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Original Article

Effects of *Nigella sativa* on disease activity, T lymphocytes and inflammatory cytokine profiles in pediatric systemic lupus erythematosus: A randomized controlled trial

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with diverse manifestations, requiring long-term treatment that can have side effects, particularly in pediatric patients. *Nigella sativa* has shown potential for improving SLE symptoms due to its anti-inflammatory and immunomodulatory effects. The aim of this study was to investigate the immunomodulatory effect of *N. sativa* oil (NSO) on disease activity, T lymphocyte activity and inflammatory cytokine profiles in pediatric SLE patients. A randomized, double-blinded, placebo-controlled clinical trial was conducted at Saiful Anwar Hospital in Malang, Indonesia. Pediatric patients with SLE were randomly assigned to receive either one gram of NSO or a placebo containing starch in capsule form as adjunct therapy alongside their SLE primary treatment. Blood samples were collected before treatment and after eight weeks of daily capsules. Disease activity was assessed using the SLE Disease Activity Index 2000 (SLEDAI-2K); flow cytometry was used to identify T helper lymphocytes, and serum cytokine levels were measured using ELISA. The statistical analysis tests were performed to compare the outcomes between groups at baseline or after the treatment, and within-group comparisons before and after the study period, as appropriate. A total of 32 patients were included in the study. A significant decrease in the SLEDAI-2K score was observed at post-treatment in both the NSO and placebo groups ($p<0.001$ and $p=0.025$, respectively). The percentage of T helper 17 (Th17) cells was significantly reduced in both the NSO and placebo groups post-treatment compared to pre-treatment ($p=0.026$ and $p=0.034$, respectively). Conversely, the post-treatment percentage of regulatory T (Treg) cells increased significantly in both groups. A significant reduction in interleukin (IL)-2 levels was observed in the NSO and placebo groups at post-treatment compared to pre-treatment ($p=0.006$ and $p=0.046$, respectively). Additionally, there were increases in IL-4 and IL-6 serum levels in both groups at post-treatment compared to pre-treatment ($p<0.05$). This study highlights that although disease activity was not significantly different between NSO and placebo groups, NSO could affect the inflammatory cytokine profiles in pediatric SLE patients.

Keywords: *Nigella sativa*, immunomodulatory, T lymphocyte, cytokine, pediatric SLE

Introduction

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder that affects multiple tissues and organs. The pathophysiology of SLE is complex, involving a combination of genetic, environmental, and immunological factors [1]. The primary causes of systemic

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inflammation in SLE include dysregulation of the immune system, an abnormal response to self-antigens, and the deposition of immune complexes, which lead to tissue and organ damage [2]. The incidence and prevalence of SLE vary worldwide, with pediatric-onset SLE representing 10–20% of all SLE cases [3]. Although pediatric SLE is less common than adult SLE, it presents significant challenges due to its severity and potential for multi-organ involvement [3].

There is growing evidence about the critical role of T-cell lymphocytes and relevant cytokines in the pathogenesis of SLE, especially in pediatric cases [4]. A study focused on immune cell phenotyping in patients with juvenile-onset SLE and identified significant disruptions in their immune cell profiles compared to healthy controls [5]. These disruptions included defects in T cells, B cells, and monocyte populations. Specifically, the study observed an increase in the total and naive $CD8^+$ T cells, total monocytes, and plasma blasts, along with a decrease in the total $CD4^+$ T cells and memory T cell and B cell populations [5]. Moreover, these immune profile dysfunctions were associated with the severity of clinical features and disease flare-ups [6].

Steroids remain the primary treatment for SLE to induce remission and prevent further organ damage, alongside the use of immunosuppressant agents [7]. However, long-term steroid use has been reported to cause serious complications, including secondary infections, cataracts, glaucoma, musculoskeletal diseases (i.e., osteoporosis, vascular necrosis, myopathy, gastroduodenal ulcers, and hypertension), and cardiovascular complications, such as myocardial infarction and cerebrovascular disease [8,9]. Therefore, alternative treatments, such as immunomodulators, are needed to mitigate these side effects. Immunomodulation involves temporarily altering specific parts of the immune system through agents that either activate or suppress its function, thereby regulating the immune system [10].

