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Original Article

Effects of *Nigella sativa* oil on Th1/Th2, cytokine balance, and improvement of asthma control in children

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Abstract

Background Asthma is a chronic inflammatory disease of the airways characterized by involvement of a variety of inflammatory cells. Asthma is associated with imbalances between Th1/Th2 cells and their characteristic cytokine profiles. *Nigella sativa* is a plant that possesses immunomodulatory and anti-inflammatory properties.

Objective To investigate the potential anti-asthmatic effect of *Nigella sativa* oil on Th1/Th2 cells, IFN-γ/IL-4 cytokines, and improvement of asthma control.

Methods Children aged 6-15 years with asthma in Dr. Saiful Anwar Hospital, Malang, were enrolled in this study. All patients were treated based on standard treatment guidelines for asthma. *Nigella sativa* oil (NSO) was given per oral as supplementary treatment at a dose of 15-30 mg/kg/day for 8 weeks, in a randomized, single-blind, controlled trial. Peripheral Th1 and Th2 cells were counted by flow cytometry and IFN-γ and IL-4 cytokines were measured by ELISA. Improvement of asthma control was assessed by the asthma control test (ACT) score.

Results Twenty-eight patients completed the study, 14 in the NSO treatment group and 14 in standard treatment group. No significant differences were found in the number of Th1 and Th2 cells, or in the Th1/Th2 ratio between groups after treatment (P=0.074, P=0.481, and P=0.265, respectively). Compared to the control, the NSO group showed a significant elevation of IFN-γ (P=0.046) and reduction of IL-4 (P=0.002). At the end of study, ACT score was not significantly different between groups (P=0.413).

Conclusion Supplementation with *Nigella sativa* oil improves IFN-γ/IL-4 balance and asthma control in children with asthma. **[Paediatr Indones. 2017;57:223-8 ; doi: http://dx.doi.org/10.14238/pi57.5.2017.223-8]**.

Keywords: *Nigella sativa; IFN-γ/IL-4; Th1/Th2; ACT score; asthma*

Asthma is a chronic inflammatory disease of the airways characterized by wheezing, difficulty breathing, or repetitive paroxysmal cough. Airway inflammation and hyperresponsiveness are central pathogenic features of asthma.[1] This process has a complex pathogenesis involving both genetic and environmental factors. One of the mechanisms underlying airway inflammation is an imbalance in T helper immune cells.[2] Experimental and clinical data suggest that the balance between the Th1 and Th2 cellular responses are central to the pathogenesis of allergic airway inflammation.[3-5] It is widely accepted that Th2 cytokines such as interleukin (IL)-4, IL-5, IL-9, and IL-13 play critical roles in orchestrating and amplifying allergic inflammation, while the Th1 cytokines, such as IFN-γ and IL-12, are thought to prevent this process.[6] In asthma, T helper type 2 (Th2) cells are functionally upregulated, while Th1 cells are inhibited, which enables Th2 cytokines to promote

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inflammation. Interleukin-4 (IL-4), secreted by Th2 cells, induces airway inflammation by activating eosinophils and promoting IgE secretion.[7] Therefore, one effective treatment for asthma is to improve Th1 immune responses and simultaneously inhibit Th2 immune responses to restore Th1/Th2 balance.[8]

One important goal of asthma treatment is to control the disease. Poor compliance with conventional asthma medications remains a major problem in achieving asthma control. Other problems are that some patients do not respond to intense asthma medications or they experience many undesired side effects due to long-term use of the medication.[8] The *Global Asthma Physician and Patient* (GAPP) Survey reported that 39% of asthma patients exchanged or stopped their asthma medication because of adverse events.[9] For this reason, introduction of novel treatment strategies is a key step for better asthma control.

