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Black cumin seed oil preparation consumption potentially improves adaptive cellular immune response among healthy volunteers

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ABSTRACT

Oxidative stress and inflammatory reactions are the pathological mechanisms for most degenerative diseases. The black cumin seed oil (BCSO) contains compounds that can act as antioxidants and immunomodulators. Consuming BCSO is thought to improve antioxidant and immunomodulatory parameters in obese people. This study investigated the effect of BCSO consumption on antioxidant and immunomodulatory activity in healthy volunteers. We conducted a quasi-experimental study on 12 healthy volunteers in Yogyakarta, Indonesia. We asked the volunteers to consume BCSO for twenty days. We measured blood pressure, body mass index (BMI), malondialdehyde (MDA) level, Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) activity, CD4Th, and IFN-γ expression before and after consuming BCSO. We carried out the average difference test of the parameters before and after consumption of BCSO by dependent t-test. The results showed that 3x1 BCSO preparation for 20 days reduced MDA levels and increased CDTh and IFN-γ. Consuming BCSO for 20 days potentially improve the adaptive cellular immune response parameters.

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1. INTRODUCTION

Obesity and air pollution are factors related to oxidative stress in the body [1], [2]. Oxidative stress causes damage at the cellular and tissue levels to evoke an inflammatory reaction and activate the immune system [3]. CD4Th cells are the prominent conductors of the adaptive immune response because they a role in regulating cellular and humoral adaptive immune response [4]. Interferon-γ (INF-γ) is a cytokine that functions as a mediator and regulator of natural and adaptive immunity [5]. IFN-γ is produced by activated T cells, CD4Th+ and CD8+T cells, and acts as a phagocytic promoter to eliminate intracellular pathogens [6]. A state of oxidative stress can cause a decreased immune system in the body [7]. Immunomodulators and herbal antioxidants are thought to be potentially helpful to prevent a decrease in the immune system due to reactive radical exposure.

Indonesia is a tropical country famous for its biological wealth. Black cumin seed oil (BCSO) is a well-known herbal medicine among Indonesians [8]. BCSO contains various unsaturated fatty acids and

essential oils with active substances such as thymoquinone, negellin, and nigelon [9]. Thymoquinone is the BCSO main compound; it is an anti-oxidative and immunomodulatory agent. Unsaturated fatty acids and thymoquinone are potent antioxidants and immunomodulators [10], [11]. The administration of BCSO containing thymoquinone can increase the antioxidant enzyme glutathione S-transferase (GST) and increase the number of Tregs in SD rats induced by dimethylbenzanthracene (DMBA) to inhibit excessive inflammatory reactions [12], [13]. The administration of 800 mg/kg BCSO orally in rats for four weeks does not adversely affect to Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase, serum bilirubin in normal albino rats [14]. Other studies suggest that Nigella sativa extract can significantly prevent hepatotoxic TB drugs in rats [15]. Consumption of BCSO in healthy volunteers is thought to reduce oxidative stress parameters (malondialdehyde) and improve immune responses, increasing CD4Th and IFN-γ.

The most common parameter of lipid peroxidation is malondialdehyde (MDA) [16]. Previous research showed that the MDA level in adolescents with obesity is higher than that in adolescents without obesity [17]. The level of MDA correlates with body weight and body mass index. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) are enzymes most often associated with liver cell damage from exposure to reactive radicals or other toxic agents [18]. Every material that enters the body undergoes a process of absorption, distribution, metabolism, and excretion. Bile excretion allows xenobiotic buildup in the liver to cause hepatotoxic effects [19]. Measurement of SGOT and SGPT enzymes can identify the safety of a substance and measure oxidative stress and inflammatory response levels. Aminotransferase activity in the blood can be detected even in a minimal amount. BCSO has passed preclinical trials in animals and has been provenefficient and safe. Preclinical research indicates that BCSO is hepatoprotective and anti-inflammatory, but the effect of giving BCSO to healthy volunteers on SGOT, SGPT, and the immune response is still limited [20]. In this study, researchers intend to conduct a quasi-experimental study to see the impactof BCSO preparation consumption on SGOT, SGPT, and adaptive cellular immune activities in healthy volunteers.

2. RESEARCH METHOD

2.1. Design

This was a pre-post test experimental research without a control group. A total of 12 healthy volunteers were asked to consume BCSO preparation for twenty days. This research was conducted according to the direction of the Helsinki declaration by following the protocol reviewed and approved by the Health research ethics committee. The Health research ethics committee of the Medicine and health science faculty, Universitas Muhammadiyah Yogyakarta, approved the research protocol (No: 166/KEP-FKIK UMY/V/2019).