Nigella sativa (*N. sativa*), known in Indonesian as *habatussauda* or *jinten hitam* (black cumin), has been used in traditional medicine to treat various diseases for centuries. The seeds contain fixed oils, proteins, alkaloids, saponins, and essential oils. Among its compounds, thymoquinone is suggested to have immunomodulatory effects [11]. It has been reported that thymoquinone can regulate inflammatory molecules, including interferons, interleukins, tumor necrosis factor-α (TNF-α), oxidative stress, regulatory T cells, and various signaling pathways such as nuclear factor kappa beta (NF-κβ), Janus kinase/signal transducer and activator of transcription (JAK-STAT), and mitogen-activated protein kinase (MAPK), at the molecular level and through epigenetic alterations [12]. Since defects in these molecules and signaling pathways play a crucial role in the development of autoimmune diseases, modulating these mechanisms could be a potential treatment strategy for conditions such as SLE [12]. Another potential mechanism of *N. sativa* in the pathophysiology of SLE is its antioxidant properties. A study reported that *N. sativa* supplementation in combination with vitamin E improved oxidative and nitrosative biomarkers in patients with SLE [13].

A study on the pristane-induced lupus mice model reported that *N. sativa* reduced cytokine expression, specifically interleukin 17 (IL-17), IL-6, and IL-23 [14]. This reduction helped prevent both the activity and chronicity index of lupus nephritis, as observed through renal histopathology [14]. Our previous study also demonstrated an increase in immunomodulatory T regulatory cells and clinical improvement in a pristane-induced SLE mice model [15]. The effects of *N. sativa* oil (NSO) extract as an adjunctive therapy in other autoimmune diseases have been shown in clinical trials involving patients with rheumatoid arthritis (RA) [16,17]. Additionally, studies have reported the anti-inflammatory, antioxidant, and immunomodulation effects of *N. sativa* [18,19]. However, a clinical trial investigating the immunomodulatory effect of NSO on a patient with SLE has not been conducted, particularly in pediatric patients. Therefore, the aim of this study was to investigate the immunomodulatory effect of NSO on the T lymphocyte activity and inflammatory cytokine profiles in pediatric patients with SLE. To the best of our knowledge, this study is the first clinical trial that evaluates the immunomodulatory effect of NSO as adjunctive therapy in pediatric SLE patients.

Methods

Study design and settings

A randomized, double-blinded, placebo-controlled clinical trial was conducted at Saiful Anwar Hospital in Malang, Indonesia, from January 2022 to January 2023. This clinical trial was

registered at clinicaltrial.gov (NCT06560775). Patients received a capsule containing either NSO or a starch placebo. All patients were treated with the primary regimen for SLE according to the standard hospital therapy protocol, which included prednisone, hydroxychloroquine, and mycophenolic acid. The adjunctive therapy (NSO or a starch placebo) began on the first day of recruitment, with one capsule administered daily for eight weeks. A pre-treatment blood sample was drawn to identify baseline laboratory parameters. Weekly monitoring calls were conducted to inquire about any adverse effects experienced by the patients and other progress-related variables. At the end of the study, the blood was re-drawn for post-treatment blood testing.

Sample size and randomization

The sample size calculation in this study used the formula for a superiority clinical trial to determine whether there was evidence of a difference in the desired outcome between NSO and placebo. Based on this calculation, the sample size (n) for each group was determined to be 13. To anticipate dropouts, an additional 20% was added to the minimum sample size, resulting in 16 patients required for each group. Purposive sampling was employed in this study.

