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EFFECTS OF BLACK SEEDS EXTRACTS SUPPLEMENTATION ON IFNγ LEVEL OF MULTIBACILLARY LEPROSY PATIENTS RECEIVING WHO-MDT

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PENGARUH SUPLEMENTASI JINTEN HITAM TERHADAP KADAR IFN-γ KUSTA TIPE MB YANG MENDAPAT TERAPI MDT-WHO

ABSTRAK

Latar belakang: Interferon-γ merupakan sitokin pengaktivasi makrofag utama, berfungsi penting dalam pertahanan alamiah dan imunitas seluler terhadap mikroba intraseluler seperti *M. leprae*. Beberapa penelitian menunjukkan bahwa Jinten Hitam dapat meningkatkan produksi IFNγ, memiliki efek potensiasi terhadap sistem imunitas seluler dan efek stimulasi terhadap sel Th1 serta memiliki efek inhibisi terhadap sel Th2. Studi ini bertujuan mengetahui pengaruh suplementasi jinten hitam terhadap kadar IFNγ kusta tipe MB yang mendapat terapi MDT-WHO.
Metode: studi eksperimental dengan desain *randomized controlled trial*. Sebanyak 44 pasien kusta MB dikelompokkan menjadi 2 kelompok: (i) kelompok plasebo, yang mendapatkan MDT-WHO dan plasebo, (ii) kelompok Jinten Hitam, yang mendapatkan MDT-WHO dan Jinten Hitam. Variabel independen adalah pemberian MDT-WHO dan Jinten hitam serta MDT-WHO dan plasebo, yang diberikan selama 2 bulan. Variabel dependen adalah kadar IFNγ.
Hasil: Rata-rata kadar IFNγ sebelum dan sesudah perlakuan pada kelompok jinten hitam secara statistik berbeda bermakna (p<0,0001) dan rata-rata nilai delta kadar IFNγ pada kelompok jinten hitam juga secara signifikan lebih tinggi (p<0,05) dibanding plasebo.
Simpulan: Suplementasi dengan ekstrak Jinten Hitam dapat meningkatkan kadar IFNγ pada pasien kusta tipe multibasiler (MB).

Kata kunci: Ekstrak Jinten Hitam, *nigella sativa*, lepra multibasiler, IFNγ

ABSTRACT

Background: *Several studies showed that black seeds enhanced the production of IFN γ, posses potent potentiating effects on the CMI/ stimulatory effect on Th1 cells, while posses suppressor effects on humoral immunity/ inhibitory effect on Th2 cells.The purpose of this study is to know the effects of black seeds extract supplementation on IFN γ level of multibacillary leprosy patientswho received MDT-WHO therapy.*
Methods: *This study is experimental research with randomized controlled trial design. A sample of 44 patients was randomized into 2 groups: (i) placebo group, receiving WHO-MDT and placebo, (ii) black seed goup, receiving WHO-MDT and black seed extracts. The independent variable was the WHO-MDT plus black seed supplementation (3000 mg), and WHO-MDT plus placebo given for 2 months . The dependent variable was IFN γ level.*
Results: *Mean level of IFN γ before and after study from black seed groups was significantly different (p<0,0001), and mean delta value of black seed group was significantly superior (p=0,005/p<0,05) to that of placebo.*
Conclusion: *Supplementation with black seeds extracts can enhance IFNγ production of multibacillary leprosy patients.*

Key words : *MB Leprosy, Black seeds extract, Nigella sativa, IFN-γ*

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INTRODUCTION

Leprosy is a chronic infectious disease that can cause deformities, disabilities and stigmatism. The use of Multidrug therapy (MDT) had lower the global prevalence from 5,2 million in the 1980s to 200.000 in 2014. However, the new case detection rate remains high; which around 220.000–250.000 people have been diagnosed with leprosy each year.Indonesia is the third country with the highest leprosy case following India and Brazil. Another strategies might be needed beside early detection and multidrug therapy. Nowadays, review and study of micronutrients impact in leprosy are developed to achieve better clinical outcomes related to treatment response and preventing household contact from developing infection.

Quality and quantity of cytokines are critical in initiation and excecution of immunity. Excessive or insufficient production of cytokines may significantly contributeto the pathophysiology of a range of disease responses and are thought to be decisive for pathological or physiological consequences. After activation, CD4 T helper cells differentiate into their Th1 type cells, secreting IL- 2, IL-12, IFN-γ and TNFα, or Th2 type cells, secreting IL-4, IL-5, IL-10 and IL-13. Indeed, the balance between Th1 and TH2 cytokines is critical for the orientation of the inflammatory responses toward cell- mediated or humoral-mediated responses. Thus, any factors that can interfere with Th1/Th2 axis might affect the outcome of the response.

