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Research Article

Immunomodulatory Effect of Nigella sativa Extract through the Improvement of IL-1β Level in Balb-c Mice Infected by Methicillin-resistant Staphylococcus aureus

Efek Imunomodulator Ekstrak Jintan Hitam (*Nigella sativa*) Melalui Peningkatan IL-1β Mencit Balb-c yang Diinfeksi *Methicillin-resistant Staphylococcus aureus*

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ABSTRACT

Nigella sativa (NS) has been used for many years as an anti-bacterial herbal treatment, such as Methicillin-resistant Staphylococcus aureus, but the mechanism in bacterial elimination is still unknown. IL-1β is a proinflammatory cytokine that helps the immune system eliminate microbes when it enters the body. The purpose of this study was to prove the immunomodulatory effects of Nigella sativa extract through increased IL-1β in dealing with MRSA infections. The design of this study was post-test only control group design using 25 male Balb-c mice infected with MRSA and randomly divided into five groups. Group K was infected with MRSA without being treated. All treatment groups were given NS extracts in different doses for 7 days before being infected with MRSA. P1 was given N. sativa extract 0.05ml, P2 (was given NS extract 0.25ml, P3 was given 0.5ml NS extract, and P4 was given 0.75ml NS. On the 8th day, blood was taken from the retroorbital plexus for IL-1β level examination using ELISA. Statistical tests were done using the One Way ANOVA test. The results showed the mean of IL-1β levels in group K was 11.65ng/L, P1 was 14.07ng/L, P2 was 16.66ng/L, P3 was 18.54ng/L, and P4 was 19.49ng/L which showed an increase in IL-1β levels along with the addition of black cumin dose although there was no difference between groups (p=0.578: ANOVA test). Nigella sativa extract is not proven effective in increasing IL-1β levels of Balb-c mice infected with MRSA

***Keywords**: IL-1β, MRSA, Nigella sativa*

ABSTRAK

Nigella sativa (*N. sativa*) atau jintan hitam telah digunakan selama bertahun-tahun sebagai pengobatan herbal anti bakteri, salah satunya untuk pengobatan *Methicillin-resistant Staphylococcus aureus* (MRSA) namun mekanismenya belum diketahui secara pasti. IL-1β merupakan salah satu sitokin proinflamasi yang membantu sistem imun mengeliminasi mikroba ketika masuk ke dalam tubuh. Tujuan dari penelitian ini untuk membuktikan efek immunomodulator ekstrak *Nigella sativa* melalui peningkatan IL-1β dalam mengatasi infeksi MRSA. Desain penelitian ini adalah *post test only control group design* menggunakan 25 ekor mencit balb-c jantan yang diinfeksi MRSA dan dibagi secara acak dalam lima kelompok. Kelompok kontrol (K) diinfeksi MRSA tanpa diterapi. Semua kelompok perlakuan diberikan ekstrak *N. sativa* dalam dosis berbeda selama 7 hari sebelum kemudian diinfeksi dengan MRSA. Perlakukan satu (P1) diberikan ekstrak *N. sativa* sebanyak 0,05ml , P2 dengan dosis 0,25ml , P3 dengan dosis 0,5ml, dan P4 dengan dosis 0,75ml . Pada hari ke-8 darah mencit diambil dari *plexus retroorbital* untuk diperiksa kadar IL-1β dengan ELISA. Didapatkan hasil rerata kadar IL-1β pada kelompok K=11,65ng/L ; P1=14,07ng/L ; P2=16,66ng/L ; P3=18,54ng/L ; dan P4=19,49ng/L yang menunjukkan adanya peningkatan kadar IL-1β seiring dengan penambahan dosis jintan hitam walaupun tidak ada perbedaan antar kelompok (p=0,578: ANOVA test). Ekstrak *Nigella sativa* tidak terbukti efektif dalam meningkatkan kadar IL-1β mencit balb-c yang diinfeksi MRSA

Kata Kunci: IL-1β, *MRSA, Nigella sativa*

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INTRODUCTION

Staphylococcus aureus (S. aureus) infection is a serious problem because of the emerging and spread of *Methicillin-Resistant Staphylococcus aureus* (MRSA), a type of multidrug resistant organism. *Methicillin-resistant Staphylococcus aureus* (MRSA) strain is known as one of the main causes of nosocomial infections in various hospitals since the 1980s with the prevalence of 50% around the world (1). The incidence of MRSA infections continues to increase in various parts of the world, for example, hospitals in Asia in early 2010 were endemic to MRSA with an estimated incidence of 28% in Hong Kong and Indonesia, whereas it was much higher (70%) in Korea (2,3).