Patients were randomly assigned to either the NSO group or the placebo group using a computer program that generated randomization tables to ensure comparability and eliminate selection bias. Each patient received a serial number based on the order of their entry into the study. Both patients and researchers were blinded to prevent bias in treatment administration, outcome assessment, and results reporting.

Patients and criteria

This study included pediatric patients aged 1–18 years with SLE from the pediatric outpatient clinic of Saiful Anwar Hospital, diagnosed according to the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria [20]. Participants were currently treated with prednisone, hydroxychloroquine, and mycophenolic acid and had not received any biological agent or cytokine inhibitors for at least two months prior to the enrollment. Meanwhile, patients with any metabolic disorders such as diabetes mellitus, Cushing's syndrome, or thyroid dysfunctions; kidney or liver diseases; chronic inflammatory diseases, including inflammatory bowel diseases; and a history of consuming antioxidant or anti-inflammatory supplements within two months before enrollment were excluded from the study.

Before enrollment, a detailed explanation of the study was provided to the children and their parents, ensuring they fully understood the clinical trial objectives, procedures, potential risks, and benefits. Parents were required to give informed consent on behalf of their children, while the children provided informed assent, demonstrating their willingness to participate.

Intervention

Patients were randomly assigned to one of two groups: the NSO group or the placebo group. Apart from receiving the primary regimen for SLE, the NSO group received a daily capsule containing 1 g of NSO, while the placebo group received a daily capsule containing 1 g of starch. Treatment was provided for eight weeks. The NSO capsules were sourced from Habbatussauda CV Rizki Abadi (registered with the Indonesian Food and Drug Authority, Registration number POM TR:183314171) [21]. The placebo capsules, containing starch, were administered daily for the same period. Both NSO and placebo capsules were identical in appearance to maintain the double-blind nature of the study. Patients were monitored for any potential adverse events. Throughout the treatment period, regular and systematic assessments were conducted via phone calls using standardized questionnaires to detect any adverse effects. Patients were asked to report any unusual or unexpected symptoms they experienced during treatment, regardless of whether they believed the symptoms were related to the study drug or not.

Data collection and study variables

Before treatment initiation, comprehensive baseline data on the patients were collected, including body mass index (BMI) (i.e., nutritional status), disease duration, treatments received, and SLE Disease Activity Index (SLEDAI) scores. Based on the anthropometric examination, the patient's nutritional status was categorized into four categories: severe acute malnutrition, undernourished, good nutritional status, and overweight. Additionally, after eight weeks of intervention, T lymphocyte profiles and cytokine serum levels from blood samples were

measured. The primary outcome of the study was the reduction in the SLEDAI score, measured using the SLE Disease Activity Index 2000 (SLEDAI-2K) [22]. The secondary outcome was the difference in T lymphocyte profiles and cytokine serum levels between the groups.

Measurement of T lymphocyte profiles

Isolation of peripheral blood mononuclear cells (PBMCs) was performed by centrifugation of the blood with Ficoll gradient density. The PBMC suspension was incubated overnight at 37°C in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 100 U/mL penicillin and 100 U/mL streptomycin, 2 mM glutamine, and 10% fetal bovine serum (FBS). Following overnight incubation, PBMCs were harvested, washed, and stained with fluorescein isothiocyanate-conjugated anti-human CD4 (BD Bioscience, San Jose, USA). Subsequently, cells were stained (BD Bioscience, San Jose, USA) with phycoerythrin (PE)-conjugated anti-human interferon gamma-γ (IFN-γ) for T-helper 1 (Th1) detection, PE-conjugated anti-human IL-4 for Th2 detection, CD25+Foxp3-PE antibodies for regulatory T-cells (Treg) detection, and CD4+IL-17A-PE antibodies for Th17 detection. Flow cytometric analysis was performed using a BD FACS Calibur flow cytometer (Becton Dickinson, California, USA), and the percentages of cell populations were analyzed using the CELL Quest Pro software (Becton Dickinson, New Jersey, USA). The T cell percentage was measured using the flow cytometry method as previously described [23,24].

