11_HASIL CEK_Akrom, Darmawan, Maulida, Syuhada_Antioxidant, Black Cumin Seed Oil, Immunomodulator

by Akrom, Darmawan, Maulida, Syuhada Antioxidants And Immunomodulatory Effect

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Antioxidants and immunomodulatory effect of black cumin seed oil in at-risk metabolic syndrome

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ABSTRACT

Introduction: Black Cumin Seed Oil (BCSO) is a traditional medicine widely used as an antioxidant and immunomodulator. Objective: The purpose of this study is to investigate the effect of dose variation of BCSO (1.5 ml/day versus 3 ml/day) on IL-10 activity and MDA levels in patients at risk of MS at Jetis 1 Public Health Center (J1PHC), Bantul Regency, Yogyakarta, Indonesia.
Methods: We conducted an analytical cross-sectional study. 66 patients at risk of metabolic syndrome (MS) were divided into two groups. In stage 1, group 1 received a 1.5 ml/day dose, and group 2 received 3 ml/day for 20 days. The clinical parameters of MS, IL-10 activity, and MDA levels were measured at the end of the study stages.
Results: The mean activity of IL-10 in the 1.5 ml/day dose group was 4.83 and in the 3 ml/day dose group was 5.49, which showed an increase of around 13.66% ($p = 0.300$). The mean MDA level in the 1.5 ml/day group was 3.92 µmol/L, which increased to 4.31 µmol/L in the 3 ml/day group or approximately 9.95% ($p = 0.802$). Statistically, IL-10 activity and MDA levels in the two-dose groups did not differ significantly.
Conclusion: The results of this study indicate that the administration of BCSO at doses of 1.5 ml/day and 3 ml/day for 20 days has the same effect on IL-10 activity and MDA levels in patients at risk of MS.

Keywords: Antioxidant, Black Cumin Seed Oil, Immunomodulator.
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INTRODUCTION

Metabolic syndromes (MS) are the risk factor of many degenerative diseases and aggravating factors in the era of the covid pandemic.[1] The imbalance of metabolic processes can lead to various clinical problems, including central obesity, hypertension, dyslipidemia, insulin sensitivity disorders, and diabetes.[2] A set of irregular metabolic process symptoms is known as metabolic syndrome (MS).[3] Several factors can affect MS, including the active radicals caused by oxidative stress.[4] Active radicals can cause cell death because they damage cellular fat and protein and lead to various diseases such as autoimmune.[5] Some major immuno-regulators play an essential role in maintaining balance and suppressing autoimmune occurrence, e.g., interleukin-10 cytokine (IL-10). IL-10 has a role in the inflammatory response as an anti-inflammatory cytokine.[6] Meanwhile, radical oxidative biomarkers have been extensively evaluated to identify the relationship between oxidative damage to macromolecules (lipids, deoxyribonucleic acid [DNA], and proteins) and disease progression.[7,8]

Malondialdehyde (MDA), as a fatty peroxidation biomarker, has increased in patients with inflammation caused by rheumatoid arthritis and MS patients.[9–11] Administration BCSO for eight weeks in arthritis patients increased IL-10 cytokine and decreased MDA.[12] The BCSO was adequate for use as adjunctive therapy in managing diabetic medicine by lowering serum creatinine and urea levels and blood sugar and HbA1c levels.[13,14,15] Meanwhile, the administration of BCSO with a dose of 5 ml/day in healthy patients for eight weeks proved to reduce blood pressure without side effects. Until now, there has been no clear evidence of BCSO effects on IL-10 and MDA as a therapeutic supplement in patients at risk of MS. BCSO is one of the natural ingredients drugs widely used in various countries, including Indonesia, so research needs to be done to find evidence.[8] The study aimed to determine the effect of BCSO on malondialdehyde levels (oxidative stress parameter) and IL-10 expression (anti-inflammatory parameter).