Black seed or *Nigella sativ*a reportedly has anti-inflammatory and anti-allergy effects.[10] For thousands of years, *Nigella sativa* has been traditionally used as a spice, food additive, preservative, and herbal remedy for various diseases.[11] In several clinical studies, *Nigella sativa* showed positive effects on clinical and biochemical markers of asthma inflammation.[12,13] In mouse models of asthma, *Nigella sativa* oil (NSO) reduced airway hyperresponsiveness, total leukocytes, macrophages, eosinophils, and serum levels of total immunoglobulin E (IgE).[14] The aim of this study was to investigate the potential anti-asthmatic effect of *Nigella sativa* on IFN-γ/IL-4 cytokines, Th1/Th2 cells, and improvement of asthma control in children with asthma.

Methods

Twenty-eight children aged 6-15 years diagnosed with asthma in the Department of Allergy and Immunology, Dr. Saiful Anwar Hospital, Malang, were recruited between February and December 2013. Asthma diagnosis and assessment of severity were performed according to the *Global Initiative for Asthma* (GINA) guidelines.[1] Subjects were randomly divided into either the treatment or the control group. This study was a single-blind, controlled trial, which means that only the subjects were blinded to the identity of the group. This study was approved by the Ethics Committee of the Brawijaya University Medical School. Written informed consent was obtained from parents/guardians of all subjects.

In this study, we used softgel capsules containing 500 mg *Nigella sativa* oil (NSO), with brand name Minyak Habbatussauda MADINAH and licensed as an herbal medicinal product in Indonesia (POM TR.123 329 761). All patients were on routine asthma medications according to GINA guidelines for standard asthma management.[1] These included inhalation of β2 agonist for intermittent asthma and β2 agonist + corticosteroid inhalation for persistent asthma. In the treatment group, NSO was given as adjunctive therapy at a dose of 15-30 mg/kg/day for 8 weeks.

Peripheral blood mononuclear cells (PBMCs) of heparinized peripheral blood from the study subjects were isolated by *Ficoll* density gradient centrifugation. The cells were cultured in RPMI 1640 medium (*Invitrogen*) supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 2 mM glutamine, and 10% fetal bovine serum (FBS) (*Gibico*, USA). The PBMCs were harvested, washed, and stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 for 30 minutes (*BioLegend*, San Diego, CA). After surface staining, the cells were stained with phycoerythrin (PE)-conjugated anti-human IFN-γ (*BioLegend*, San Diego, CA) for Th1 detection, and PE-conjugated anti-human IL-4 (*BioLegend*, San Diego, CA) for Th2 detection, at 4°C for 30 minutes. Data were acquired on a FACS Calibur and analyzed using flow cytometry software.

Serum specimens were obtained from peripheral venous blood that was centrifuged at 3000 rpm for 5 min. The measurement of serum IFN-γ and IL-4 level was done by human ELISA kit (*Novateinbio Human IFN-γ ELISA Kit* and *Novateinbio Human IL-4 ELISA Kit*).

For assessment of asthma control, we used the *Asthma Control Test* (ACT) questionnaire. The ACT was one of the best-validated instruments to measure asthma control, consisted of five questions on a total scale of 5–25, with each question scaled from one to five. Full control was defined as a total ACT score of 25.[1]

Statistical analysis was performed by *SPSS for Windows* version 16.0 (*SPSS Inc*., USA). Data were

expressed as mean (SD). Statistical analyses were performed using paired and unpaired T-test to evaluate the differences between groups. The correlation coefficient was generated by Pearson's correlation. Statistical significance was defined to be P values <0.05.

Results

The clinical characteristics of participants are summarized in **Table 1**. There were 14 children in the NSO treatment group and 14 children in the standard treatment group. During our study, all patients completed the study and there were no treatment side effects observed. The subjects' mean ages were similar in both groups. The subjects were predominantly female and most had good nutritional status. More than 50% percent of subjects had a family history of atopic disease, such as rhinitis, asthma, eczema, urticaria, and conjunctivitis. The most common clinical manifestation was cough and dyspnea. The assessment of asthma severity revealed 50% with intermittent asthma and 50% with mild persistent asthma, in each group.