Measurement of cytokine levels

After being centrifuged at 3000 rpm for 10 minutes, the serum samples were then analyzed for several cytokine levels (IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-17, and IL-10) using the enzyme-linked immunosorbent assay (ELISA) method with all kits from BD Immunoassay ELISA Kit (BD Biosciences, San Jose, United States). Each cytokine was expressed in picograms per milliliter (pg/mL).

SLE Disease Activity Index 2000 (SLEDAI-2K)

The SLEDAI-2K scoring system assessed disease activity in SLE across several key domains, including constitutional symptoms (4 points), musculoskeletal (8 points), skin (10 points), mucous membranes (2 points), renal (6 points), neurological (8 points), hematological (6 points), and serological (4 points). The presence and severity of symptoms in each domain contribute to the overall SLEDAI-2K score, offering a comprehensive measure of disease activity. The scoring system ranged from 0 to 105, and the disease activity was categorized as mild (0–5), moderate (6–14), or severe (>14) [22].

Statistical analysis

The quantitative and qualitative data were presented as mean±standard deviation (SD) for normally distributed data and as median for non-normally distributed data. Normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was tested using Levene's test. Characteristics between the two groups were compared using the Student t-test, Chi-squared, and multinomial logistic regression test as appropriate. To compare the mean values between groups at baseline, the independent Student t-test or Mann-Whitney U-test was employed as appropriate. Within-group comparisons of mean values before and after the intervention were conducted using paired Student t-test or Wilcoxon test as appropriate. All statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered significant.

Results

Characteristics of the patients

A total of 32 pediatric patients with SLE were recruited, all of whom completed the study, as presented in **Table 1**. The mean ages in the NSO and placebo groups were 12.61 and 14.17 years, respectively (**Table 1**). The majority of the patients were female, with only one male patient in each group. The mean BMI in the NSO and placebo groups were 19.28±5.92 and 20.02±5.76 kg/m^2, respectively. Half of the NSO group were undernourished, while more than one-third of the placebo group had severe acute malnutrition. On average, the disease duration was 8.19

months for the NSO group and 7.5 months for the placebo group. All patients received treatments with prednisone, hydroxychloroquine, and mycophenolate mofetil. The mean SLEDAI scores were 13.56±6.03 in the NSO group and 14.18±4.99 for the placebo group, with a larger proportion of patients in both groups exhibiting severe disease activity. All baseline characteristics showed no significant differences between the NSO and placebo groups (**Table 1**).

Table 1. Characteristics of the pediatric patients with systemic lupus erythematosus (SLE) patients (n=32)

Characteristic	Study group		*p*-value
	NSO group (n=16)	Placebo group (n=16)	
	n (%)	n (%)	
Age (year), mean±SD	12.61±3.81	14.17±2.54	0.141[a]
Sex			1.000[b]
Male	1 (7)	1 (7)	
Female	15 (93)	15 (93)	
Body mass index (BMI) (kg/m²), mean±SD	19.28±5.92	20.02±5.76	0.481[a]
Nutritional status			
Severe acute malnutrition	4 (25)	6 (37.5)	0.809[c]
Undernourished	8 (50)	2 (12.5)	0.638[c]
Good nutritional status	2 (12.5)	5 (31)	
Overweight	2 (12.5)	3 (18)	0.853[c]
Disease duration (month), mean±SD	8.19±3.60	7.50±5.04	0.350[a]
Treatment received			
Prednisone	16 (100)	16 (100)	
Hydroxychloroquine	16 (100)	16 (100)	
Mycophenolate acid	16(100)	16(100)	
SLEDAI-2K score, mean±SD	13.56±6.03	14.18±4.99	
SLEDAI-2K score, mean±SD	13.56±6.03	14.18±4.99	0.413[a]
Classification of SLEDAI-2K			
Mild	2 (12.5)	4 (25)	0.560[c]
Moderate	3 (18)	4(25)	
Severe	11 (68)	8 (50)	0.745[c]

