

The effect of black cumin seed oil consumption on the platelets and leukocytes number in healthy smokers in rural area Yogyakarta



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ABSTRACT

Background: The high number of smokers in rural areas is a public health problem in Indonesia. Tobacco smoke exposure is associated with the incidence of cardiovascular disease and cancer. Smoking increases the activity of the hematological system, characterized by an increase in the number of platelets and leukocytes. Platelet and leukocytes are essential cells in thrombus formation and hemostasis. As an immunomodulatory supplement, black cumin seed oil (BCSO) has been used in various studies, affecting the blood cell formation system. The purpose of the study was to determine the effect of administration of BCSO preparation for 30 days on platelet and leukocytes.

Methods: The 15 patients were placed into two groups in this open-label Randomized Controlled Trial. Group 1 received a placebo (3x1 capsules per day), and group 2 received BCSO (3x1 capsules per day). For 30 days, the intervention was carried out. Blood was obtained for analysis on the 31st day. The average amount of platelets and leukocytes in each group was compared using one-way ANOVA.

Results: The test results are said to have no difference in platelets and leukocytes number between groups with 30 days of BCSO consumption, with their respective values being 0.848 in the placebo group and 0.211 in the treatment group.

Conclusions: The homeostatic condition of subjects who consumed BCSO 3x2 capsules/day for 30 days remained stable and balanced.

Keywords: Black cumin seed, Healthy smoker, Homeostatic, Leukocytes, Platelets.

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INTRODUCTION

The black cumin seed oil (BCSO) capsule contains many linoleic, linolenic fatty acids, and essential oils. The active substances of the essential oil are thymoquinone, nigelin, and nigellone.¹ In vitro and in vivo, thymoquinone has been shown to have anti-cancer, antibacterial,² antioxidants,³ anti-diabetic,⁴ chemoprevention,⁵ and anti-hepatotoxic,^{6,7} effects. Meanwhile, thymoquinone has an immunomodulatory effect on T helper with Suppressor T cells,^{8,9} increases macrophage activity through toll-like receptors (TLR) and Suppressor T cells.⁷ and reduces platelet aggregation by inhibiting cyclooxygenase and lipoxygenase pathways.^{8,10}

Smoking has become a particular concern in the world.¹¹ Indonesia occupies the first position in the country with the most significant percentage of male smokers aged 15 years and over globally,

namely 66%.¹² A person who smokes ten or more cigarettes per day has an average life expectancy of 5 years shorter and 20 times higher risk of developing lung cancer than those who have never smoked.¹³ Smoking increases the number of erythrocytes, leukocytes, platelets, and hemoglobin in the blood.¹⁴ The previous study showed that acute cigarette smoke exposure increases the secretion of inflammatory mediators from various cell types, including epithelial cells, macrophages, and neutrophils.¹⁵ Cigarette smoke contains gaseous components and particles that can cause free radicals, such as carbon monoxide, nitrogen oxide, and hydrocarbon compounds, during particle components such as tar, nicotine, benzopyrene, phenol, and cadmium.¹⁶ One of the substances in cigarettes is nicotine, which can stimulate hormones that cause blood cell accumulation and

platelet aggregation, causing blood vessels to become sticky.^{17,18}

This study is a phase 1 clinical trial in which the subjects used are healthy-smoker volunteers. The goal of phase 1 is to determine the size of a dose that is acceptable, meaning that it does not cause serious side effects. Based on these descriptions, this study was conducted to determine the impact of 30 days of administration of BCSO on the leukocyte and platelet counts in healthy-smoker volunteers.

METHODS

Research Design and Variables

An observational analytic investigation was carried out. We reviewed the laboratory data from the trial of giving black cumin oil capsules versus placebo in healthy smokers by the health worker of Jetis I Public Health Center. Subjects

consumed 3x1. BCSO and a placebo 3x1 for 30 days. This pilot test was conducted in Jetiš I Primary Health Care, Bantul, Yogyakarta, namely Sindet and Blawong I Hamlets. Measurement of vital signs, filling case report form, and blood collection was carried out at residents' homes. In addition, a complete blood count was carried out at the clinical laboratory of Nur Hidayah hospital. We observed the study's primary outcome, namely the number of leukocytes and platelets, and the secondary outcomes, namely the hemogram profile, liver function, kidney function, and vital signs.

Population and Subjects

The population in this study were healthy adult males, smokers, and willing to sign the informed consent. The exclusion criteria were if the subject had a history of chronic disease, experienced an allergic reaction to BCSO, and had psychiatric disorders. We recruited 18 subjects according to the selection criteria and obtained informed consent from each subject. We exclude participation if the test subjects did not attend the study until the end and were unavailable for a blood sample on the 31st day. At the end of study 15 active smokers met the selection criteria. There were three subjects who were dropped out for various technical reasons. At the time of testing, a subject traveled out of town, so he stopped taking medication, and two subjects were not willing to take blood samples on day 31. The number of subjects is following the recommendations of the Food and Drug Supervisory Agency of the Republic of Indonesia and based on the minimum sample size of the Lemeshow formula.¹⁹

Materials and Instruments

This study used BCSO soft capsules and placebo capsules. BCSO soft capsules are produced by the traditional medicine industry (CV Al Afiat). They have received a TR number from the Food and Drug Supervisory Agency of the Republic of Indonesia. The production process of BCSO soft capsules follows good manufacturing product (GMP) guidelines. The certified pharmaceutical industry prepared both types of tablets. The official certificate was obtained from the Drug and Food Regulatory Agency of

the Republic of Indonesia. Blood pressure measuring devices and thermometers to monitor participants' vital signs. Blood collection devices and complete blood count procedures were used to check the number of leukocytes and platelets with the hematology analyzer. The analyzer works because the blood sample is washed 200 times and then mixed with hemolyzing Solution, then the tool will calculate the hemoglobin and white blood cells. We use the case report form to record the daily progress of the participants.