Methicillin-resistant Staphylococcus aureus (MRSA) strains have been known to be resistant to various antibiotics in all beta-lactam groups, and more than 2 non-beta-lactam antimicrobials such as macrolides (erythromycin), ampicillin, protein synthesis inhibitors (tetracycline, chloramphenicol) and quinolones. Not only vancomycin but also linezolid and ceftarolin are still effective for the treatment of MRSA infections (4,5). However, at this time there is also a discovery on strains of *S. aureus* that are resistant to vancomycin (6). The widespread bacterial resistance to existing drugs encourages the importance of extracting antibacterial sources from the natural material which modulates the immune response (7).

Black cumin (*Nigella sativa*) has long been used in several countries, especially in the Middle East and in several other Asian countries, including Indonesia. The active compound of the black cumin is monoterpen molecule that is extracted from the residue of black cumin oil containing thymoquinone (TQ) and thymohydroquinone (THQ). The substance has properties to enhance the body's immune system, anti-bacterial, and anti-inflammatory. Both TQ and THQ allegedly can form irreversible complexes with nucleophilic amino acids in proteins of bacteria causing the inactivation of the protein (8). *Nigela sativa* oil is rich in unsaturated fatty acids, linolenic acid, and stearidonic acid. Various studies have shown that *N. sativa* has anti-inflammatory and immunomodulatory properties (9,10). Study on the immunomodulatory effects of *N. sativa* had been conducted, and it is proven that *N. sativa* can improve the immune response in humans, by increasing IgM production in murine lymph cell culture at low concentrations otherwise can reduce IgM levels at high concentrations (11).

The invasion of *S. aureus* bacteria to the body will stimulate phagocytosis by macrophages. Macrophages, dendritic cells, and other cells recognize microbes and give respond by producing cytokines. One of these cytokines was IL-1β which work on the endothelial venules at the site of infection and initiate the process of migrating leukocytes into the tissues for bacterial elimination (1,7,11).

This research was conducted to identify the potential of black cumin extract as an immunomodulator through increased IL-1β in Balb-c mice infected by MRSA. The reason researchers chose IL-1β to be evaluated in this study was because IL-1 is one of the early pro-inflammatory cytokines that is expected to stimulate the immune system in eliminating extracellular bacteria such as MRSA

METHOD

This study is experimental studies with posttest only control group design conducted in the Faculty of Medicine, Universitas Jenderal Soedirman (Unsoed) Purwokerto for eight months.This research applied animal ethics in handling the experimental animals. Ethical clearance number 3968/KEPK/VIII/2019 was obtained from the Health Research Ethics Commission before the study was begun. The study population was male Balb-c strain mice aged eight weeks and weighed 20-30 grams. Mice were obtained from the Pharmacology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada (UGM) Yogyakarta. Experimental animals were placed in cages and given food and drink ad libitum. Before treatment, mice underwent an adaptation for one week, and no mice were found sick or died.

Black cumin seeds were obtained from the Ting Bao herbal shop in Purwokerto, Banyumas, Central Java. Extracts were made at the Laboratory of Pharmaceutical Biology, Department of Pharmacy, Faculty of Health Sciences, Unsoed. Black cumin seeds were extracted using maceration and vacuum evaporator methods with 96% ethanol solvent, and the results were diluted with *Carboxyl Methyl Cellulose* (CMC) until a concentration of 20 mg/ml was reached.

MRSA ATCC 43300 test bacteria were obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Diponegoro (UNDIP) Semarang with a concentration of 10^7cfu/ml.