Table 1. Baseline characteristics of subjects

Characteristics	NSO treatment group (n= 14)	Standard treatment group (n= 14)
Mean age (SD), years	8.79 (2.940)	8.71 (3.771)
Gender, n		
Male	5	6
Female	9	8
Nutritrional status, n		
Good	12	13
Underweight	2	1
Family history of atopic disease, n		
Yes	10	9
No	4	5
Clinical manifestation, n		
Cough	2	2
Dyspnea	4	5
Cough + dyspnea	8	7
Asthma classification, n		
Intermittent	7	7
Mildly persisten	7	7

No significant differences between groups were found in the numbers of Th1 cells after 8 weeks of treatment [19.335 (SD 7.328) *vs.* 20.577 (SD 13.273), P=0.074]. But the number of Th1 cells tended to increase in both groups post-treatment. There was also no significant differences between groups in the numbers of Th2 cells after 8 weeks of treatment [21.875 (SD 12.871) *vs.* 18.478 (SD 11.729), P=0.481], (**Figure 1**). Th1/Th2 ratio showed no significant difference in the standard and NSO treatment groups [1.459 (SD 1.292) *vs.* 1.994 (SD 2.135), P=0.265], but Th1/Th2 ratio was higher in the NSO treatment group.

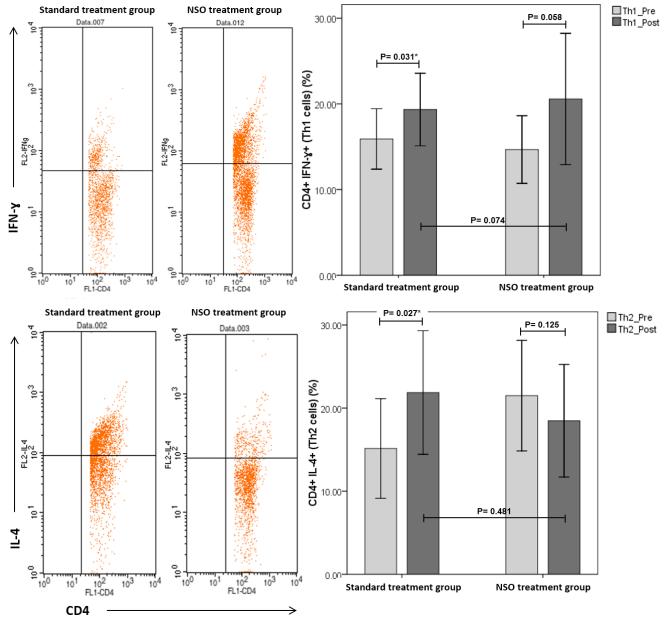

Figure 1. The number of Th1 and Th2 cells in the standard and NSO treatment groups

At the end of study, serum IFN-γ levels were significantly increased in the NSO group, while IL-4 was markedly decreased in the NSO group, compared to the standard treatment group (P=0.046 and P=0.002, respectively). At baseline, there were no significant differences in serum IFN-γ and IL-4 levels between groups before treatment (P=0.575 and P=0.470, respectively). In addition, there was no significant difference in IFN-γ/IL-4 ratio between groups both before and after treatment (P=0.275 and P=0.130, respectively) (**Table 2**).

Table 2. Serum levels of IFN-γ and IL-4 cytokines

Variables	Pre-treatment			Post-treatment		
	Standard treatment group	NSO treatment group	P value	Standard treatment group	NSO treatment group	P value
Mean IFN-γ (SD), pg/mL	10.083 (3.190)	12.495 (4.367)	0.575	9.786 (3.273)	20.035 (6.416)	0.046
Mean IL-4 (SD), pg/mL	1.303 (0.519)	1.413 (0.331)	0.470	1.434 (0.512)	1.107 (0.207)	0.002
Mean IFN-γ/IL-4 ratio (SD)	8.959 (4.738)	10.421 (4.663)	0.275	9.476 (3.834)	20.516 (5.700)	0.130

After 8 weeks of treatment, the mean ACT score was not significantly different in the standard and NSO treatment groups (P=0.692). But, there were significant increases in ACT scores between pre- and post-treatment in the standard treatment group [17.57 (SD 1.222) *vs.* 19.36 (SD 1.151), respectively, P<0.0001)] and the NSO treatment group [16.57 (SD 2.533) *vs.* 20.29 (SD 1.816), respectively, P<0.0001)] (**Figure 2**).

