Study of Black Cumin Seed Oil (BCSO) (*Nigella sativa L.*) as an Immunomodulator in The Healthy Active Smoker Volunteer

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Abstract. A Black cumin seed oil (BCSO) contains many unsaturated fatty acids and essential oils that are antioxidative. The Black Cumin Seed Oil (BCSO), both in vitro and in vivo, has been used as an immunomodulatory compound and is expected to increase lymphocytes number and IL-2 expression. The purpose of this study is to find out how BCSO influences the percentage of peripheral blood lymphocytes and IL-2 expression in active smokers. A total of 36 healthy, active volunteer smokers aged> 18 years were divided into four groups. Group 1 (placebo) was given a 3x1 capsule/day placebo; group 2, 3, and 4 were given a dose of BCSO 3x1, 3x2, and 3x3 capsule/day. Placebo and BCSO interventions were provided for 30 days. A flow cytometer determined the percentage of lymphocytes and IL-2 expression. There were no significant differences between groups. Based on IL-2 test subjects' average rate, the lowest IL-2 expression was in the placebo group (1.67%) and increased in the BCSO group (3.54%, 3.49%, and 3.72%). The effect of BCSO administration on the percentage of lymphocytes and IL-2 expression were not statistically significant.

Keywords: BCSO, lymphocytes, interleukin-2 expression, healthy-active smokers

1. Introduction

Black Cumin Seed Oil (BCSO) has been widely used as a potent immunomodulator, antioxidant, antidiabetic, antihypertensive, anti-inflammation, anticancer, analgesic, antimicrobial, spasmolytic, hepatoprotection, a bronchodilator, and antioxidant with its main ingredients, namely thymoquinone, nigellone, and unsaturated fatty acids [1-4]. The BCSO is thought to reduce the immunosuppressive effects of exposure to cigarette smoke or exposure to smoke from motorized or diesel vehicles. Motor vehicle fumes from burning petroleum and cigarette smoke are harmful to health. Smoke from motor vehicles and cigarette smoke contains toxic, carcinogenic, and immunosuppressive compounds [5]. Smoking directly can expose at least 60 potent carcinogens to epithelial tissue that has the potential to cause DNA damage to the larynx, bronchi, and lung epithelial cells [6]. The most common compounds in cigarette smoke are nicotine, 7,12-dimethylbenz[a]anthracene (DMBA), carbon monoxide, carbon
dioxide, benzopyrene, tar, and nitrogen oxide[7]. The content of cigarette smoke can affect the innate and adaptive immune system [8]. Nicotine and tar have immunosuppressive effects on innate and adaptive immune responses. Tobacco products that contain high concentrations of tar and nicotine cause significant immunological impact [9]. Nicotine and tar can suppress the expression of IL-2 in peripheral blood mononuclear cells (PBMC) by reducing signaling in T-cell activation mediated by antigens [10-12].

The immunomodulator can stimulate the development and activity of lymphocytes[13]. Immunomodulators are substances that can help improve the function of the immune system[14-15]. The synthetic immunomodulators drugs have many unexpected effects, such as immunosuppressants (azathioprine, chlorambucil) and hepatotoxic. Immunostimulants (levamisole and arginine) agents can increase uric acid levels, urticaria, and agranulocytosis [16]. Further research needs to be done to prove the immunomodulatory activity of extracts and isolates of medicinal plants, which are expected to have smaller side effects [15,17]. Previous studies on using BCSO as an immunomodulator have suggested a significant increase in the number of lymphocytes and T lymphocytes [18]. Research with mice proved that black cumin seed extract has potential as an immunomodulator, and n-hexane extract has high potential as an immunostimulator compared to other extracts [19-21]. The extracts of *Nigella sativa* can significantly increase splenocyte proliferation, secrete Th2, suppress proinflammatory mediators in Th1[22]. The previous study showed the effect of thymoquinone prevention on tracheal and lung inflammatory responses [23]. Based on the description, this study was conducted to determine the impact of BCSO as an immunomodulator on lymphocyte percentage and IL-2 expression in active smokers.

