential roles in the pathogenesis of diseases. Subpopulation of T cell, especially CD4+, CD8+ and T regulatory cells (Treg) was contributed to the pathogenesis, progression, and protection of many

autoimmune diseases (7,8). Dysregulation of immunomodulatory cells, such as imbalances between Th1/Th2 and Th17/regulatory T [Treg] cells was linked to progression of diseases (9). NS is one of the herbs in several studies proven to reduce inflammation and modulate the immune response in several autoimmune diseases (10). Several studies have shown that NS has been proven to have anti-inflammatory and immunomodulatory capabilities by reducing the production of inflammation immune cytokine (11,12). High level of thymoquinone contained in NS can act as a free radical scavenger. NS is even able to increase the production and activity of immunomodulator such as Treg(13,14).

A few systematic reviews and meta-analyses have been assessed the effectiveness of *N.sativa*(2,13,15–18) but none has assessed the immune-inflammatory capabilities of NS supplementation. Given the absence of a unifying and elucidatory analysis, we sought to systematically review and metaanalyse the effects of NS supplementation on immune-inflammatory parameters such as Th1, Th2, Th17, Treg, CD4, CD8, CD4/CD8 ratio. Thus, the finding may help the decision-making process of a physician as to the usage of NS supplementation to improve immune-inflammatory status.

2. Methods

The data in this meta-analysis are reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (19).

2.1 Search Strategy

We performed a comprehensive searching in PubMed/Medline, SCOPUS, and Google Scholar databases to collect the relevant studies published from inception until June 6, 2020. The search strategy was developed using the following keywords and medical subject heading (MeSH) terms: “*Nigella sativa*” OR “black seed” OR “black caraway” OR “thymoquinone” AND “immunomodulator” OR “anti-inflammation” or “anti-inflammatory” AND “clinical trial” OR “clinical” OR “patient.” No restriction was conducted based on language or publication date. To avoid missing any study, the reference lists of all eligible articles, related reviews, and meta-analyses were also reviewed. The references cited by all the selected original research articles and reviews were searched for additional articles that might have been missed. Any document reporting a measure of NS supplementation in clinical trials based on primary data was considered. Studies were excluded if they did not contain primary data, used unreferenced sources. Duplicate data, commentaries, letters, correspondence, and editorials are excluded. Unpublished records such as conference papers, theses, and patents were not included in this meta-analysis.

2.2 Eligibility Criteria

This systematic review and meta-analysis were conducted according to the PRISMA guidelines. Studies included in the meta-analysis had to satisfy the following criteria: (i) the study was a clinical observation in humans; (ii) the study reported parameters before and after the intervention in both placebo and treatment groups; (iii) no language or time frame restriction; and (iv) the study included baseline information regarding inflammation indicators, such as CD4, CD8, CD4/CD8 ratio, Th1 (CD4+ IFN- γ +), Th2 (CD4+ IL-4+), Th17(CD4+ IL17+), Treg (CD4+ CD25+). All articles of any design (randomized controlled trials, non-randomized controlled trials, case-control studies, and cross-sectional studies) were included except for narrative review, comment, opinion piece, or methodological report.

2.3 Exclusion Criteria

The exclusion criteria of this meta-analysis were as follows: (1) being non-clinical trials studies; (2) conducted on animals; (3) not being placebo-controlled trials; (4) lack of sufficient data for the outcomes of interest in NS or control group.

2.4 Data Extraction

Two independent reviewers (T.M and A.C) abstracted relevant data from the selected trials. This process was verified by another independent reviewer (S.A). The following data were extracted from the included studies by using standardized protocol: first author's name; year of publication; study location; target population; sample size; age of participants; number of participants in each groups; type and dose of NS supplements; study duration; and the main results.

2.5 Quality Assessment of Studies

The methodological quality of enrolled studies was assessed using the scoring system developed by Jadad et al.(20), where total score ranges 0–5 points based on the 5 items: 1) randomization, 2) appropriate method for randomization, 3) blinding, 4) appropriate method for blinding, and 5) description of dropouts and withdrawals. 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 (T.M and A.C). Each paper's quality was independently assessed by two experts and disagreements were resolved through consensus.