NSO: *Nigella sativa* oil; SLEDAI-2K: SLE Disease Activity Index 2000
[a] Analyzed with independent Student t-test
[b] Analyzed with Chi-squared test
[c] Multinomial logistic regression test

Effect of *N. sativa* oil (NSO) on SLEDAI-2K scores

Significant decreases in the mean SLEDAI-2K score were observed at the end of the study compared to the initial score in both groups ($p<0.001$ and $p=0.025$ for NSO and placebo groups, respectively) (**Figure 1**). No significant difference in mean SLEDAI-2K scores was found between the NSO and placebo groups at post-treatment (12.12±6.15 vs 8.68±6.71; $p=0.442$) (**Figure 1**).

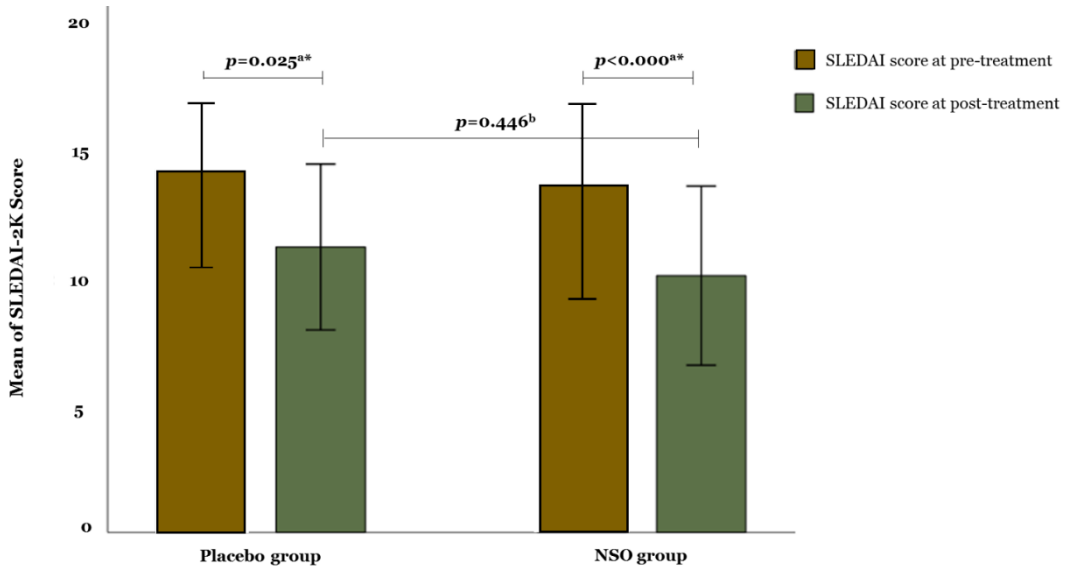

Figure 1. Comparisons of mean scores of SLEDAI-2K pre- and post-treatment between *Nigella sativa* oil (NSO) and placebo groups. *Statistical significance at $p<0.05$; [a] Analyzed with paired Student t-test; [b] Analyzed with unpaired Student t-test.

Effect of *N. sativa* oil (NSO) on T cell lymphocyte profiles

Comparison of T lymphocyte profiles in the NSO and placebo groups suggested that Th17 percentages significantly declined post-treatment (p=0.026 and p=0.034, respectively) (**Table 2**). The Treg percentages significantly increased after eight weeks of intervention, with p=0.017 and p<0.001 for NSO and placebo groups, respectively. There were no significant differences in T cell lymphocyte percentages between pre- and post- treatment in both groups (**Table 2**).