2. Methods and Materials

2.1. Research design

This type of research is an open-label clinical trial. This research is a phase I clinical trial. This phase evaluates the method of drug administration, drug dosage, frequency of administration, side effects, and maximum tolerable dose (MTD) [24-26]. This research was carried out in the Jetis I Public Health Center (J1PHC) in Bantul, Yogyakarta, starting in September 2019 until October 2019. This study has received ethical clearance or information passed the proper review from the Research Ethics Commission of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta No. 166 / EP-FKIK-UMY / V / 2019.

2.2. Research instruments and subjects

Case Report Form (CRF) data collection sheet is used to collect patient demographic data, physical activity, and smoking behavior. The BCSO used in this study was packaged in soft capsules containing black cumin oil, where one capsule contained ± 450 mg (excluding capsule shells). The Subject in this study were healthy-active smokers, adult males> 18 years old, and willing to become research respondents after filling out informed consent. Exclusion criteria were respondents who use herbs other than BCSO, suffer from chronic diseases, and experience allergies after giving BCSO.

2.3. Research procedure

Patient recruitment is carried out at the residents' homes in the Sindet and Blawong. After explaining the aims, benefits, and significance of the study, prospective subjects who stated that they were willing to become volunteers were asked to fill in informed consent and signatures as a form of approval for the administration of BCSO. A total of 36 respondents who met the inclusion criteria were grouped randomly into four groups. Group 1 was the control group. The Subjects were given placebo 3x1 capsules/day for one month. In contrast, groups 2, 3, and 4 were the treatment group assigned BCSO for one consecutive month with different doses. BCSO was administered orally at a dose level of 3x1, 3x2, and 3x3 capsules/day for 30 days. Interventions with placebo and BCSO are given for 30 days. Monitoring is carried out once a week by direct interview method to residents' homes to evaluate the BCSO administration's effects. On the 31st day, respondents were reassembled for a complete blood sample, lymphocyte count, and IL-2 expression after the intervention are
determined. A hematology analyzer examines a lymphocyte. A flow cytometer determines IL-2 expression. Active smokers are people who do the activity of smoking cigarette smoke directly, both smokers mild, moderate, and severe [25]. Activity within a specific time duration requires skeletal muscles' energy and movement in MET/week (Metabolic equivalent/week) [26].

2.4. Data analysis
Respondent characteristics were served in the form of frequency distribution tables. Bivariate analysis was performed to determine differences in the average number of lymphocytes and IL-2 expression between groups. The results are significantly different if the p-value <0.05 in both the One Way Anova test and the Kruskal Wallis test [27].

3. Result and discussion

3.1. Subject characteristic
The respondents' socio-demographic characteristics consisted of age, sex, marital status, education, and occupation. Table 1 illustrates the socio-demographic characteristics of the respondents in this study.

| No. | Characteristic of Respondent       | N (%)   |
|-----|-----------------------------------|---------|
| 1.  | Sex                               |         |
|     | Male                              | 36(100%)|
| 2.  | Marital status                    |         |
|     | Marriage                          | 24(66.7%)|
|     | No marriage                       | 12(33.33%)|
| 3.  | Education                         |         |
|     | Elementary school                 | 4(11,1%) |
|     | Yunior intermediate school        | 6(16,7%) |
|     | Senior height school              | 21(58,3%)|
|     | University                        | 5(13,9%) |
| 4.  | Job                               |         |
|     | Civil servants                    | 1(2,8%)  |
|     | National Private                  | 5(13,9%) |
|     | Small businessman                 | 6(16,7%) |
|     | Farmers and Labor                 | 18(50%)  |
|     | Others                            | 6(16,7%) |
| 5.  | Age (year)                        |         |
|     | <37 year                          | 16(44,4%)|
|     | ≥37 year                          | 20(55,6%)|
| 6.  | Smoking behavior (year)           |         |
|     | < 20 year                         | 14(38,9%)|
|     | ≥ 20 year                         | 22(61,1%)|
|     | Number cigarette (stick/day)      |         |
|     | < 9 stick/day                     | 17(47,2%)|
|     | ≥ 9 stick/day                     | 19 (52,8%)|
| 7.  | Physical activity (MET/week)      |         |
|     | < 2733 MET/week                   | 21(58,3%)|
|     | ≥ 2733 MET/week                   | 15(41,7%)|
From Table 1, it is known that the Subject's characteristics are all men. Most are married, work as laborers, have a high school education, and are above 37 years old. Subjects have smoked for more than 20 years and amounted to > 9 cigarettes/day. It is known that most of the Subjects have high physical activity and have an ideal BMI. Research data prove that smoking behavior in men is higher than in women; men tend to have the desire to smoke every day with an average cigarette consumption higher than women[7]. The prevalence of smokers in men is 64.9%[27]. The duration and intensity of physical activity can affect blood parameters. One method that can be used to estimate physical activity according to the calorie need is MET (Metabolic Equivalent). The respondents' average physical activity is 2924.64 MET/week, where this number is classified in the high activity category[28]. The average body mass index (BMI) of respondents in this study is 24.24 Kg/m² and ranked in the excellent grade. The BMI of smokers shows a decrease compared to nonsmokers[29]. The nicotine can interfere with eating behavior. Nicotine affects the nicotinic beta four receptors in the brain that play a role in eating behavior. It can suppress a person's appetite so that the smoker's body is thinner than nonsmokers[30].

