Effect of *Nigella sativa* Extract on Bacteriology Parameter of *Multibacillar leprosy* Patients Administering MDT-WHO: Three Months Observation

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Abstract: MDT-WHO implementation has decreased leprosy global prevalence. However, number of new cases detected is still high. Therefore, new appropriate strategy, besides early detection and WHO-MDT treatment which are the main strategies in leprosy eradication, is needed. The aim of this study was to investigate the effects of *Nigella sativa* extract on bacteriology parameters of multibacillar leprosy patients administering MDT-WHO. It was Double Blind - Randomized Controlled Trial study which was conducted in Jepara, Central Java Province, Indonesia during February to June 2016. This study was performed on 60 eligible multibacillar leprosy patients administering MDT-WHO. Bacterial index (BI) was measured using Ridley logarithmic scale, whereas morphology index (MI) was measured as percentage of solid bacteria to the all forms of bacteria. There was no subject who dropped out. The characteristics of subjects were similar between the two groups, except for the MDT duration at baseline, where MDT duration in control group was longer. There was no significant difference in BI proportion and MI proportion in the two groups after treatment. Average BI in month 2 and 3 tend to be lower than control although not statistically significant. There were significant differences of average BI decline between control and treatment groups in month 2 (p=0.000) and month 3(p=0.005). There were statistically significant differences of average MI and average MI decline between control and treatment groups in month 1 (p=0,000, p= 0.001, respectively). This study demonstrated that *Nigella sativa* extract affected average MI and BI decline differences. Its efficacy as adjuvant therapy might be confounded by duration of MDT-WHO.

1 INTRODUCTION

Leprosy is still world burden. Leprosy is infectious disease that does not only affect medical condition, but also social, economy, culture, and national defense aspects. In most of the cases, leprosy does not cause disability when it first manifests. Conditions contributing to disability and deformity are preventable when early detection can be performed (Kemenkes RI, 2012).

Multi Drug Therapy recommended by World Health Organization (MDT-WHO) has decreased leprosy global prevalence from 5.2 million cases in 1980 to 200,000 cases in 2014. However, new cases detected in 2014 were still high that is about 220,000-250,000 new cases are diagnosed every year (Smith & Aerts, 2014). Indonesia is a country with the third highest leprosy prevalence in the world after India and Brazil. Central Java is the province which has the second highest new cases of leprosy in Indonesia (Kemenkes RI, 2012). Therefore, new appropriate strategy, besides early detection and WHO-MDT treatment which are the main strategies in leprosy eradication, is needed. To date, there are some studies that have investigated micronutrients as adjuvant therapy in leprosy treatments as well as to prevent contact from getting infection (Smith & Aerts, 2014; Vazquez et al., 2014; Rahfiludin, 2011).

Lepromatous or multibacillar (MB) leprosy has lower cellular immunological reaction against *M. leprae*. Multibacillar leprosy was characterized by high bacillary load and high number of skin lesions. Thus, untreated MB patients are the main source of leprosy transmission (Walker & Lockwood, 2006).

The presence of acid-fast bacilli on skin tissue scrapings is considered as one of the main signs of

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leprosy and sufficient to establish a diagnosis of leprosy. In 1982, WHO recommended the use of MDT as a standard treatment for leprosy and introduced an operational classification of leprosy which differentiated leprosy into PB and MB based on Reitz serum smear. Bacterial Index (BI) and Morphological Index (MI) are used for the serum Reitz smear assessments. Bacterial Index is a semi-quantitative measure of leprosy bacillus density in the smear. BI helps in determining the type of leprosy and assessing the outcomes of the treatment. Morphology Index is a percentage of the solid form to all forms of leprosy bacillus. The Morphology Index is useful for assessing the transmission and the outcomes of the treatment and determining drug resistance (Kemenkes RI, 2012).

Black cumin/ black seed (*Nigella sativa*) belongs to *Ramunculaceae* family and is popular as herbal medicine.(6) The efficacies of *Nigella sativa* that have been widely known by the community included as anti-parasite, antimicrobial, anti-inflammatory, improving liver and kidney function, treatment for respiratory and digestive disorders, and immune-modulator. Some studies showed that continue *Nigella sativa* administration can improve immune responses. *Nigella sativa* oil has potential potentiation effect to cellular immune response through Th1 modulation and Th2 suppression, meanwhile the other contents can suppress cell-mediated immune system (humoral) (Salem, 2005; Boskabady et al., 2011).

To our knowledge, there has not been any research article evaluating the effect of *Nigella sativa* extract on bacterial parameter in MB leprosy patients. Therefore, this study sought to study the effects of *Nigella sativa* on bacteriological parameters of MB leprosy patients.

2 METHODS

The double blind-randomized control trial was conducted from February to June 2016 in Donorejo Hospital, Pati Regency, Central Java Province, Indonesia. Donorejo Hospital was referral hospital for leprosy.