2.6 Statistical Analysis

NS supplementation and control group will be pooled and evaluated for the mean differences and standard deviations (SDs) of the following outcomes: (i) Th1, (ii) Th2, (iii) Th17, (iv) Treg, (v) CD4, (vi) CD8, (vii) CD4/CD8 ratio. The fixed-effect model is used to estimate the overall effect size for homogeneous data. The random-effect model is used to estimate the overall effect size for heterogeneous data. Heterogeneity was examined using Cochrane's Q test (significance point at $p < 0.05$). The risk of bias in reporting the cumulative incidence was independently calculated by the authors. The publication bias of each study was assessed through Egger's test. A p value < 0.05 in Egger's test was suggestive of publication bias. All statistical analyses were done using Review Manager (RevMan) 5.4 (Computer program, The Cochrane Collaboration, London, UK) and Comprehensive Meta-Analysis (CMA) 3.3 (Computer program, New Jersey, USA). $p < 0.05$ was considered as statistically significant.

2.7 Ethical Approval

This meta-analysis did not require ethical approval since all of the data retrieved for the studies were already available in the public domain.

3. Results

3.1 Study Selection

A total of 146 publications were identified using the search strategy previously described in the method part. After excluding duplicated papers, 98 publications were admitted to the screening step. By reviewing the title and abstracts of the remaining articles, 78 publications which were irrelevant to the study objectives were excluded. Subsequently, 20 full-text articles were carefully reviewed for eligibility and 5 clinical trials were excluded because of the following reasons: four studies had no relevant outcome reported, and one article included a duplicate data. Finally, 15 studies were considered eligible for the meta-analysis. The PRISMA flow diagram (21) for the study selection process presented in Figure 1.

3.2 Study Characteristics

Out of selected papers, a total of 509 patients with age ranged between 4 to 50 years old were included in our investigation. Among 15 articles included in analysis, 11 were performed in Indonesia (22–31), 2 in Turkey (32,33), 1 in Iran (14), and 1 in Egypt (34). All studies involved in this research were considered to

have high quality (Jadad score ≥ 3), except four studies (29,32–34). Most of the study participants were children (22–31), only three studies used adult population (14,32,33). Patients with asthma (22–31), beta thalassemia (34), allergic rhinitis (33), rheumatoid arthritis (14), and healthy volunteer (32) participated in these studies. *Nigella sativa* form and dosage varied between 15mg/kgBW/day and 2 g / day in these studies, including either capsule or powder form taken orally. Characteristics of studies entered into meta-analysis are presented in Table 1.

3.3 Effect of *Nigella sativa* on Th1, Th2, Th17, and Treg

The effect of *Nigella sativa* supplementation was shown in standardized mean difference (SMD) between *Nigella sativa* and control group from several studies. Analysis from two studies showed that *Nigella sativa* supplementation did not significantly reduce Th1 cells compared to control (SMD -0.04 [-0.56, 0.47], $p = 0.87$). Similar result was shown in Th2 cells from the analysis of three studies (SMD -0.08 [-0.49, 0.34], $p = 0.72$).

A significant reduction was found in Th17 cells following supplementation with *Nigella sativa* (SMD -0.58 [-1.08, -0.34], $p = 0.02$) compared to control group from the analysis of three studies. Marginally significant increased Treg cells was found from the analysis of four studies in favour of *Nigella sativa* supplementation compared to control group (SMD 0.32 [0.01, 0.63], $p = 0.05$) (figure 2).

3.4 Effect of *Nigella sativa* on CD4, CD8, and CD4/CD8 ratio

Nigella sativa supplementation showed significantly increased CD4 compared to control (SMD 2.29 [0.27, 4.32], $p = 0.03$) from the analysis of four studies, but failed to show significant increase of CD8 compared to control group from the analysis of four studies (SMD 1.58 [-0.44, 3.61], $p = 0.12$). The ratio of CD4/CD8 was analyzed from three studies. The result showed the significantly higher ratio in *Nigella sativa* supplementation compared to control group (SMD 0.65 [0.30, 1.00], $p = 0.0003$) (figure 3).