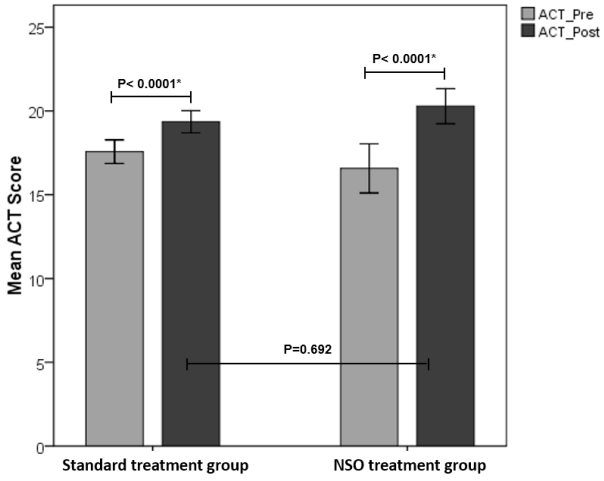

Figure 2. The mean ACT scores in the standard and NSO treatment groups, pre- and post-treatment

Although the results of this study showed an increase of IFN-γ, decrease of IL-4, and improvement of ACT score in the NSO group, we found no significant correlations between IFN-γ, IL-4, IFN-γ/IL-4 ratio, and ACT score. The number of Th1 cells, Th2 cells, and the Th1/Th2 ratio also showed no correlation with ACT score (**Table 3**).

Table 3. Correlation between biochemical markers and ACT score in the NSO group

Variables	r	P value
IFN-γ	0.081	0.681
IL-4	0.018	0.927
IFN-γ/IL-4 ratio	0.049	0.803
Th1	0.070	0.722
Th2	0.152	0.278

Discussion

Nigella sativa (NS) or commonly known as black seed is a one of medicinal plants that belongs to Ranunculaceae Family. The seeds and oil of NS have been widely used for the treatment of various diseases. NS has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities such as anticancer and immunomodulatory, analgesic and anti-inflammatory, anti-allergy, antimicrobial, anthelmintics, spasmolytic, bronchodilator, gastroprotective, hepatoprotective and antioxidant properties.[15] A randomized, double-blind, placebo-controlled trial conducted by Koshak *et al.* showed that NSO improved the mean ACT score in children with asthma.[13] Our previous study reported that the administration of *Nigella sativa* oil along with immunotherapy and probiotic therapy to asthmatic children, significantly increased their ACT scores.[12] Another study described that children with asthma who were given NSO showed a significant reduction in pulmonary index (PI) and improvement of peak expiratory flow rate (PEFR).[11] Boskabady *et al.* also reported that prophylactic therapy with an aqueous extract of NS could improve the severity of asthma symptoms.[16] However, in our study, both groups had significantly improved ACT scores pre-

and post-treatment. These improvements were not significantly different between the two groups.