Mice were randomly divided into five groups. Control group (K) was mice infected with MRSA without any treatment, treatment 1 (P1) was mice treated with 50mg/kgbb/day or 0.05ml *N. sativa* extract for seven days and infected with MRSA 0.2ml (10^7cfu/ml), treatment 2 (P2) was mice treated with 250mg/kgbb/day or 0.25ml *N. sativa* extract for seven days and infected with MRSA 0.2 ml (10^7cfu/ml), treatment 3 (P3) was mice treated with 500mg/kgbb/day or 0.5ml *N. sativa* extract for seven days then infected with MRSA, and treatment 4 (P4) was mice treated with 750mg/kgbb/day or 0.75ml *N. sativa* extract for 7 days then infected with MRSA. MRSA inoculation was done through intraperitoneal (i.p) injection. The treatment of mice and the process of blood sampling were carried out at the Laboratory of Parasitology, Faculty of Medicine, Unsoed.

The independent variable in this study was the dose of *N. sativa* extract while the dependent variable was IL-1β levels. Blood of the mice was taken 24 hours after MRSA injection and followed by IL-1β level examination. Mice were then terminated. IL-1β examination was carried out in the Research Laboratory, Faculty of Medicine, Unsoed by using Bioassay Technology Laboratory Mouse IL-1β ELISA Kit.

The research data were processed and analyzed using the SPSS 22.0 for Windows Data were presented in the form of mean and standard deviation. Normality test with the Shapiro-Wilk on the measurement results of IL-1β levels taken 24 hours after MRSA infection showed normal data distribution, so the differences in IL-1β levels were analyzed using the One Way ANOVA.

RESULTS

The results showed that increasing dosage of *N. sativa*

extract resulted in a higher mean of IL-1β level, but the difference was not statistically significant (Table 1). This could be due to the large variation in each group. Further studies are needed to identify and control other factors that influence IL-1β levels.

Tabel 1. Mean of IL-1β of each treatment group

Group	Mean ± SD (ng/L)	p-value
K	11,65 ± 5,15	0,578
P1	14,07 ± 8,75	
P2	16,66 ± 3,07	
P3	18,54 ± 14,43	
P4	19,49 ± 5,76	

Note: K = mice infected with MRSA and not treated, P1=mice treated with *Nigella sativa* extract 0.05ml for 7 days and infected with MRSA, P2=mice treated with *Nigella sativa* sativa extract 0.25ml and infected with MRSA, P3=mice treated with *Nigella sativa* extract 0.5ml and infected with MRSA, and P4=mice treated with *Nigella sativa* extract 0.75ml and infected with MRSA

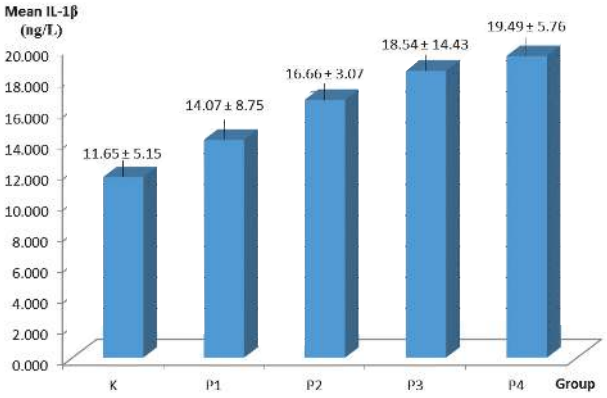

Figure 1. Bar chart of IL-1β level

Note: K=mice infected with MRSA and not treated, P1=mice treated with *Nigella sativa* extract 0.05ml for 7 days and infected with MRSA, P2=mice treated with *Nigella sativa* extract 0.25ml and infected with MRSA, P3=mice treated with *Nigella sativa* extract 0.5ml and infected with MRSA, and P4=mice treated with *Nigella sativa* extract 0.75ml and infected with MRSA

This study result showed an increase in IL-1β levels along with the addition of black cumin dose even though statistically there was no significant difference among groups (p=0,578: ANOVA test).