METHODS

Research design and subjects

We conducted an analytical cross-sectional study. We examined biological materials and analyzed data from based-data from open-label clinical trials of providing additional therapy for preparations containing black cumin seed oil in patients at risk for metabolic syndrome (MS) at the Jetis 1 Community Health Center (J1PHC). The study complied with the Helsinki Declaration in involving humans as the subjects. The protocols and informed consent of the study have been reviewed and approved by the research ethics committee of the Faculty of Medicine and Health Sciences of Muhammadiyah University of Yogyakarta (UMY) in 2016 with the number of certificates of ethical research eligibility:

279/EP- FKIK-UMY/V/ 2016.[15]

A total of 66 patients at Jetis 1 PHC, Bantul District, Yogyakarta Province, at risk of MS who had met the study requirements, expressed their willingness to be the subject of the study, and filled informed consent, were divided into two groups (groups 1 and 2) of 33 subjects each. We signed the issue by simple manual randomization technique.

The inclusion criteria are patients at risk of MS who demonstrated one or more of the following MS-related clinical problems (1) increased fasting glucose (fasting glucose ≥100 mg/dL), (2) increased blood pressure (blood pressure ≥ 130/85 mmHg), (3) elevated blood triglyceride levels (≥ 150 mg/dL) or received specific therapy for abnormal lipids, (4) decreased HDL cholesterol (<40 mg/dL in males and <50 mg/dL in females). The exclusion criteria in this study are (1) dropping outs during the study, (2) pregnant women, (3) patients taking corticosteroids, undergoing anti-tuberculosis therapy, clinically diagnosed with cancer, severe kidney disease, and consuming immunomodulatory agents, and (4) allergic to BCSO.

In groups 1 and 2, each subject received BCSO capsules at a dose of 1.5 ml/day and 3 ml/day in the first 20 days, followed by a wash-out period for seven days. Subsequently, the doses were exchanged (group 1 with 3 ml/day and group 2 with 1.5 ml/day) on the second 20 days. Blood sampling for the IL-10 activity and MDA level tests was performed on the day after the intervention.

Blood Sampling

The peripheral blood was taken from the cubital vein by a trained analyst. Blood sampling was done three times: day 0 (before treatment), day 21, and day 49 (after treatment). Blood collected on day 0 was used to examine essential clinical characteristics (baseline). The blood taken was centrifuged for approximately 15 minutes with a relative centrifuge force (RCF) of about 1,500 g so that the serum sample was obtained for analysis. Unused serum samples were stored at temperatures less than 60°C until analysis was performed. All the samples obtained were secretly coded only known by the researcher to be blind to reduce the bias of the research results. Blood samples were divided into parts 1 for blood chemistry (glucose, LDL cholesterol, HDL, and triglyceride levels), part 2 for MDA level examination, and section 3 for IL-10 expression examination.

Measuring subjects' essential clinical characteristics

Baseline examination of glucose level, LDL cholesterol, HDL cholesterol, and triglyceride was done at Jetis I PHC with spectrophotometric equipment by trained personnel. Trained doctors performed blood pressure checks.

Measuring IL-10 activity

The collected and secretly coded sera were analyzed to determine IL-10 activity using flow cytometry (flow cytometer B.D. Facscalibur) performed by trained analysts. The principle of this method is the illumination of cells or other particles as they flow separately through a narrow slit of exposed light, and the character of light-absorbing fractionation will be detected by the detector and processed by the computer. IL-10 activity measurements were performed on the 21st and 49th blood samples by trained analysts in the Clinical Pathology Laboratory of UGM Faculty of Medicine, Yogyakarta.

Measuring antioxidant parameter: MDA level

Measurement of MDA levels was performed by visible spectrophotometric method (spectrophotometer 5010-V5+®). The principle of action is to use an MDA reaction with thiobarbituric acid (TBA) absorption, which is read with a maximum wavelength of 532 nm. MDA levels were measured on blood samples on the 21st and 49th days by trained analysts in the Clinical Pathology laboratory of UGM Faculty of Medicine, Yogyakarta.