2.2. Research subjects

A total of 12 healthy volunteers met the following inclusion criteria: healthy volunteers as evidenced by a health certificate from an authorized hospital, men and women aged 17-55 years, and willing to be the subject of research by signing informed consent. Volunteers who dropped out (not cooperative) during the research and are pregnant women were excluded. The sample calculation used a hypothesis test on the average of two dependent variables [21].

2.3. Research instruments and materials

We tested soft capsules containing standardized black cumin seed oil (BCSO) with thymoquinone (2.72%) and fatty acids in the form of caprylate (0.15%), caprat (0.1%), laurat (0.18%), myristate (12.27%), palmitate (0.28%), s tearate (7.99%), o leic (0.07%), l inoleate (2.85%), e icosanoate (3.15%), e ococciate (0.25%), eicosanoenenoate (0.03%), arachidonate (0.03%), eicopentanoat (0.03%), behenat (0.06%), dokoheksanoat (0.04%), teracosanoate (0.02%) and placebo in the form of fish oil capsules [22].

2.4. Research variables and operational definitions

The independent variable included the BCSO administration in the treatment group orally in soft capsules for 20 days. The dependent variables were CD4Th and CD4CD25Treg number, IFN-gen gene expression, MDA level, and SGOT-SGPT activity. Each soft capsule contained 0.5 ml of black cumin seed oil and was given two to three times daily. CD4Th and CD4CD25Treg number and IFN-γ expression are the percentages of CD4Th, CD4CD25Treg, and IFN-γ expression in peripheral blood lymphocyte cells determined by immunoassay with a flow cytometer. MDA levels were determined by chromatography. SGOT-SGPT activity is the enzyme's activity in human serum as measured by the Aero set System. Parameter checking was carried out on days 0 and 21.

2.5. Research procedure

2.5.1. Preparation of test volunteers and BCSO consumption

We recruited 12 healthy volunteers who met the study requirements. Subjects were declared healthy by a health certificate. Volunteers were asked to fill in a Case Report Form during the research. On the day 0 or before treatment, clinical condition, CD4Th and CD4CD25Treg, SGOT and SGPT activity, MDA level, and IFN-γ gene expression were examined. Then volunteers were given BCSO capsules for 20 days, as the guideline for phase I clinical trials issued by the Food and Drug Supervisory Agency of the Republic of Indonesia. On the 21st day, the CD4Th and CD4CD25 Treg number, MDA levels, SGOT and SGPT activity, and IFN-γ expression were re-examined.

2.5.2. Examination of the MDA levels and SGOT and SGPT activity

Peripheral blood was drawn from the cubital vein by trained analysts, as stated in the protocol reviewed and approved by the Universitas Ahmad Dahlan (UAD) research ethics committee. SGOT-SGPT activity and MDA level were examined on days 0 and 21 of treatment from the collected blood.

2.5.3. CD4Th and CD4CD25Treg number, and IFN-γ gene expression examination

Peripheral blood is drawn from the cubital vein by analysts. The Analysts have been trained in the procedure, as stated in the protocol. IFN-γ gene expression was examined from the blood collected using flow cytometry using the procedure as previously done [23]. Briefly, The procedure was as follows: i) the specimen was pipetted into the falcon tube by 50 µL; ii) 5 µL of CD4Th, CD4CD25Treg, and IFN-γ anti-human FITC reagent was added; iii) then, they were mixed homogeneously on a vortex mixer, then incubated for 15 minutes in a dark room at 20-25 °C; iv) 50 µL of 10x FACS analysis solution was diluted with 450 µL of distilled water and then mixed homogeneously; v) after the incubation time was complete, 450 µL of the diluted FACS (lx) reagent was added to the sample; vi) they were mixed homogenously and then incubated for 15 minutes in a dark room at 20-25 °C; vii) Finally, after the incubation period was complete, the sample was analyzed using the flow cytometer FACS tool [24].