Eligible subjects for this study were those who gave their consent after thorough explanation and met inclusion and exclusion criteria. Subjects were men and women aged 20-60 years who had not received MDT-WHO or had been on MDT-WHO for at least 1 month, did not have leprosy reaction, did not pregnant or breastfeed, and did not consume any drugs with immune-suppressant or immune-modulator effects in the past 1 month. All subjects who did not follow research protocols or experienced drug eruption due to MDT and *Nigella sativa* are excluded.

Multibacillary leprosy was diagnosed according to WHO classification. Ritz serum from both ear lobules and active lesion were stained with acid-fast stain and observed under microscope to obtain MI and BI indexes. BI was calibrated using Ridley algorithm and scaled from 0 to +6 points. MI was counted from the percentage of solid mycobacterium to all mycobacterium observed in the smear. MI was expressed as percentage.

Subjects who were enrolled to this study were randomized into two groups, i.e. treatment group and controlled group. Subjects in controlled group were asked to take two capsules of 500 mg *Nigella sativa* oil, three times daily for 3 months. Subjects in control group were asked to take placebo capsules in the same frequency and duration. Each placebo capsules contain 1000 mg lactose and had the same appearance as *Nigella sativa* oil capsules. All of subjects received WHO-MDT. Evaluation of BI and MI through Reitz serum smear was performed in month 1, 2, and 3.

This study was approved by Health Ethics Committee of Medical Faculty, Diponegoro University and Kariadi Central Hospital, Semarang, Indonesia.

3 RESULTS

During study period there were 65 patients who clinically diagnosed having MB leprosy and only 60 subjects had positive Reitz serum. Therefore, only 60 patients were enrolled to this study. Through randomization, the subjects were divided equally into treatment and control groups. There was no dropped out subject. All of subjects' characteristics, except MDT duration, were similar in the two groups. The MDT duration in the treatment group (Mean ± SD, 1.67 ± 1.3) was significantly shorter than in the control group (Mean ± SD, 2.53 ± 1.1), $p = 0.007$.

Table 1. Average BI and MI in month 0, 1, 2, and 3

	Treatment			Control			*P*
	N	Mean ± SD	Median (Min – Max.)	N	Mean ± SD	Median (Min – Max.)	
BI Index							
Month 0	30	4.53 ± 0.97	4 (3 – 6)	30	3.87± 0.70	4 (3 – 5)	0.007[a]
Month 1	30	2.43 ± 0.94	3 (1 – 4)	30	2.13± 0.86	2 (1 – 3)	0.188[a]
Month 2	30	0.23± 0.63	0 (0 – 2)	30	0.47± 0.73	0 (0 – 2)	0.097[a]
Month 3	30	0	0 (0 – 0)	30	0.03 ± 0.18	0 (0 – 1)	0.317[a]
MI Index							
Month 0	30	32.00± 27.72	15 (10 – 80)	30	12.00 ± 9.61	10 (0 – 60)	0,000[a]
Month 1	30	6.33 ± 5.56	10 (0 – 20)	30	4.67± 5.07	0 (0 – 10)	0,000[a]
Month 2	30	0	0 (0 – 0)	30	0	0 (0 – 0)	
Month 3	30	0	0 (0 – 0)	30	0	0 (0 – 0)	

Table 2. Average of BI and MI decline difference

	Treatment		Control		*P*
	Mean ± SD	Median (Min – Max.)	Mean ± SD	Median (Min – Max.)	
Δ IB month 1	2.10± 0.88	2 (0 – 3)	1.73± 0.69	2 (1 – 3)	0.064[a]
Δ IB month 2	4.30± 0.95	4 (2 – 6)	3.40± 0.86	3 (2 – 5)	0.000[a]
Δ IB month 3	4.53 ± 0.97	4 (3 – 6)	3.83 ± 0.70	4 (3 – 5)	0.005[a]
Δ IM month 1	25.67± 25.28	10 (0 – 70)	7.33± 9.80	10 (0 – 50)	0.001[a]
Δ IM month 2	0	0 (0 – 0)	0	0 (0 – 0)	
Δ IM month 3	0	0 (0 – 0)	0	0 (0 – 0)	

There was no significant difference of bacterial index proportion at baseline (month 0) between treatment group and control group. There were BI proportion changes in both groups in month 1, 2, and 3. However, the changes were not statistically significant (data was not shown). The average BI of treatment group in month 0 is significantly higher from the control group (p= 0.007). However, in the following months the difference became insignificant, and even average BIs in treatment group tended to be lower in month 2 and 3 as shown in Table 1.

There was no significant difference of average BI decline (Δ BI) in month 1 between two groups. However, the comparison was statistically significant in month 2 (p= 0,000) and month 3 (p= 0,005) as shown in the Table 2.

At baseline there was statistically significant difference of MI proportion between the two groups (p= 0.037). After 1 month observation, significant difference of MI proportion was not observed (p= 0.394). Statistics analysis could not be obtained in month 2 and 3 due to all subjects in group treatment were in MI 0% (data was not shown).

Average MI at baseline and month 1 were significantly different between the two groups as shown in the Table 1. Average of MI decline (Δ IM) of treatment group in month 1 was significantly higher than control group (p= 0.001) as shown in the Table 2. Bivariate analysis could not be obtained in month 2 and 3 due to average MI in both groups were 0.