Statistical analysis

Data on IL-10 activity measurements and MDA levels were analyzed by paired-sample t-test with SPSS software. A p-value less than 0.05 is considered significant. Statistical test results are presented with mean values in tables or graphs as research data.[16]

RESULTS

Subjects

At the beginning of the study, there were 66 subjects, but not all of them completed the procedure of research intervention. In group 1, the total subjects who were able to complete the study intervention procedure at stage 1 (BCSO 1.5 ml/day) and stage 2 (BCSO 3 ml/day) were 31 of 33 subjects (94%). Two issues (6 %) were unable to continue the study procedure because of the side effects. One point (3%) had elevated blood pressure, and another (3%) experienced nausea and vomiting. Similarly, in group 2, the total subjects who completed the research intervention were 31 subjects (94%), two subjects (6%) were unable to continue the study. One issue (3%) felt the side effects of gastric pain, and one other (3%) resigned because the subjects performed kidney stone surgery. Thus, the total subjects who completed the study were 62 patients.

Baseline characteristics

Baseline data indicate the clinical condition of the study subjects: elevated LDL levels 91.9%, hyperglycemia 70.97%, hypertension 59.7%, hypertriglyceridemia levels 54.8%, and low serum HDL levels 37.1% involved in this research. Most subjects had a BMI <25 (58.06%). The summary of baseline descriptions of subject characteristics is presented in Table 1.

As shown in Table 1, it is known that there is no difference in clinical characteristics between the two subject groups.

IL-10 activity data

The dot plot graph of IL-10 activity/expression examination results by flow cytometry method is presented in Figure 1. The results of flow cytometry examination of IL-10 expression after BCSO intervention with doses of 1.5 ml/day and 3 ml/day are presented in Fig. 1.

It can be seen that a group with a 3 ml/day dose treatment had a lower expression than the 1.5 ml/day group. The results of the statistical analysis of the IL-10 examination are shown in Table 2.

Table II shows that the mean expression of IL-10 was 4.83% in the group receiving 1.5 ml/day and 5.49% in the 3 ml/day

Table 1. Demographic (sex, age) and clinical characteristics (BMI, hyperglycemia status, hypertension status, dyslipidemia status) of the patients at risk of MS baseline.

Characteristic	Groups		p
	1.5 ml/day BCSO group (n=31)	3 ml/day BCSO Group (n=31)	Total (%)
Sex			
Male (%)	7/31 (22.6)	6/31 (19.35)	13/62 (21)
Age group			
≤60 years (%)	24/31(77.4	22/31(70.97	46/62 (74)
BMI			
≤25 kg/m2 (%)	18/31(58.06)	18/31(58.06)	36/62(58.06)
Hyperglycemia			
(RBS ≥ 200 mg/dl)	22/31(70.97	22/31(70.97	44/62(70.97
Hypertension			
BP systole ≥140 mmHg	17/31(54.83)	20/31(64.52)	37/62 (59.7)
BP diastole ≥90 mmHg	4/31(12.90)	6/31(19.35)	10/62 (16.12)
dyslipidemia			
Triglycerides ≥150 mg/dl	17/31(54.83)	17/31(54.83)	34/62(54.83)
LDL ≥100 mg/dl	30/31(96.7)	27/31(87.1)	57/62(91.93)
HDL ≤ 40 mg/dl	11/31(35.48)	12/31(38.71)	23/61(37.1)

Note BMI, Body Mass Index; BP, Blood Pressure; RBS, Random Blood Sugar; LDL, Low-Density Lipoproteins; HDL, High-Density Lipoprotein.

Table 2. IL-10 expression in patients at risk of MS after receiving BCSO at a dose of 1.5 or 3 ml/day for 20 days.

BCSO Dosage	IL-10 activity (%)		% change	*P*
	Mean	SD		
1,5 ml/day BCSO group	4,83	3,49	13,66	0,300
3 ml/day BCSO group	5,49	3,58		

Paired T-test; SD, Standard Deviation

Table 3. MDA levels of patients at risk of MS after receiving BCSO at a dose of 1.5 or 3 ml/day for 20 days.