2.6. Data analysis

We analyzed the data using the SPSS version 16 free edition program. Univariate analysis was done to describe the subjects' characteristics. We performed the mean difference test of CD4Th and CD4CD25Treg number, and MDA levels, SGOT and SGPT activity, and IFN-γ expression on days 0 and 21 in a single group through a dependent t-test with a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Characteristics of research subjects

This study involved 12 healthy volunteers. The demographic and clinical characteristics of volunteers are presented in Table 1. The average blood pressure (systolic and diastolic) and pulse were normal. According to Joint National Committee (JNC) VIII, normal blood pressure is <120/<80 mmHg, and American Heart Association (AHA) states that regular pulse rate is 60-100 times per minute. All mean values of clinical parameters (blood sugar, cholesterol, triglycerides, SGOT, SGPT, blood urea nitrogen (BUN), and creatinine level) in the pre-BCSO preparation consumption were within the regular average rates. According to WHO, the standard body mass index (BMI) is 18.50-24.99 kg/m2, International obesity task force (IOTF) is 18.5-22.9 kg/m2, and the Ministry of Health of the Republic of Indonesia is 18.5-25 kg/m2. Based on the research data, it is known that the participants' average BMI is obese.

Table 1. Characteristics of healthy volunteers who consumed BCSO preparation

Characteristics	Parameter measurement (Mean±SD)	min-max
Age (year)	24.58±5.18	18.00–48.00 year
Gender (male/female)	12/24	-
Systolic blood pressure (mmHg)	116.22±15.91	95.00–160.00
Diastolic blood pressure (mmHg)	73.61±11.80	55.00–105.00
Pulse (times/minute)	75.58±6.67	64–96
Body weight (Kg)	57.60±13.25	39.12–87.35
Body mass index (BMI) (Kg/m2)	27.41±5.46	19.15–39.20
Random blood sugar (mg/dl)	92.27±14.60	70.56–133.43
SGOT (µ/L)	20.80±14.70	10.90–89.80
SGPT (µ/L)	20.56±21.48	6.50–115.6
Cholesterol (mg/dl)	185.97±27.16	113.00–252.00
Triglyceride (mg/dl)	115.27±68.91	45.00-489.00
Blood urea nitrogen (mg/dl)	7.98±2.12	3.30–19.40
Creatinine (mg/dl)	0.74±0.14	0.68–1.60

3.2. Effect of BCSO consumption on antioxidant and immunity parameters

The measurement results of oxidative stress parameters, antioxidant capacity, immune response, and clinical conditions before and after 20-day BCSO consumption are presented in Table 2. The table shows that the parameters observed both before and after BCSO consumption were within normal limits, except BMI. Before consuming BMI, the average BMI of healthy volunteers was 27.38±5.24 Kg/m2, which is classified as obese. After consuming BCSO for 20 days, volunteers experienced a decreased BMI and reached a standard BMI value of 23.07±4.44 Kg/m2 and were statistically significant ($p<0.01$). Along with the decrease in BMI, after consuming BCSO, volunteers also had lower MDA levels and SGPT activity. Before consuming BCSO, volunteers had MDA levels of 4.81±1.24 and SGPT activity of 20.56±21.48. In contrast, after consuming BCSO, MDA levels dropped to 3.90±0.84 mg/dL, and SGPT activity also declined to 17.50±17.86 and was statistically significant ($p<0.05$). However, BCSO consumption did not significantly reduce triglyceride, cholesterol, blood sugar, and blood pressure levels. Cholesterol, triglyceride, and blood sugar levels after consuming BCSO were lower but not statistically significant ($p>0.05$).

Table 2. The antioxidant and adaptive immune response parameters before and after 20-day BCSO preparation consumption in healthy volunteers

BCSO preparation consumption (n=12)		
Characteristics	Pre	Post
Systolic blood pressure (mmHg)	116.22±15.91	112.77±13.22
Diastolic blood pressure (mmHg)	73.61±11.80	70.97±8.52
Body mass index (Kg/m2)	27.38±5.24	23.07±4.44**
SGOT (µ/L)	20.80±14.705	20.16±13.20
SGPT(µ/L)	20.56±21.48	17.50±17.86*
Cholesterol (mg/dl)	185.97±27.16	181.22±23.90
Triglyceride (mg/dl)	115.27±68.91	107.33±73.70
Random blood sugar (mg/dl)	92.27±14.60	90.33±18.05
MDA_(mg/dl)	4.81±1.24	3.90±0.84**
CD3CD4CD25 number (%)	25.93±7.58	12.59±4.02**
CD3CD4 number (%)	42.44±8.67	49.77±7.44**
IFN-γ expression (%)	2.89±2.18	4.39±2.39**