Leprosy has clinical manifestation spectrum related to host immune response against *M.leprae*. Its characterized by two polar disease form. In polar tuberculoid leprosy there is a strong cell mediated immune response (CMI) which limits the disease to a few lesions with little evidence of bacilli. Th1 cytokine like TNFα and IFN-γ are expressed stronger in tuberculoid pole/pausibacillar spectrum. In contrast, at another spectrum (lepromatous pole), patients exhibit a defective CMI response to *M. leprae* there by causing a high bacillary load and lots of skin lesions, making untreated multibacillary patients becoming main reservoir and transmission of *M.leprae*. Th2 cytokines like IL-4, IL-5 and IL-10 elevated anc becoming characteristic of lepromatous or multibacillar patiens, with low level of Th1 cytokines, including IFN-γ.

Interferon is the principal macrophage-activating cytokine and serves critical functions in immunity against intracellular microbes, including *M.leprae*. Interferon enhances production of reactive oxygen species and nitrogen intermediates by macrphage dan indusce macrophage to kill and limiting intracelluler pathogen growth. Interferon involved in the formation of granulomas containing well-differentiated, bactericidal macrophages that play an essential role in preventing the extension and dissemination of mycobacterial infections. The protective functions of IFN γ has been emphasized in several reports that describe the deleterious effect of mutation and deletions in IFN γ genes and their receptors on mycobacterial infections.

Seeds of *Nigella sativa*/black seed, which belongs to *Ranunculaceae* family already well known and widely used in traditional medicines. One of the precious properties of black seeds is the immunomodulatory effects of its constituents. Several studies showed that black seeds enhanced the production of IFN γ, posses potent potentiating effects on the CMI/ stimualtory effect on Th1 cells, while posses suppressor effects on humoral immunity/ inhibitory effect on Th2 cells

The relationship between black seeds extract supplementation and IFN γ level of multibacillary leprosy patients is still unknown. Therefore, in the present study the effect of black seeds extract supplementation on IFN γ level of multibacillary leprosy patients is examined.

METHODS

The study design was experimental research with randomized controlled trial design. A sample of 44 patients was randomized into 2 groups. They were selected by consecutive sampling and subjected to the double blind treatment. All patients received the standard WHO-MDT regimen for leprosy, while 1 group received black seeds supplementation and another group received placebo. Standard drug used was WHO multidrug therapy, while black seeds used in this study were manufactured and standardized by the pharmaceutical industry Habasa Al-Afiat, Indonesia. Inclusion criterias were newly diagnosed multibacillary leprosy patients or no longer than 3 months on multidrug therapy, not having systemic, autoimmune or other infection

Table 1. Characteristic data regarding gender, age, education and body mass index variable

	Black seeds group			Placebo group			*p*
	n (%)	*Mean* ± SD	*Median* (Min-max)	*n* (%)	*Mean* ± SD	*Median* (Min-max)	
Gender							
Male	15 (68.2)			15 (68.2)			1.00[a]
Female	7 (31.8)			7 (31.8)			
Age		37 ± 14.0	35 (18–64)		43 ± 12.0	45 (18–60)	
Education level							
Uneducated	4 (18.2)			3 (13.6)			0.661[a]
Elementary school	11 (50.0)			15 (68.2)			
Junior high school	5 (22.7)			3 (13.6)			
Senior high school	2 (9.1)			1 (4.5)			
Body mass index		19.93 ± 3.46	20.20 (10.94–24.44)		19.53 ± 2.41	19.21 (16.61–26.16)	0.656[b]
Underweight	6 (27.3)			9 (40.9)			
Normoweight	16 (72.7)			12 (54.5)			
Overweight	0 (0.0)			1 (4.5)			
Obese	0 (0.0)			0 (0.0)			
Family history with leprosy							
Positive	20 (90.9)			19 (86.4)			1.000[a]
Negative	2 (9.1)			3 (13.6)			

disease, noton any immunosuppresan or immunomodulator drugs/supplement and were willing to sign informed consent. Exclution criteria were patients on reactional states or pregnancy.

The independent variable was the WHO-MDT plus black seed supplementation (3000 mg), andWHO-MDT plus placebo given for 2 months. The dependent variable was IFN γ level .

Analysis of the data was calculated by using SPSS (SPSS Inc, Chicago, IL) to know the differenceslevel of IFNγ serum before and after the study. A *p value* less than 0.05 was considered significant. Approval of the Medical Ethic Committees was obtained before the start of the study (sebutkan Ethical Clearance No...../.../....???). Only patients who had given informed consent were included in the study.