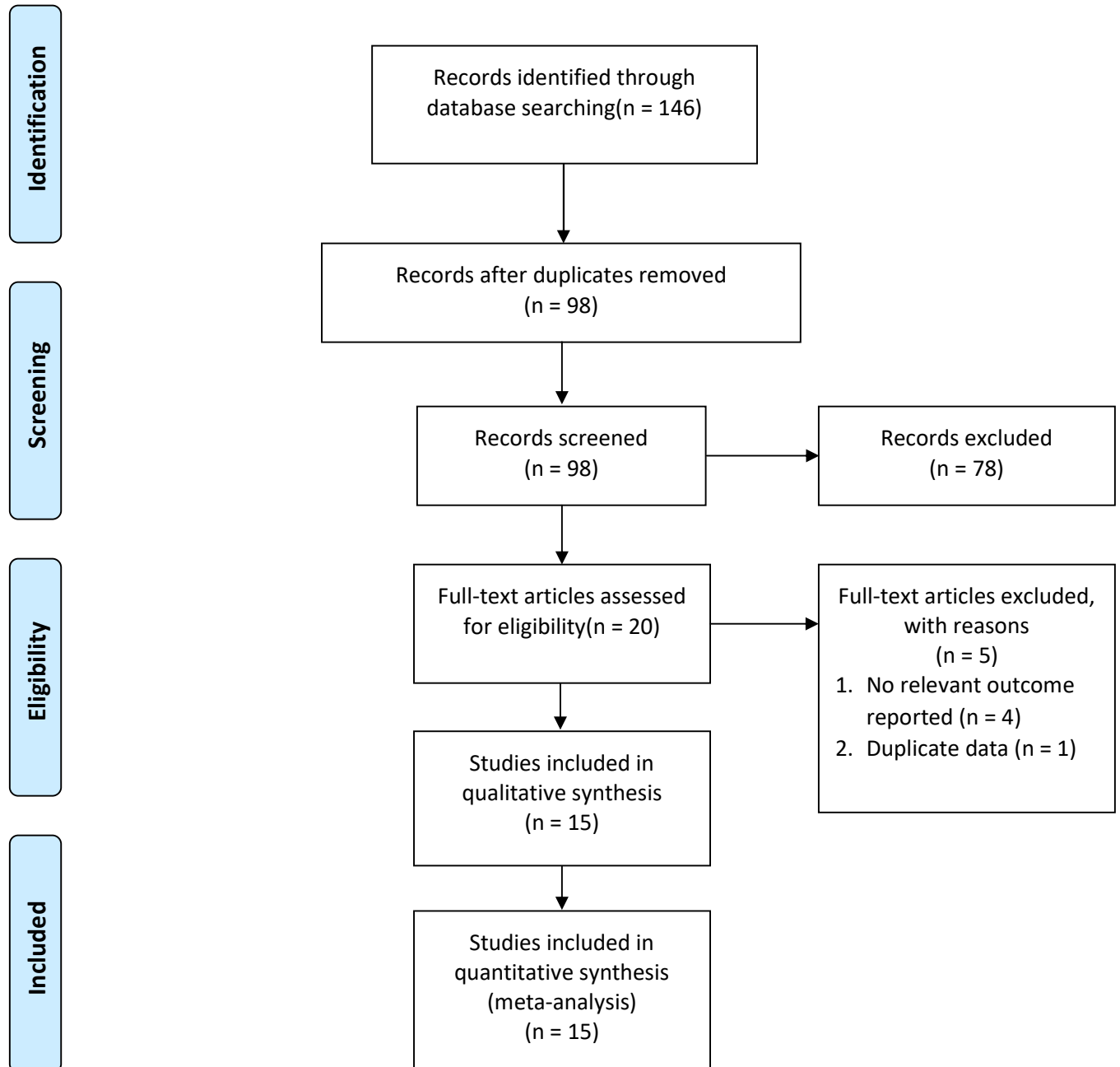


Figure 1. PRISMA Flow Diagram for the included studies.