3.2. Effect of BCSO administration on lymphocyte percentage and IL-2 expression of healthy-volunteers smokers

BCSO intervention was given for 30 days. Blood sampling at residents' homes was carried out by medical personnel from Nur Hudayah Hospital Bantul on the 31st day. Data on the measurement of lymphocyte counts and IL-2 expression are presented in Table 2.

Table 2. The One Way ANOVA analysis of the average lymphocyte of healthy respondents smokers who consumed BCSO in the work area of the J1PHC, Bantul, in the September-October 2019 period

| Group (n) | Lymphocytes (%) (mean±SD) | IL-2 expression (%) (mean±SD) |
|-----------|---------------------------|-------------------------------|
| Plasebo 3x1 (8) | 36.58±7.36 | 1.67±1.20 |
| BCSO 3x1 (9) | 38.77±9.72 | 3.54±2.92 |
| BCSO 3x2 (11) | 34.53±9.74 | 3.49±2.47 |
| BCSO 3x3 (8) | 30.54±10.50 | 3.72±2.50 |
| Total | 35.16±9.51 | 3.15±2.43 |
| P | 0.34a | 0.36b |

1 BCSO capsule = 450 mg BCSO
1 placebo capsule = 450 mg placebo
aData analysis used One Way Anova
the normal range of lymphocytes is 20-40%[31]

Description of the percentage of IL-2 expression in active smokers analyzed by flow cytometer:
Participants are healthy, active smokers. At homeostatic conditions, About 20%-40% of total leukocytes are lymphocytes, 80% of total lymphocytes are T lymphocytes, and 20% are B lymphocytes [31]. Based on the data in Table II and Figure 1, it is known that the lymphocyte number in all groups is within normal limits. Consumption of BCSO for 30 days with three dose variations did not affect lymphocyte count (p>0.05). Consumption of BCSO for 30 days was also not shown to affect IL-2 expression (p> 0.05). Based on this data, it can be seen that the consumption of BCSO for 30 days does not affect the immune system in healthy participants. Lymphocytes are the body's specific defense system. Immunostimulant increases lymphocyte proliferation in splenic tissue, increase leukocytes, and CD4 + activity. Increasing CD4 + activity is associated with a IL-2 production [22,31]. The content of cigarette smoke is immunosuppressive and immunotoxic. DMBA compounds have been shown to suppress the process of erythropoiesis in the bone marrow and reduce lymphocyte proliferation in splenic tissue [32].

4. Conclusion
The effect of giving Black Cumin Seed Oil (BCSO) on the percentage of lymphocytes in active smokers was not statistically significant. The result of the administration of Black Cumin Seed Oil
(BCSO) on the IL-2 expression rate was not statistically significant. Still, the average IL-2 expression showed an increase in the group given BCSO treatment compared to a placebo control group.

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