Table 2. Comparison of T cell lymphocyte profiles pre- and post-treatment between *Nigella sativa* oil (NSO) and placebo groups

Parameters	Study phases		*p*-value
	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	
T helper 1 (Th1) (%)			
NSO group	11.04 (2.52–31.96)	1.95 (0.81–4.64)	0.088[a]
Placebo group	5.69 (1.25–9.62)	0.89 (0.45–2.35)	0.053[a]
p-value	0.214[b]	0.083[b]	
T helper 2 (Th2) (%)			
NSO group	0.13 (0.04–0.22)	0.22 (0.04–0.91)	0.054[a]
Placebo group	0.10 (0.05–0.47)	0.07 (0.03–1.60)	0.609[a]
p-value	0.266[b]	0.819[b]	
T helper 17 (Th17) (%)			
NSO group	8.10 (3.60–13.44)	5.35 (2.24–10.10)	0.026[a*]
Placebo group	9.56 (3.59–16.88)	2.63 (1.79–5.52)	0.034[a*]
p-value	0.780[b]	0.673[b]	
Regulatory T (Treg) (%), mean±SD			
NSO group	9.86±8.59	13.60±7.23	0.017[c*]
Placebo group	8.47±6.07	12.17±5.81	<0.001[c*]
p-value	0.642[b]	0.780[d]	

[a] Analyzed with Wilcoxon test
[b] Analyzed with unpaired Student t-test
[c] Analyzed with paired Student t-test
[d] Analyzed with Mann-Whitney U test
[*] Statistical significance at p<0.05

Effect of *N. sativa* oil (NSO) on inflammatory cytokine profiles

After the intervention, significant reductions in IL-2 levels were observed in both groups compared to pre-intervention, with p=0.006 and p=0.046 for NSO and placebo groups, respectively (**Table 3**). In contrast, after the intervention, the levels of IL-4 and IL-6 increased significantly in both NSO and placebo groups compared to pre-treatment (**Table 3**). The levels of IL-4 and IL-17 after post-treatment were significantly different between the NSO and placebo groups, while the other cytokine parameters had no difference (**Table 3**).

Table 3. Comparisons of inflammatory cytokine profiles pre- and post-treatment between groups

Parameters	Study phase		*p*-value [a]
	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	
IFN-γ (pg/mL)			
NSO group	15.04 (5.79–45.02)	27.07 (17.03–37.24)	0.234
Placebo group	8.43 (1.58–39.28)	16.23 (7.71–30.00)	0.569
p-value[b]	0.323	0.171	
TNF-α (pg/mL)			
NSO group	122.86 (67.40–605.53)	113.06 (33.9–546.26)	0.234
Placebo group	98.47 (46.57–467.70)	133.80 (42.80–779.47)	0.569
p-value[b]	0.642	0.564	
IL-2 (pg/mL)			
NSO group	7.09 (1.40–11.28)	0.81 (0.10–0.81)	0.006[*]
Placebo group	7.43 (0.56–14.75)	0.91 (0.28–3.76)	0.046[*]
p-value[b]	1.000	0.897	
IL-4 (pg/mL)			
NSO group	0.29 (0.14–1.61)	14.11 (6.83–18.53)	0.006[*]
Placebo group	0.36 (0.15–0.52)	9.49 (2.15–12.53)	0.013[*]
p-value[b]	0.926	0.010[*]	
IL-6 (pg/mL)			
NSO group	6302.35 (2446.75–21261.65)	23999.90 (12470.28–24011.54)	0.023[*]
Placebo group	5477.42 (2314.00–15491.92)	15856.69 (12650.48–23999.18)	0.034[*]

Parameters	Study phase		*p*-value [a]
	Pre-treatment	Post-treatment	
	Median (IQR)	Median (IQR)	
p-value[b]	0.696	0.323	
IL-10 (pg/mL)			
NSO group	26.13 (13.37–53.23)	24.55 (16.06–40.53)	0.408
Placebo group	35.34 (11.63–48.51)	20.32 (16.36–32.80)	0.959
p-value[b]	0.669	0.752	
IL-17A (pg/mL)			
NSO group	2.22 (0.56–1.93)	5.92 (0.19–9.77)	0.409
Placebo group	3.69 (1.18–9.99)	9.79 (2.28–15.26)	0.203
p-value[b]	0.341	0.004*	