Table 1. Characteristic of the included studies

First Author and Year	Sample Size	Country	Target Population	Age (years)	NS Form and daily dose	Duration	Outcome
Barlianto, 2017 (24)	28	Indonesia	Asthma in children	8.75	15-30mg/kgBW/day oil capsule	8 weeks	Th1, Th2
Barlianto, 2018 (23)	28	Indonesia	Asthma in children	8.75	15-30mg/kgBW/day oil capsule	8 weeks	Th17, Treg
El-Shanshory, 2018 (34)	50	Egypt	Beta Thalassemia in children	6 - 18	2g/day powder	3 months	CD4, CD8
Fattory, 2015 (25)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day capsule	56 weeks	CD4/CD8 ratio
Isik, 2010 (33)	24	Turkey	Allergic rhinitis	34	2g/day orally	30 days	CD4, CD8
Kardani, 2013 (27)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day powder or capsule	14 weeks	Th17
Kartini, 2014 (28)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day powder or capsule	56 weeks	Th2
Kaya, 2003 (32)	60	Turkey	Healthy Volunteer	24	30mg/kgBW/day orally	4 weeks	CD4, CD8, CD4/CD8 ratio
Kheirouni, 2016 (14)	43	Iran	Rheumatoid Arthritis	42.26	1000mg/day oil capsule	8 weeks	CD4, CD8, CD4/CD8 ratio
Muhyi, 2015 (26)	31	Indonesia	asthma in children	4 - 14	15mg/kgBW/day	56 weeks	Th17
Olivianto, 2013 (29)	28	Indonesia	asthma in children	Not mentioned	Not mentioned	14 weeks	Treg
Ratih, 2015 (30)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day capsule	56 weeks	Th1
Sugiono., 2013 (35)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day powder or capsule	14 weeks	Th2
Sumantri, 2015 (31)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day capsule	56 weeks	Treg
Susanti, 2013 (22)	31	Indonesia	Asthma in children	4 - 14	15mg/kgBW/day capsule	14 weeks	Treg

KgBW, kilogram body weight; NS, *Nigella sativa*; Th1, T helper 1; Th2, T helper 2; Th17, T helper 17; Treg, T regulator.

3.5 Publication bias

Potential publication bias was assessed via Eggers' regression symmetry test. No significant publication bias were found on Th17 ($p=0.45$), Th2 ($p=0.18$), CD4 ($p=0.29$), CD8 ($p=0.3$), and CD4/CD8 ratio (0.63). However, there was a significant publication bias on Treg ($p=0.004$)

a								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Barlianto 2017	20.577	13.273	14	19.334	7.328	14	47.6%	0.11 [-0.63, 0.85]
Ratih 2015	11.999	9.511	15	13.813	9.536	16	52.4%	-0.19 [-0.89, 0.52]
Total (95% CI)			29			30	100.0%	-0.04 [-0.56, 0.47]

b								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Barlianto 2017	21.875	12.871	14	18.478	11.729	14	31.1%	0.27 [-0.48, 1.01]
Kartini 2014	15.961	8.941	15	19.135	11.875	16	34.3%	-0.29 [-1.00, 0.42]
Sugiono 2013	0.4	0.242	16	0.445	0.282	15	34.6%	-0.17 [-0.87, 0.54]
Total (95% CI)			45			45	100.0%	-0.08 [-0.49, 0.34]

c								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Barlianto 2018	20.64	7.03	14	24.79	12.01	14	32.7%	-0.41 [-1.16, 0.34]
Kardani 2013	0.355	0.234	16	0.422	0.258	15	35.5%	-0.27 [-0.97, 0.44]
Muhyi 2015	6.653	5.916	15	12.674	4.746	16	31.8%	-1.10 [-1.86, -0.33]
Total (95% CI)			45			45	100.0%	-0.58 [-1.08, -0.08]

d								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Barlianto 2018	21.24	9.71	14	21.35	6.31	14	17.9%	-0.01 [-0.75, 0.73]
Kheirouni 2016	30.69	8.52	23	23.69	7.91	20	25.0%	0.83 [0.21, 1.46]
Olivianto 2013	0.084	0.054	14	0.0805	0.054	14	17.9%	0.06 [-0.68, 0.80]
Sumantri 2015	13.145	8.658	15	11.607	8.395	16	19.7%	0.18 [-0.53, 0.88]
Susanti 2013	0.13	0.083	16	0.105	0.054	15	19.5%	0.35 [-0.37, 1.06]

Figure 2. The effect of *Nigella sativa* on (a) Th1, (b) Th2, (c) Th17, and (d) Treg. Comparison between *Nigella sativa* and control group.