4 DISCUSSION

MDT duration between the two groups was significantly different at baseline suggested that MDT duration could confound interpretation of *Nigella sativa* administration effect in this study. One or two doses of rifampisin killed 10^5 organisms, decreased organism numbers into 10^4 in MB leprosy, and eliminated most of them. Rifampisin was the most effective anti-leprosy drug and could decrease MI of lepromatous leprosy into 0% in 5 weeks (Bryceson et al., 1990). Therefore, significant MI and BI decline in this study might be due to MDT or *Nigella sativa* administration.

Administration of *Nigella sativa* capsules 1000 mg three times daily could significantly decrease the average of BI decline difference on month 1, 2, and 3 compared to baseline. The average BI decline difference in treatment group (4.53 ± 0.97) was higher than the control group (3.83 ± 0.69). However,

BI proportion difference between the two groups in month 1, 2, and 3 were not statistically significant. Those suggested that *Nigella sativa* did not result in different BI outcomes.

At baseline, there was significant difference of MI proportion in both groups. However, after 1 month therapy, the proportion comparison became not statistically significant. This suggested that *Nigella sativa* did not significantly decreased MI decline in treatment group compared to MI decline in control group.

Immunity mechanisms relied on Accessory Immune System (AIS) reactivation which was responsible for leprosy and stimulation of T or B lymphocytes. T cells regulated T cytotoxic cells induction, B lymphocyte function and macrophage function by releasing cytokines. Th1 cells release IFN-γ which would activate macrophages to kill bacteria or to inhibit microbe growth and would trigger T cytotoxic cell response resulting in self-healing disease (Williams & Kupper, 2012). In contrast, Th2 cells facilitated humoral immune response and inhibited cellular immune response which caused progressive infection. There were cross regulatory cytokines. Th1 released IFN-γ which down regulated Th2. Th2 released IL-4, IL-5, and IL-10 which inhibited and down regulated Th1 and macrophages. (Williams et al., 2012; Abulafia & Vignale, 2001).

Studies had demonstrated that *Nigella sativa* extract had immune-modulator effect. *Nigella sativa* had strong potentiation effect on cellular immunity (mediated by T cell), as well as suppression effect on humoral immunity which was mediated by B lymphocytes. A study which was conducted more than a decade ago, showed that *Nigella sativa* could increase human immunity when was used regularly. Most of subjects who administered *Nigella sativa* for 4 weeks showed increasing T CD4 to CD8 ratio by 55% and increasing natural killer cell function by 30% (Salem, 2005).

Boskabady et al. studied about *Nigella sativa* immune-modulator effects in albumin desensitized guinea pigs and observed the increase of IFN-γ and the decrease of IL-10, suggested that *Nigella sativa* had inhibition effect on Th2 cell and its cytokines and stimulation effect on Th1 and its cytokines (Boskabady, 2011). Study on cytomegalo-virus infected murine showed that serum IFN-γ level was associated with undetected virus on the 10^{th} day (Salem, 2000). Study on allergic rhinitis patients showed that supplementation with *Nigella sativa* 2 gram daily for 30 days would increase PMN fagocytosis activity and intracellular killing (Isik et al., 2010).

In lepromatous leprosy, immune response against *M. leprae* was dominated by T-suppressor cells ($CD8^+$, $CD28^-$), with only small amount of $CD4^+$. Naive T cells commonly could not be activated immediately. In this type of leprosy, high T2 activity also observed, which should increase IL-4, IL-5, and IL-10 productions. These cytokines would stimulate B lymphocytes transformation into antibody-secreting cells which produced antibody.(Bryceson et al., 1990; Williams & Kupper, 2012; Abulafia & Vignale, 2001) Therefore, *Nigella sativa* supplementation to MB leprosy patients administering MDT-WHO would improve patients' prognosis.

It was possible to increase *Nigella sativa* clinical efficacy and effect on lipid metabolism by means of increasing the dose. However, it would increase the gastrointestinal side effect. Daily 40 mg/kg *Nigella sativa* administration was well tolerated by adults and children. There was no side effect observed, except for children who received 80 mg/kg dose (Kalus et al., 2003) Other toxicity study demonstrated that 1 g/kg *Nigella sativa* supplementation for 28 days did not elevate liver enzyme and did not cause toxicity effect on the liver function (Dollah et al., 2013).

There were some limitations in this study. There was inhomogeneity of MDT duration at baseline due to the difficulty of finding new untreated cases as the study subjects. In addition, in Donorojo Hospital, Reitz serum examination were only performed on serum from right and left ear lobules, whereas this study only included patients from Donorejo Hospital. The other limitation of this study was the study only gave one supplementation dose to subjects.

5 CONCLUSIONS

This study had not proved *Nigella sativa* effect on decreasing BI and MI of MB leprosy patients. However, there was significant difference of average MI and BI decline between treatment and control group. Further studies should consider the subjects homogeneity especially for MDT duration, enroll new untreated cases only, use various *Nigella sativa* doses, and be performed in larger field.

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