NSO: *Nigella sativa* oil
[a] Analyzed with Wilcoxon test
[b] Analyzed with Mann-Whitney U test
* Statistical significance at $p<0.05$

Discussion

SLE is an autoimmune disorder characterized by chronic inflammation affecting various tissues and organs. The disease's progression is driven largely by intricate interactions among immune cells and cytokines, which lead to an abnormal immune response [5]. T lymphocyte and cytokine profiles in SLE underscore a complex interplay of immune dysregulation, characterized by increased proinflammatory responses and impaired regulatory mechanisms. A study reported increased IL-2, IL-10, and IL-21 cytokine serum levels in children with SLE, which correlated positively with SLEDAI-2K scores [25]. Another study demonstrated uncontrolled expansion of T helper cell subsets—Th1, Th2, and Th17—in both lupus-prone mice and SLE patients, contributing to the promotion of autoantibody-producing plasma cells through T–B cell interactions [26]. Tregs, a subset of follicular T cells acting as immunomodulators, were significantly increased in both percentage and frequency in SLE patients, potentially serving as a compensatory mechanism against heightened T helper cell activity. However, the T helper/Treg ratio was much lower in patients with SLE [27]. Understanding the T lymphocyte and cytokine profiles in SLE is pivotal for unraveling disease mechanisms and identifying potential therapeutic targets.

In recent years, there has been growing evidence supporting the exploration of alternative immunomodulators as complementary therapies in SLE. *N. sativa*, commonly known as black seed or black cumin, has potential therapeutic effects in autoimmune diseases, including SLE, due to its primary active component, thymoquinone. Thymoquinone is reported to have anti-inflammatory and immunomodulatory activities [13]. In this clinical trial involving pediatric patients with SLE, we reported similar results. Both the NSO and placebo groups showed a significant increase in Treg percentages after 8 weeks of intervention compared to pre-treatment. Additionally, the percentage of Th17 cells was notably reduced in both groups. However, there was no significant difference in Th1 and Th2 cell percentages before and after treatment. Our previous study involving asthmatic children also demonstrated significant findings [24]. After eight weeks of NSO treatment, there was a marked decrease in Th17 cells and an increase in Treg percentages compared to the control group. Furthermore, the Th17/Treg ratio was lower in the NSO group compared to the control group [24]. In the pristane-induced lupus mice model, similar findings demonstrated that treatment with *N. sativa* significantly reduced anti-double-stranded deoxyribonucleic acid (anti-dsDNA) and significantly increased the Treg cell counts compared to the control group [15]. Furthermore, clinical trials in patients with RA have reported the immunomodulatory effects of NSA on T lymphocytes [21,28].

Several studies have reported that thymoquinone reduces the production of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IFN-γ, by inhibiting Nuclear Factor-kappa B (NF-κB) activation, a transcription factor that plays a crucial role in the expression of inflammatory genes [29, 30]. In a study involving Wistar rats with collagen-induced arthritis, oral administration of thymoquinone significantly lowered levels of proinflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ, and prostaglandin E2) while increasing the anti-inflammatory cytokine IL-10 [31]. *N. sativa* was reported to reduce the cytokine expression of IL-17, IL-6, and IL-23 in a pristane-induced lupus mice model. It was also observed to prevent renal tissue injury, as confirmed by a histopathological examination [32]. In this study, a significant reduction of IL-2

was observed in both groups at post-treatment compared to pre-treatment. However, contradictory results emerged with increased serum levels of IL-4 and IL-6 in both the NSO and placebo groups after intervention compared to pre-treatment. No significant differences in other cytokine profiles were reported at the end of the observation period compared to pre-treatment.