4. Discussion

The pathogenesis of allergic and autoimmune disease are associated with an underlying chronic inflammatory process determined by a balance of pro-inflammatory and anti-inflammatory mediators, Many studies of *Nigella Sativa*(NS) have revealed the positive effects on clinical outcomes, cytokines, and balancing immune cells (36,37). Previous studies reported a favorable effect of NS on immune response and pro and anti-inflammatory cytokines (36). Immune response include items on CD4, CD8,Th1, Th2, Th17 and Treg. In present study, there was no significant differences in the number of Th1 and Th2 cells after supplementation of NS. The studies that analyses Th1 and Th2 all were in asthmatic patients. In asthma, T helper type 2 (Th2) cells are functionally upregulated, while Th1 cells are inhibited, which enables Th2 cytokines to promote inflammation (38). An in vivo study reported that an aqueous extract of NSincreased IFN-g and decreased IL-4 cytokines (39). Majdawaliehet *al* showed that NS reduces the secretion of Th2 cytokines by splenocytes(38). Other study described that NS supplementation has no significant effect in Th1 and Th2 cytokines secreted from splenic mononuclear cells (40). Clinical study from Barlianto*et al* showed insignificantly increased of Th1 and the ratio of Th1 / Th2 (24). The inconsistent results most likely due to different condition including dose, mode of administration. Clinicalstudies should be done more with more participants to shed more light on the immunomodulatory effects of NS on Th1 and Th2.

a								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
El-Shanshori 2018	2,007.64	90.34	25	1,319.88	74.56	25	22.2%	8.17 [6.41, 9.93]
Isik 2010	40.91	7.14	12	43.71	7.8	12	25.6%	-0.36 [-1.17, 0.45]
Kaya 2003	41.1	1.1	30	39	1.5	30	26.1%	1.58 [0.99, 2.16]
Kheirouni 2016	54.04	8.99	23	49.13	6.43	20	26.1%	0.61 [-0.00, 1.22]
Total (95% CI)			68	66			100.0%	0.65 [0.30, 1.00]

b								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
El-Shanshori 2018	1,145.31	77.58	25	727.09	42.81	25	23.2%	6.57 [5.12, 8.02]
Isik 2010	30.55	5.6	12	31	6.87	12	25.3%	-0.07 [-0.87, 0.73]
Kaya 2003	29.9	1.4	30	28.5	1.3	30	25.8%	1.02 [0.48, 1.56]
Kheirouni 2016	25.26	7.66	23	29.87	4.12	20	25.7%	-0.72 [-1.34, -0.10]
Total (95% CI)			68	66			100.0%	0.65 [0.30, 1.00]

c								
Study or Subgroup	NS Group			Control Group			Weight	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Fattory 2015	1.288	0.755	15	0.935	0.214	16	23.3%	0.63 [-0.10, 1.35]
Kaya 2003	1.5	0.1	30	1.45	0.1	30	46.2%	0.49 [-0.02, 1.01]
Kheirouni 2016	2.43	1.01	23	1.7	0.41	20	30.6%	0.91 [0.27, 1.54]
Total (95% CI)			68	66			100.0%	0.65 [0.30, 1.00]

Figure 3. The effect of *Nigella sativa* on (a) CD4, (b) CD8, and (c) CD4/CD8 ratio. Comparison between *Nigella sativa* and control group

There are also Treg and Th17, CD4 and CD8 as an immune response pathway. IL-4, Th17 indicates increased inflammation while IFN- γ and Treg has the opposite effect (9,41). Administration of NS to patients with allergic autoimmune disease was associated with a reduction in Th17 (23,26,27), an indicator of the inflammatory reaction underlying pathogenesis of autoimmune disease(9). Recent animal and human evidences suggest that T cells have a role in controlling the development of autoimmune diseases, such as RA (42,43). In this meta-analysis, the effect of NS supplementation on Th17 was significant, suggesting that the effect of NS is beneficial for reducing proinflammatory pathway activity.

Interestingly, this meta-analysis study showed that NS increased CD4+ T cells. This effect Strong evidence from animal studies indicated that CD4+ T cells may have a protective effect in autoimmune diseases. El-shanshoryet al(34) showed that NS enhanced cell-mediated immunity where CD4+ T cells increased in beta-thalassemia major patients. Similar results was also found in RA patients after NS oil supplementation, with a marked negative association between CD4+ and the changes in diseases activity (14). Other study showed that NS treatment could result in increased numbers of CD4+ cells in splenocytes of cytomegalovirus infected mice (44). However, there is no other report to address the relationship between CD4+ T cells and disease activity in the patients, despite many reports indicating a disease inducer role for CD4+ T cells (35).