RESULTS

Fourty two patients met the inclusion criteria were collected. Those patients were divided into 2 groups, (i) placebo group, receiving WHO-MDT and palcebo, (ii) black seed goup, receiving WHO-MDT and black seed extracts. Both group consist of 15 men and 7 women each. Mean age of black seed group was 37±14 years with a range from 18 to 64, while placebo group was 43±12 years with a range from 18 to 60. The level of education of black seed group was not educated in 4 (18.2%), elementary school in 11 (50%), junior high school in 5 (22.7%) and senior high school in 2 (9.1%) patients, while placebo group was not educated in 3 (13.6%), elementary school in 15 (68.2%), junior high school in 3 (13.6%) and senior high school in 1 (4.5%) patients. The mean body mass index (BMI) of black seed group was 19.93±3.46 and placebo group was 19.53±2.41 (table 1?). Family history of leprosy was positive in 20 (90.9%) patients from black seed groups and 19 (86.4 %) in palcebo groups. There was no significant difference in those characteristic between two groups.

Mean level of IFN γ before and after study from black seed groups was significantly different (p<0.0001), which mean level of IFN γ before study was 4.24 pg/ml and after study was 7.57 pg/ml

Tabel 2. Wilcoxon test of differences in IFN gamma levels before and after study from black seed group

IFN γ	*n*	Mean ± SD	Median	Min–Max	*p*
Before study	22	4.24 ± 4,93	3.50	0.31–25.16	<0.0001[a]
After study	22	7.57 ± 5.57	6.51	0.45–28.68	

Tabel 3. Wilcoxon test of differences in IFN gamma levels before and after study from placebo group

IFN γ	*n*	Mean ± SD	Median	Min–Max	*p*
Before study	20	6.59 ± 3.75	5.41	3.18–19.12	0.639[a]
After study	20	7.49 ± 6.96	3.98	1.27–22.15	

Tabel 4. Mann Whitney test of difference between the delta level IFN gamma of black seed extracts and placebo group

	n	Mean ± SD	Median	Min–Max	*p*
Black seed group	22	3.33 ± 2.16	3.5	(-1.27)–8.28	0.005[a]
Placebo group	22	0.91 ± 7.36	-0.96	-11.14–17.42	

(table 2).

Mean level of IFN γ before study from placebo groups was 7,49 pg/ml and after study was 6.59 pg/ml. This difference was not statistically significant (p=0.639) (table 3).

Mann Whitney test showed that mean delta value of black seed group was significantly superior (p=0.005/ p<0.05) to that of placebo (table 4).

DISCUSSION

This study investigated the effect of black seed extracts on IFNγ level of multibacillary leprosy patients. We analyse IFN γ level before and after study from two multibacillary leprosy groups on WHO-MDT less than 3 months. One group received black seed extracts while other received placebo.

In this study there was increasement on IFN γ level from both group. But the increasement wasno significant in placebo group which only receiving MDT-WHO and placebo. This study match with study by Joshi *et al* which found that more WHO-MDT treated multibacillary patients produced IFN γ than did untreated patients, but there was no significant difference in the level between untreated and WHO-MDT treated multibacillary patients.

In our study we found that theres was significant differences between IFNγ level before and after study from black seed extract groups. We also found significant differences on delta IFNγ level between black seeds extract and placebo groups.

The effect of black seed extracts on IFNγ level of multibacillary leprosy patients has not been studied previously. One proposed mechanism of action from black seed extracts is its regulation of Th1 and Th2 balance. Some study found that black seeds extracts posses potent potenstiating effects on the cellular (T cell-meditaed) immunity, while posses suppressor efects on B cell-mediated (humoral) immunity. Study by Boskabady (2011) on effect of black seed extracts on ovalbumin sensitized guinea pigs found that the black seed extracts enhanced the production of IFNγ and caused inhibitoy effect on IL-4, indicating the inhibitory effect on Th2 cells and stimulatory effect on Th1 cells. The effect of black seed oil on murine cytomegalovirus infection showed the most striking inhibition of virus titers in the spleen and liver which was showed by undetectable virus titer on day-10 and an increase in the serum level of IFNγ. Study of black seeds' adjuvant effects to improve allergic rhinitis patients showed statistically significant increase in the phagocytic and intracellular killing activities of PMNs. El Gazzar *et al* (2006) also examined effect of black seed in mouse model of allergic lung inflammation which showed a marked decrease in Th2 cytokines in black seeds terated sensitized mice.

In leprosy, the quality and quantity of the innate and adaptive immune response determine the outcome of infection; whereas the pro inflammatory cytokine IFNγ provides protection against mycobacteria, the anti-inflammatory cytokines IL-10 has been showd to be associated with dampening Th1 cells' responses toward mycobacteria. Thus, changes in the IFNγ/IL-10 ratio may provide information about potential disease development or response to treatment. It is hoped that black seeds extract supplementation on multibacillary leprosy patienst receiving WHO-MDT can inprove disease prognostic and lower disease transmission risk by increasing patients' low cellular immunity.

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