DISCUSSION

The results showed an increase in the average IL-1β level along with the increasing dose of *N. sativa* extract, however difference test using ANOVA showed no significant difference among groups. Previous studies also identified that black cumin extract could act as immunomodulator. Aldi and Suharti showed that administration of black cumin ethanol extract dose of 50mg/kg BW, 100mg/kg BW, and 200mg/kg BW in mice given antigen (Goat Eritrosit 5%) could increase the number of lymphocytes, monocytes, and reduce the number of segment neutrophils significantly (12). Sari and Purnawati also found that *N. sativa* extract dose of 5.2gr/day could increase macrophages nitric oxide (NO) production of Balb-c mice infected by *Salmonella thyphimurium* (13). In contrast to research by Zainiyah that black cumin extract could reduce pro-inflammatory cytokines IL-6 in endothelial dysfunction of preeclampsia-induced Balb-c mice (14). Research conducted by Muhyi found no significant differences in the number of Th-17 cells and neutrophils in asthmatics who only received immunotherapy and placebo compared to those that received immunotherapy and *N. sativa* extract and those who received immunotherapy and probiotics (15).

The difference in the nature of *N. sativa* extract in related to the immunoregulatory effects in this study to other studies may be caused by several factors including the way of extraction, the number of samples, the composition of the active ingredients of black cumin plants, the dose of black cumin extract, and the age of the plant that significantly affects plant components and the results (16). Other studies found that *N. sativa* has anti-bacterial activity against MRSA in vitro (17). Sulistiawati stated that *N. sativa* could serve as an immunomodulator and also anti-inflammatory depending on the accompanying disease conditions because of the number of substances contained in black cumin plants (9).

In this research, *N. sativa* extract along with the increasing doses showed differences in IL-1β levels in Balb-c mice that were infected with MRSA but not statistically significant. It is possible to happens because the strong anti-MRSA effect of *N. sativa* extract causes the number of germs in the host body to decrease so that the inflammatory response becomes lower. Previous study stated that *N. sativa* could inhibits the growth of certain bacteria in vitro such as *Staphylococcus aureus*. Thymoquinone and thymohydroquinone contain in *N. sativa* act as antibacterial by causing inactivation of the bacteria protein. This explains why IL-1 levels among treatment groups was not significantly difference (8). This study showed that *N. sativa* extract did not increase IL-1 levels. Besides because *N. sativa* has a strong anti-bacterial effect, this is possible because the role of *N. sativa* in overcoming MRSA infection is not through increasing IL-1β. In another study *N. sativa* could modulate inflammation, cellular and humoral adaptive immune responses. The study focused on the immunomodulatory effect of *N. sativa* seed extract on sheep macrophage functions *in vitro*. The monocytes derived macrophages (MDM) were cultured with *N. sativa* seed extract and their morphological changes, phagocytic activity, nitric oxide production, and microbicidal activity were investigated. Significant increases in phagocytic activity to Candida albicans yeast and bacteria per individual MDM were observed in cells cultured with seed extract. It could be concluded that *N. sativa* seed extract can enhance macrophages important innate immune functions that could control infectious diseases and regulate adaptive immunity (18). Because the increasing of the potential microbial killing by macrophages due to administration of *N. sativa* extract, it is possible that the release of IL-1 cytokines that invite another macrophag to overcome infection become not really necessary. So it may explain why IL-1 cytokine in this study was not significantly difference.

One of the active compounds of the black cumin is Thymoquinone (TQ). On humoral immunity, Mohany and colleagues investigated Thymoquinone effects on pesticide induced immunotoxicity in male albino rats. Among several biochemical and histopathological changes, Imidacloprid insecticide treatment caused a

significant decline in total Ig levels (especially IgGs) and a significant inhibition of antibody hemagglutination. Intraperitoneal injection of TQ reversed the immunological effects, leading to significantly increased total Ig levels and antibody hemagglutination. The findings of this study provided hints that TQ may potentially modulate the outcome of humoral immune responses (19). Another thing that causes IL-1 levels in this study is not statistically significant is maybe because *N. sativa* extract contains various compounds, one of which is have anti-inflammatory effect so that it can overcome infection in host cells without causing severe tissue damage.

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