Studies in human indicate that CD8+ T cells play an important role in the establishment of germinal centers, observed in nearly 50% of autoimmune patients, rheumatoid arthritis, which may have a decisive role in the initiation and maintenance of the disease process. In a later study, the same authors had fractionated NS proteins by ion exchange chromatography and showed that some proteins have suppressive and other stimulatory properties in lymphocyte cultures (45). It may be speculated that an important number of cytokines induced by NS produce immunomodulatory changes. Kheirouri et al (14) showed that NS oil

supplementation resulted in significant augmentation of Treg cell percentage in autoimmune RA patients in comparison to the placebo group or baseline. More interestingly, augmentation of CD4+ CD25+ Treg cell percentage was positively correlated to CD8+ T cell percentage. Even though previous study indicated that CD4+ CD25+ regulatory T cells limited CD8+ T-cell effector differentiation (46). Other study have demonstrated that CD8+ responses to immune stimulation suppressed by CD4+ CD25+ Treg cells during both acute and chronic, asymptomatic phase of HIV infection (47). CD4+ CD25+ T cells, upon isolation and polyclonal T cell stimulation, were anergic and capable of inhibiting CD8+ T cell responses (48). Altogether, the results from the current study and previous findings indicated that increases in the number or activity of CD4+ CD25+ T cells was leading to suppressed CD8+ T cell responses or quantity and subsequent alleviation of disease activity. Interestingly, a significant positive correlation was observed between changes in CD4+ CD25+ T cells and changes in the CD4+ /CD8+ ratio (14). This may be due to the negative association between CD4+ CD25+ and CD8+. Therefore, the existences of consistency between the two findings reinforce and strengthen the findings.

5. Conclusion

Our findings suggest that NS seed supplementation may have beneficial effect on immune-inflammatory parameters especially CD4+ T cells, CD4/CD8 ratio, Treg, and Th17. To the best of our knowledge, this is the first meta-analysis that evaluates the effect of NS supplementation on immune mediator parameters. However, some limitations existed in this meta-analysis. Several factors that cannot be standardized between studies may greatly affect the meta-analysis results. These factors include non-standardized NS formulation since there is a lack of regulation for nutraceutical products, in addition to the difference in dose, disease type and severity, and supplementation duration between research studies. Therefore, further clinical trials with similar intervention period, similar type of disease and severity, more subjects, and standardized NS preparation are needed.

6. Acknowledgment

The authors thank to Faculty of Medicine, Universitas Airlangga, Dr Soetomo Academic Hospital, and Faculty of Medicine, Brawijaya University, Indonesia for supporting this research.

7. References

- [1] Golkar P, Nourbakhsh V. Analysis of genetic diversity and population structure in *Nigella sativa* L. using agronomic traits and molecular markers (SRAP and SCoT). *Ind Crops Prod.* 2019;
- [2] Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pac J Trop Biomed.* 2013;
- [3] Islam MT, Khan MR, Mishra SK. An updated literature-based review: phytochemistry, pharmacology and therapeutic promises of *Nigella sativa* L. *Oriental Pharmacy and Experimental Medicine.* 2019.
- [4] Srinivasan K. Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: Traditional uses, chemical constituents, and nutraceutical effects. *Food Qual Saf.* 2018;
- [5] 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.
- [6] Samarghandian S, Farkhondeh T, Samini F. A Review on Possible Therapeutic Effect of *Nigella sativa* and Thymoquinone in Neurodegenerative Diseases. *CNS Neurol Disord - Drug Targets.* 2018;