Note: *=p<0.05; **=p<0.01; ***=p<0.00

Consumption of BCSO improves immune response in healthy volunteers. The results show sharply decreased CD4CD25Treg levels after consuming BCSO for 20 days, from 25.93±7.58% to 12.59±4.02% ($p<0.05$). Further, BCSO consumption for 20 days increased CD4Th count and IFN-γ expression ($p<0.05$). The volunteers had a lower CD4Th count and IFN-γ expression before the consumption, namely 42.44±8.67 vs. 49.77±7.44 % and 2.89±2.18 vs. 4.39±2.39, for CDTh and IFN-γ, respectively ($p<0.05$). Based on the datafrom this study, BCSO could reduce oxidative stress and improve immune response.

This research found that BCSO consumption for 20 days increased the adaptive immune response by increasing the number of CD4Th and the expression of IFN-γ and decreasing the number of CD4CD25Treg. The results are in line with previous studies [22]. Thymoquinone increased macrophage phagocytic activity by inducing the enzyme neu4sialidase. Neu4sialidse plays a role in delivering the response from the toll like receptor-4 (TLR-4) of the macrophages [25]. TLR-4 is a receptor on the surface of macrophages [26]. The signal from TLR activates a non-specific immune response, which stimulates the production of transcription factors that produce various proteins and then macrophages to release many cytokines that play a role in the adaptive immune response. Activating macrophages will produce cytokines such as IL-12 and IL-2[27], which activate T cells to secrete IFN-γ [28].

The results show that 20-day BCSO preparation consumption reduced MDA levels and SGPT activity because the BCSO can act as antioxidants and hepatoprotective. These results are consistent with previous studies [29]. Thymoquinone, the main active ingredient of BCSO, has been reported to prevent liver damage in mice through antioxidant and anti-inflammatory mechanisms [30]. In vivo, the administration of BCSO preparation containing thymoquinone can increase the antioxidant enzyme and increase the number of Tregs [31]. It has been proven that there is a relationship between GST level and cell damage level, showing that GST is cell repair and cell destruction prevention [32]. Increased levels of the antioxidant GST are thought to be through the Nrf2 activation [33]. Thymoquinone is an inducer of antioxidant response; the inducer reacts with cysteine on Keap1, resulting in the release of Nrf2 from Keap1. GST as an antioxidant can protect liver cells from the influence of free radicals so that the SGOT-SGPT enzymes are within the normal range [34]. Nigella sativa as an antioxidant can prevent damage to the liver cells membrane due to oxidative stress. Because SGPT and SGOT do not circulate blood, their activity in the blood will decrease

and go towards normal. Some studies mention that using black cumin seed oil can reduce SGPT and SGOT in ethanol-induced mice [35], [36].

Thymoquinone prevents ethanol-induced liver damage in white rats by decreasing IL-6, tumor necrosis factor alpha (TNF-α), MDA and increasing the activity of antioxidants enzymes, and liver protein through antioxidant and anti- inflammatory mechanisms [37]. Free radicals on immunocompetent cells, especially macrophages and CD4 T lymphocytes, cause cells to experience a decrease in cytokine activity and production. Decreased macrophage activity in producing IL-1 and IL-12 will inhibit the proliferation and differentiation of CD4 T cells into Th1 subsets. Decreased Th1 cell activity will then cause a decrease in the production of cytokines IL-2 and IFN- γ [4]. Increased IFN-γ expression in healthy volunteers shows that BCSO can improve the immune system. BCSO can modulate the immune system by playing a role in maintaining the body's homeostatic conditions and helping to correct immune system imbalances [38].

4. CONCLUSION

The possibility that consumption of BCSO preparations for 20 days can increase the adaptive cellular immune response, namely increasing the number of CD4Th and IFN-γ, cannot be ascertained but cannot be ignored. Elucidated the effect of consuming BCSO preparations on the adaptive cellular immune response, it is necessary to conduct research using a better research design, namely a randomized clinical controlled trial.

We recognize that this study has weaknesses. Some of the weaknesses of this study include the number of subjects being small, the testing time being short, the subjects being less than 50 years old, and there is no representative of the subjects with age >50 years and no controlled group. Adhering clinical trial guidelines from the Food and Drug Supervisory Agency of the Republic of Indonesia, subjects are limited to between 6-15 subjects in phase one clinical trials. Further research on patients with hypertension or at high risk of cardiovascular diseases and diabetes mellitus with a proper trial design needs to be done to test the effectiveness of this BCSO preparation.

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