BCSO Dosage	MDA level (µmol/L)		% change	*P*
	Mean	SD		
1,5 ml/day BCSO group	3,92	5,00	9,95	0,802
3 ml/day BCSO group	4,31	11,10		

Note: As shown in Table 3, the mean MDA levels in the dose group of 1.5 ml/day were 3.92 µmol / L and increased to 4.31 µmol / L or 9.95%. Statistically, the increase in this activity did not differ significantly (p = 0.802).

dose group, or an increase of 13.66%. Statistically, this increase in activity did not differ significantly (p = 0.300).

MDA level data

Results of MDA examination after the intervention in subjects with doses 1.5ml/day and 3 ml/day can be seen in Table 3. The results of statistical analysis on MDA levels are shown in Table 3.

DISCUSSION

Effects of BCSO dose variation on IL-10 activity

Mild inflammation is induced by immune system disruption in MS settings, where pro- and anti-inflammatory conditions are imbalanced, with increased activity of pro-inflammatory cytokines (IL-6, IL-18, and TNF-) and decreased activity of anti-inflammatory cytokines (IL-10).[17] Increased pro-inflammatory cytokines are linked to several pathologic conditions associated with MS, including insulin resistance, diabetes, impaired cardiovascular function, and impaired lipid metabolism.[18] IL-10, as an anti-inflammatory cytokine by inhibiting macrophages and dendritic cells that play a role in controlling nonspecific immune and cellular immune reactions, maybe one of the pathways inhibiting the development of MS disease and its complications by inhibiting the formation of pro-inflammatory cytokines.[19]

Many studies and literature mention the benefits of BCSO supplementation in MS-related conditions, especially in controlling blood glucose levels. The ability of BCSO as a complement to hyperglycemia therapy may affect the pro-inflammatory and anti-inflammatory status, including IL-10.[20] However, dosing of 1.5 ml/day and 3 ml/day did not significantly affect the increase in IL-10 in MS patients. The supplementation of BCS powder with doses of 2 g / day and 3 g / day for three months as adjunctive therapy

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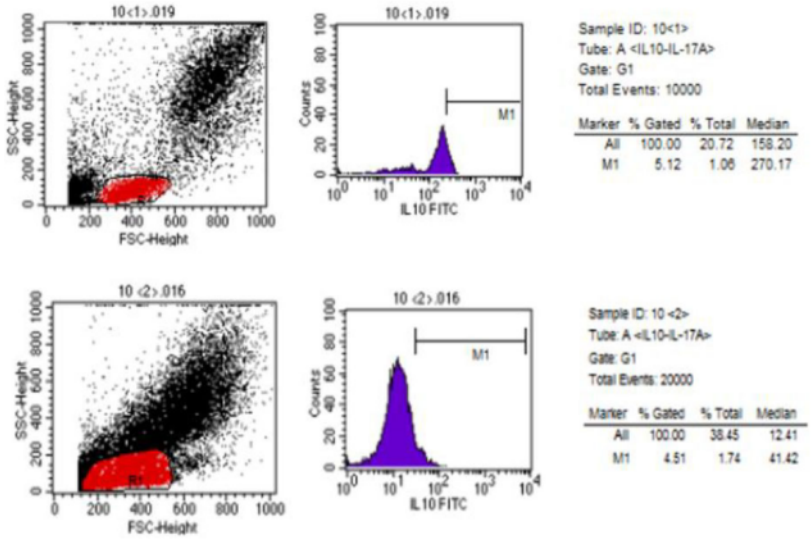

Figure 1. The examination results of IL-10 expression by flow cytometry method in patients at risk of MS after receiving BCSO at a dose of 1.5 or 3 ml/day for 20 days.

in type 2 diabetes mellitus patients showed similar benefits compared to baseline.[10]