The increase in IL-4 and IL-6 in patients with SLE can be attributed to the immunomodulatory effects of *N. sativa*. *N. sativa* has been shown to favor the Th2 immune response, which is characterized by the production of cytokines such as IL-4. This enhancement can lead to increased activation and differentiation of B cells, promoting antibody production. In the context of SLE, where the immune system is already dysregulated, this can exacerbate the production of autoantibodies [33]. The active compounds in *N. sativa*, such as thymoquinone, can stimulate B cells, leading to their proliferation and the increased production of cytokines like IL-4. This cytokine is crucial for B cell differentiation and function, which includes the production of autoantibodies characteristic of SLE [34]. While *N. sativa* is known for its anti-inflammatory properties, it also has immunomodulatory effects that can lead to an increase in certain proinflammatory cytokines, such as IL-6. This cytokine plays a significant role in the inflammatory processes and immune response regulation in SLE [35]. *N. sativa* can activate macrophages and T cells, which are sources of IL-6. The interaction between *N. sativa*'s components and the immune system is complex and involves both suppression and stimulation of different immune pathways. This dual role can lead to an increase in IL-4 and IL-6 levels as part of the body's attempt to regulate the immune response, which in the case of SLE, may result in heightened disease activity [36,37].

In a study on Egyptian patients with SLE, supplementation with *N. sativa* and vitamin E for three months significantly decreased the antinuclear antibodies, anti-dsDNA levels, and SLEDAI score [38]. In this clinical trial, both NSO and control groups showed significant improvements in the SLEDAI-2K score after 8 weeks of treatment compared to their initial scores. However, no significant difference in the mean SLEDAI-2K score was observed between the NSO and control groups at the end of the study. The improvement in disease activity in both groups can be due to the complex nature of immune modulation by *N. Sativa*, individual variability in patient responses, multifactorial pathogenesis of SLE, and limitations in study design. SLE is a multifactorial disease involving various immune cells, cytokines, and pathways. *N. sativa* might not effectively target all the relevant mechanisms involved in SLE pathogenesis [39]. For example, while it may modulate certain cytokines, it may not adequately address other critical aspects of the disease, such as T cell dysregulation or complement activation. Furthermore, the design and duration of clinical trials can significantly influence outcomes [39,40].

This study had several limitations. First, the effect of NSO treatment was observed for only eight weeks. This short duration may not capture potential long-term benefits or identify the appropriate therapeutic dose needed to significantly improve disease activity. Second, the small sample size may not adequately represent the broader population of pediatric patients with SLE. To address this, multicenter studies in various regions worldwide are necessary to evaluate the effects of *N. sativa* across a diverse patient population. Future research with larger sample sizes, longer treatment durations, and more precise targeting of immune pathways will be essential to fully understand the potential benefits and limitations of *N. sativa* in SLE treatment.

Conclusion

The supplementation of NSO in conjunction with primary SLE treatment was found to be as effective as standard treatment in improving the SLEDAI-2K score. NSO could modulate the T lymphocytes and cytokine profiles in pediatric SLE patients. However, further investigation is needed to confirm the potential therapeutic benefits and mechanisms of NSO in managing pediatric SLE.

Ethics approval

This study was approved by the ethics committee of Universitas Brawijaya, Malang Indonesia (400/089/K3/302/2021). Parents or legal guardians provided permission for their child's participation in research after receiving full information about the study's purpose, procedures, risks, benefits, and alternatives. Key ethical considerations included obtaining the child's assent, providing age-appropriate information, addressing potential risks, and respecting cultural

differences in family dynamics. Researchers ensured that the process of obtaining informed consent was conducted with the highest ethical standards, prioritizing the welfare and rights of the child. Both parents and children were free to decline participation without any negative consequences and could withdraw from the study at any time without penalty.

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Competing interests

All the authors declare that there are no conflicts of interest in this study.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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