Several mechanisms of action have been suggested for *Nigella sativa*, which may explain its beneficial effects in asthma. One such mechanism is the regulation of Th1/Th2 cellular balance. In allergy conditions, such as in asthma, regulation of Th1 and Th2 cells is a key process contributing to asthma pathogenesis.[3] Th2 cells promote the macrophage activity and regulate the proinflammatory response, whereas Th1 cells inhibit the activity of Th2. The Th1 cells produce IL-2 and IFN-γ, whereas Th2 cells produce IL-4 and IL-10.[6] Interleukin-4 (IL-4), secreted by Th2 cells, induces airway inflammation by activating eosinophils and promoting IgE secretion.[7] Majdalawieh *et al.* described that an aqueous extract of *Nigella sativa* reduces the secretion of Th2 cytokines by splenocytes.[17] An in vivo study also reported that an aqueous extract of *Nigella sativa* increased IFN-γ and decreased IL-4 cytokines, but histopathological findings in lung parenchyma of ovalbumin-sensitized guinea pigs were not improved.[14] Clinical study conducted by Salem *et al.* reported that there was significant increase in the serum IFN-γ and improvement in the ACT score after 12 weeks of *Nigella sativa* supplementation in asthmatic patients.[18]

This study showed that NSO treatment increase of IFN-γ and decrease of IL-4 serum level. It indicates that there was stimulatory effect on Th1 cells and inhibitory effect on Th2 cells. The underlying mechanism of Th1/Th2 cytokine modulation by *Nigella sativa* may be attributed to the inhibition of the signal transducer and activator of transcription-6 (STAT6) signaling pathway.[19] STAT proteins are a group of transcription factors that transmit signals from cytoplasmic milieu of cells to nucleus. The activation of STAT6 is pivotal in naive CD4+T (Th0) cell differentiation to Th2 pathways. At the same time, STAT-6 induction inhibits Th1 differentiation, both by increasing IL-4 production and by inhibiting the master Th1 transcription factor.[20,21]

However, we found no significant differences in the numbers of Th1 and Th2 cells in both groups after 8 weeks of treatment (**Figure 1**). But the number of Th1 cells and Th1/Th2 ratio tended to be increased in the NSO treatment group. It was reported that *Nigella sativa* can potentially inhibit Th2 immune responses, but there was no clear evidence that *Nigella sativa* decreased Th2 cell population. Several clinical studies reported a significant increase in the percentage of CD4+ and CD8+ T cells producing IL-4 in asthmatic children.[22,23] This finding suggests that IL-4 is not only produced by CD4+ Th2 cells, but also by CD8+ T cells. While it is well established that CD4+ T lymphocytes play a crucial role in the initiation, progression, and persistence of asthma, the role of CD8+ T cells is less understood.[23] CD8+ T cells form functionally similar subsets which exhibit similar cytokine profiles as Th1 and Th2 cells, known as Tc1 and Tc2. Evidence from animal studies suggest that CD8+ T cells are capable of regulating IgE production through the induction of IL-12 and IL-18 production in dendritic cells, and that CD8+ T cells may act to moderate Th2 polarization within the localized lymph nodes during allergic sensitization.[24]

Despite the many published studies evaluating immune functions of *Nigella sativa* products, this plant is not yet in clinical use for treatment of asthma. With regards to immunomodulatory effects of *Nigella sativa* and its constituents, this plant has a potential therapeutic effect on asthma. However, more studies are required for clinical application of *Nigella sativa*.

In conclusion, the results indicate that there is no different in ACT scores and Th1/Th2 balance between NSO and standard treatment group, but there is decrease of IL-4 and increase of IFN-γ serum level in asthmatic patients with NSO supplementation.

Acknowledgements

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Conflict of Interest

None declared.

References

1. Global Initiative for Asthma (GINA). Global Asthma Strategy of Management and Prevention Revised. Cape Town: National Heart, Lung and Blood Institute; 2014. p.1-15
2. Vock C, Hauber HP, Wegmann M. The other T helper cells