BCSO contains antioxidants, especially thymoquinone attire substances that have anti-inflammatory effects.[21] It may be that the optimal ability of the anti-inflammatory effect has been achieved at a dose of 1.5 ml/day so that the increased amount of BCSO has not significantly affected the activity of IL-10.[15] The thymoquinone content in BCSO has an essential role in the immune system by inhibiting the synthesis of pro-inflammatory cytokines, MCP-1, TNF-α, IL-1β, and COX-2, but with a lower inhibitory effect on COX-1 expression and PGE2 production. In addition, the thymoquinone may also increase the expression of p21 WAF1, inhibit histone deacetylase activity, and induce histone hyperacetylation.[22] The anti-inflammatory capabilities in BCSO are expected to inhibit pro-inflammatory pathways through a combination of anti-inflammatory action and proapoptosis that can be applied to the management of inflammatory therapies in cancer. This will affect the pro-and anti-inflammatory balance in MS patients by shifting the increase of anti-inflammatory cytokines, including IL-10. Based on the study results, there was no significant difference between the use of BCSO at doses of 1.5 ml/day and 3 ml/day of IL-10 activity in patients at risk of MS.[23]

Effects of BCSO dose variation on MDA levels

MDA comprises double-bond carbons of polyunsaturated fatty acids and is easily oxidized but can produce lipid radicals, especially lipid hyper-peroxides, triggering a chain-oxidative reaction.[24] The adverse effect of increased MDA level includes cell membrane damage due to altered structural integrity of the membrane, inactivation of membrane enzyme bonds, inactivation of surface receptor molecules leading to cell functional regulatory errors, and oxidized LDL involvement foam cell formation leading to atherosclerosis.[25] Previous research shows that the effect of BCSO on the response of oxidative stress substances to rheumatoid arthritis patients showed decreased MDA levels.[26] Increased MDA levels at higher BCSO dose concentrations may be associated with the activity of thymoquinone and alpha-hederin, which can induce apoptosis of cancer cells by increasing ROS in cancer cells to produce residual MDA due to cell lipid metabolism.[27]

MS plays a role in the formation of MDA by increasing oxidative radicals due to metabolic failure. Hyperglycemia plays a role in increasing lipid catabolism due to the body's compensation in generating energy through the gluconeogenesis pathway and resulting in increased metabolism of fat that produces many oxidative lipid radicals.[4,29] BCSOs with antioxidant activity counteract the oxidative lipid radicals formed and reduce fat catabolism by inhibiting hyperglycemia and lipid profile dysfunction with potential mechanisms such as insulin secretion and glucose absorption, gluconeogenesis, and gene expression.[30] Based on the study results, it was found that there was no significant difference in the use of BCSO between the doses of 1.5 ml/day and 3 ml/day in MDA activity status in patients at risk of MS.[31] This study has limitations related to uncontrolled variables, such as diet, physical activity/exercise, and medication compliance, affecting the controlled variables observed.

CONCLUSION

Provision of BCSO at doses of 1.5 ml/day and 3 ml/day for 20 days had the same effect on IL-10 activity and MDA levels in patients at risk of MS ($p > 0.05$). A 1.5 ml/day dose has the same benefit as a 3 ml/day dose on the anti-inflammatory cytokine activity (IL-10) and the radical oxidant (MDA) level. Future studies related to BCSO dose effects in patients at risk for MS with more diverse time and doses are needed to obtain more decisive conclusions.

CONFLICT OF INTEREST

There is no potential conflict of interest in this research.

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ETHICS APPROVAL

The research ethics committee approved this study of the Faculty of Medicine

and Health Sciences of Muhammadiyah University of Yogyakarta (UMY) in 2016 with approval number 279/EP- FKIK-UMY/V/ 2016.

AUTHOR CONTRIBUTION

AA and ED prepared the research design, data collection, processing and analysis, and drafting. NM and S were involved in data collection and processing, as well as drafting articles. All authors reviewed draft articles.

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