- [7] Petrelli A, Van Wijk F. CD8+ T cells in human autoimmune arthritis: The unusual suspects. *Nature Reviews Rheumatology*. 2016.
- [8] Skapenko A, Leipe J, Lipsky PE, Schulze-Koops H. The role of the T cell in autoimmune inflammation. *Arthritis Research and Therapy*. 2005.
- [9] Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmunity Reviews*. 2014.
- [10] Hussain DA, Hussain MM. Nigella sativa (black seed) is an effective herbal remedy for every disease except death – a Prophetic statement which modern scientists confirm unanimously: A review. *Adv Med Plant Res*. 2016;Vol.4(2)(April):27–57.
- [11] Guergouri FZ, Benboubetra M, Sobhi W. Antioxidant activity of Algerian Nigella sativa total oil and its unsaponifiable fraction. *J Phytopharm*. 2017;
- [12] Singh Z, Karthigesu IP, Singh P, Kaur R. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: A review. *Iranian Journal of Public Health*. 2014.
- [13] Majdalawieh AF, Fayyad MW. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. *International Immunopharmacology*. 2015.
- [14] Kheirouri S, Hadi V, Alizadeh M. Immunomodulatory Effect of Nigella sativa Oil on T Lymphocytes in Patients with Rheumatoid Arthritis. *Immunol Invest*. 2016;
- [15] Daryabeygi-Khotbehsara R, Golzarand M, Ghaffari MP, Djafarian K. Nigella sativa improves glucose homeostasis and serum lipids in type 2 diabetes: A systematic review and meta-analysis. *Complementary Therapies in Medicine*. 2017.
- [16] Hallajzadeh J, Milajerdi A, Mobini M, Amirani E, Azizi S, Nikkhah E, et al. Effects of Nigella sativa on glycemic control, lipid profiles, and biomarkers of inflammatory and oxidative stress: A systematic review and meta-analysis of randomized controlled clinical trials. *Phytotherapy Research*. 2020.
- [17] Sahebkar A, Soranna D, Liu X, Thomopoulos C, Simental-Mendia LE, Derosa G, et al. 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*. 2016.
- [18] Sahebkar A, Beccuti G, Simental-Mendia 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.
- [19] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*. 2009.
- [20] Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Control Clin Trials*. 1996;

- [21] Moher D, Liberati A, Tetzlaff J AD. PRISMA 2009 Flow Diagram. The PRISMA statement. 2009.
- [22] Susanti N, Barlianto W, Kalim H, Kusuma H. Asthma clinical improvement and reduction in the number of CD4 CD25 foxp3 Treg and CD4 IL-10 cells after administration of immunotherapy house dust mite and adjuvant probiotics and/or Nigella Sativa powder in mild asthmatic children. *IOSR J Dent Med Sci.* 2013;(7):50–9.
- [23] Barlianto W, Wulandari D, Chusniyah M, Chandra Kusuma HMS, Prawiro SR. Improvement of th17/treg balance and asthma control test score by nigella sativa supplementation in asthmatic children: A new approach to managing asthma. *Turkish J Immunol.* 2018;
- [24] Barlianto W, Rachmawati M, Irawan M, Wulandari D. Effects of Nigella sativa oil on Th1/Th2, cytokine balance, and improvement of asthma control in children. *PaediatrIndones.* 2017;57(5):223–8.
- [25] Fattory H, Tri Endharti A, Barlianto W, Olivianto E, Chandra Kusuma H. EfekImunoterapi, Probiotik, Nigella Sativa terhadapRasio CD4+/CD8+, Kadar Immunoglobulin E, danSkoringAsma. *J KedoktBrawijaya.* 2015;
- [26] Muhyi A, Barlianto W, Chandra Kusuma H. EfekPemberianImunoterapi, Probiotik, Nigella sativa terhadap Th17, Neutrofil, danSkoringAsma. *J KedoktBrawijaya.* 2015;
- [27] Astrid Kristina Kardani AKK. The Effect of House Dust Mite Immunotherapy, Probiotic and Nigella sativa in The Number of Th17 Cell and Asthma Control Test Score. *IOSR J Dent Med Sci.* 2013;
- [28] Kartini R. EfekPemberianProbiotik, Nigella Sativa, Imunoterapiterhadap CD4+IL-4+, CD8+IL-4+ danSkoringAsma. *J KedoktBrawijaya.* 2014;
- [29] Olivianto E, Barlianto W, Kusuma HMSC. Effect of Nigella sativa (blackseed) and probiotics on expression of CD4CD25 Fox P3+ in children with mild asthma receiving immunotherapy. *PaediatrRespir Rev.* 2013;
- [30] Ratih I, Chandra Kusuma H, Barlianto W, Olivianto E. PengaruhImunoterapi, ProbiotikdanJintenHitamterhadap CD4+IFN γ , Eosinofil, danSkorAsma. *J KedoktBrawijaya.* 2015;
- [31] ChristinneSumantri D, Sumarno S, Barlianto W, Olivianto E, Chandra Kusuma H. PerbedaanSkor ACT, CD4+CD25+Foxp3treg, CD4+IFN- γ padaPemberianImunoterapi, Probiotikdan Nigella Sativa. *J KedoktBrawijaya.* 2015;
- [32] Kaya MS, Kara M, Ozbek H. Çörekotu (Nigella sativa) tohumuninsanhücre selbağışıklıksisteminin CD3+, CD4+, CD8+ hücrelerivetoplamlökosit sayısüzzerine etkileri. *Genel Tıp Derg.* 2003;13(3):109–12.
- [33] Işık H, Çevikbaş A, Gürer ÜS, Kiran B, Üresin Y, Rayaman P, et al. Potential adjuvant effects of nigella sativa seeds to improve specific immunotherapy in allergic rhinitis patients. *Med PrincPract.* 2010;
- [34] El-Shanshory M, Hablas NM, Aboonq MS, Fakhreldin AR, Attia M, Arafa W, et al. Nigella sativa improves anemia, enhances immunity and relieves iron overload-induced oxidative stress as a novel promising treatment in children having beta-thalassemia major. *J Herb Med.* 2019;