in asthma pathogenesis. JAllergy. 2010:519298.
3. Deo SS, Mistry KJ, Kakade AM, Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. Lung India. 2010;27:66–71.
4. Toldi G, Molvarec A, Stenczer B, Muller V, Eszes N, Bohacs A, *et al.* Peripheral Th1/Th2/Th17/regulatory T-cell balance in asthmatic pregnancy. Int Immunol. 2011;23:669–77.
5. Ling MF, Luster AD. Allergen-specific CD4 T cells in human asthma. Ann Am Thorac Soc. 2016;13:25-30.
6. Abbas AK, Lichtman AH, Pillai S. Basic immunology: functions and disorders of the immune system. 5th ed. St. Louis: Elsevier; 2016. p.132-6.
7. Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. Nat Rev Immunol. 2010;10:838-48.
8. Bosnjak B, Stelzmueller B, Erb KJ, Epstein MM. Treatment of allergic asthma: modulation of Th2 cells and their responses. Respir Res. 2011;12:114.
9. Canonica GW, Baena-Cagnani CE, Blaiss MS, Dahl R, Kaliner MA, Valovirta EJ, *et al*. Unmet needs in asthma: Global Asthma Physician and Patient (GAPP) Survey: global adult findings. Allergy. 2007;62:668-74.
10. Tembhurne SV, Feroz S, More BH, Sakarkar DM. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. J Med Plants Res. 2014;8:167-177.
11. Ahmad J, Khan RA, Malik MA. Study of *Nigella sativa* oil in the management of wheeze associated lower respiratory tract illness in children. African J Pharm Pharmacol. 2009;3:248-51.
12. Kardani AK, Fitri LE, Barlianto W, Olivianto E, Kusuma HC. 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;6:37-47.
13. Koshak A, Wei L, Koshak E, Wali S, Alamoudi O, Demerdash A, *et al*. *Nigella sativa* supplementation improves asthma control and biomarkers: a randomized, double- blind, placebo-controlled trial. Phytother Res. 2017;31:403-9.
14. Boskabady MH, Keyhanmanesh R, Khamneh S, Ebrahimi MA. The effect of *Nigella sativa* extract on tracheal responsiveness and lung inflamation in ovaalbumin- sensitized guinea pigs. Clinics (Sao Paulo). 2011;66:879-87.
15. Ahmad A, Asif H, Mohd M, Shah AK, Abul K, Nasir AS, Zoheir A, Damanhouri, Firoz A. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 2013;3:337-352.
16. Boskabady MH, Farhadi F. The possible prophylactic effect of *Nigella sativa* seed aqueous extract on respiratory symptoms and pulmonary function tests on chemical war victims: a randomized, double-blind, placebo-controlled trial. J Altern Complement Med. 2008;14:1137- 44.
17. Majdalawieha AF, Hmaidan R, Carr RI. *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. J Ethnopharmacol. 2010;131:268–75.
18. Salem AM, Bamosa AO, Qutub HO, Gupta RK, Badar A, Elnour A, Afzal MN. Effect of *Nigella sativa* supplementation on lung function and inflammatory mediatorsin partly controlled asthma: a randomized controlled trial. Ann Saudi Med. 2017;37:64-71.
19. Maier E, Duschl A, Horejs-Hoeck J. STAT6-dependent and –independent mechanisms in Th2 polarization. Eur J Immunol. 2012;42:2827–33.
20. Guo HW, Yun CX, Hou GH, Du J, Huang X, Lu Y, *et al*. Mangiferin attenuates Th1/Th2 cytokine imbalance in an ovalbumin-induced asthmatic mouse model. PLoS One. 2014;9:e100394.
21. Machura E, Mazur B, Rusek-Zychma M, Bar´c-Czarnecka M. Cytokine production by peripheral blood CD4+ and CD8+ T cells in atopic childhood asthma. Clin Dev Immunol. 2010;2010:606139.
22. Ito C, Okuyama-Dobashi K, Miyasaka T, Masuda C, Sato M, Kawano T, *et al*. CD8+ T cells mediate female-dominant IL-4 production and airway inflammation in allergic asthma. PLoS One. 2015;10:e0140808.
23. Betts RJ, Kemeny DM. CD8+ T cells in asthma: friend or foe? Pharmacol Ther. 2009;121:123-31.
24. Wang X, Wang J, Xing CY, Zang R, Pu YY, Yin ZX. Comparative analysis of the role of CD4(+) and CD8(+) T cells in severe asthma development. Mol Biol. 2015;49:482-90.