- [35] Louisa T Sugiono LTS. The Effect of House Dust Mite Immunotherapy, Probiotic and *Nigella sativa* in The Number of CD4+ IL-4 + Cell, Total IgE level and Asthma Control Test (ACT) Score. IOSR J Dent Med Sci. 2013;
- [36] Boskabady MH, Mohsenpoor N, Takaloo L. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. *Phytomedicine*. 2010;
- [37] Shahzad M, Yang X, RazaAsim MB, Sun Q, Han Y, Zhang F, et al. Black seed oil ameliorates allergic airway inflammation by inhibiting T-cell proliferation in rats. *PulmPharmacolTher*. 2009;
- [38] Majdalawieh AF, Hmaidan R, Carr RI. *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. *J Ethnopharmacol*. 2010;
- [39] Boskabady MH, Keyhanmanesh R, Khamneh S, Ebrahimi MA. The effect of *Nigella sativa* extract on tracheal responsiveness and lung inflammation in valbuminsensitized guinea pigs. *Clinics*. 2011;
- [40] Büyüköztürk S, Gelincik A, Özşeker F, Genç S, Şavran FO, Kiran B, et al. *Nigella sativa* (black seed) oil does not affect the T-helper 1 and T-helper 2 type cytokine production from splenic mononuclear cells in allergen sensitized mice. *J Ethnopharmacol*. 2005;
- [41] Packard KA, Khan MM. Packard, Kathleen A., and Manzoor M. Khan. "Effects of histamine on Th1/Th2 cytokine balance. *IntImmunopharmacol*. 2003;3(7):909–20.
- [42] Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *ClinExpImmunol*. 2009;
- [43] Wong CP, Stevens R, Long B, Li L, Wang Y, Wallet MA, et al. Identical β Cell-Specific CD8 + T Cell Clonotypes Typically Reside in Both Peripheral Blood Lymphocyte and Pancreatic Islets . *J Immunol*. 2007;
- [44] Salem ML, Hossain MS. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. *Int J Immunopharmacol*. 2000;
- [45] Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *Int J Immunopharmacol*. 1999;
- [46] McNally A, Hill GR, Sparwasser T, Thomas R, Steptoe RJ. CD4+CD25+ regulatory T cells control CD8+ T-cell effector differentiation by modulating IL-2 homeostasis. *ProcNatlAcadSci U S A*. 2011;
- [47] Fogle JE, Mexas AM, Tompkins WA, Tompkins MB. CD4 CD25 T Regulatory Cells Inhibit CD8 IFN- γ Production During Acute and Chronic FIV Infection Utilizing a Membrane TGF- β -Dependent Mechanism. *AIDS Res Hum Retroviruses*. 2010;26(2).
- [48] Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4+CD25+ T cells with regulatory properties from human blood. *J Exp Med*. 2001;



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.