Research Procedures

Participants were recruited from two hamlets in the Jetiš I Primary Health Care working area, namely Blawong and Sindet Hamlets. Recruitment of participants was done randomly using selection criteria. Simple random sampling was done by writing down the number of each member of the population on a piece of paper, then taking a number of pieces of paper with your eyes closed. We obtained informed consent from all participants individually. An explanation of the objectives, direct benefits for participants, and possible risks are given separately. Health practitioners of Jetiš I Primary Health Care were also informed about the public use of the research.

We randomly assigned the Subjects into two groups, namely placebo (n=6) and treatment (n=9). Three placebo subjects have dropped out. The placebo group received three times one placebo capsule per day, whereas the placebo capsules were without any active ingredients. In contrast, the treatment group received BCSO with three soft capsules per day. Both BCSO and placebo capsules were administered for 30 days, three times daily. Demographic data, smoking habits, and lifestyle were obtained through interviews using a list of questions. Height and weight were checked before and after the intervention. Trained health workers visit "participants' homes to monitor blood pressure, body temperature, respiration rate, health complaints, and side effects 2x/ week. Health workers used WhatsApp, a smartphone-based messenger application, as the media to report if they experienced health developments and side effects. On the 31st day, blood samples were taken,

and the number of platelets and leucocytes was examined at The Clinical Laboratory of Nur Hidayah Hospital. Demographic and lifestyle data are collected using the CRF.

Statistical Analysis

The data were evaluated, presented descriptively, and then bivariate analysis was performed. We used dependent and independent t-test to analyze the difference in the mean number of platelets and leukocytes, random blood sugar, triglyceride, blood pressure, and body mass index among groups with a significance level of 0.05.

Research Ethics

The study was authorized by the local Institutional Review Board and was done following Good Clinical Practice. It was necessary to explain the research purpose and objective to all possible subjects, the anticipated advantages and uses, the repercussions for responders, and the rights and obligations of the themes previously discussed. The written informed consents from each subject before entering the have been obtained. The study protocol and interview guidelines have been reviewed and approved by the Research Ethics Committee, the Universitas Muhammadiyah Yogyakarta (078/EP-FKIK-UMY/III/ 2021).

RESULT

Subject characteristic

Table 1 summarized the demographic characteristics of research subjects by gender, education, marital status, occupation, smoking status, age, duration of smoking, number of cigarettes consumed, and number of leukocytes and platelets. There were 15 subjects followed to the end in this study. Three participants stated that they had withdrawn from the study due to technical reasons, so they could not participate until the end (Table 1).

Table 2 shows the subjects' general examination results before the intervention. Male smokers made up 100% of the subjects, the majority of whom graduated from high school (58.3%), were married (66.7%), and worked as laborers (47.2%). The average age of the

participants was 38.69 ± 11.659 years, the average duration of smoking was 20.92 ± 10.81 years, the average number of cigarettes consumed per day was 10.61 ± 7.68 , the average platelet count was 244.42 ± 57.50 cells/mm³, and the average leukocyte count was 9.02 ± 1.96 (Table 2).

Table 1. Demographic characteristics of the subjects.

Demographic	Categories	Frequency (n = 15)	Percentage (%)
Sex	Male	15	100
	Female	0	0
Education	Primary school	1	11.1
	Junior high school	3	16.7
	High school	9	58.3
	University	2	13.9
Marital status	Married	10	66.7
	Not Married	5	33.3
Occupation	Government worker	1	6.6
	Private worker	2	13.3
	Entrepreneur	3	20
	Peasant	2	13.3
	Labor	7	46.8
Smoking status	Smoker	15	100
	Non-smoker	0	0

Table 2. Baseline clinical characteristic of subject.

Examination	Unit	Mean ± SD
Age	year	38.69 ± 11.659
Smoking duration	year	20.92 ± 10.808
Cigarette consumption	stick(s)/day	10.61 ± 7.680
Total leucocyte	cell/mm ³	9.02 ± 1.96
Thrombocyte	cell/mm ³	244.42 ± 57.50
Hemoglobin	%	14.66 ± 1.35
Erythrocyte	Cell/mm ³	5.01 ± 0.45
SGOT	IU/L	22.47 ± 7.54
SGPT	IU/L	22.98 ± 17.50
Random blood glucose	mg/dl	142.91 ± 60.32
Triglyceride	mg/dl	174.44 ± 92.79
BMI (body mass index)	kg/m ²	24.47 ± 4.92
Blood pressure systolic	mmHg	136.00 ± 18.75
Blood pressure in the diastole	mmHg	86.08 ± 12.47
Pulse	bpm	81.21 ± 11.45
Temperature	Celsius degree	35.91 ± 0.73

Effect of 30 days BCSO Consumption

Table 3 describes healthy smokers' blood pressure, hemogram, and blood chemistry after consuming 3x1 BCSO capsules or a placebo for 30 days (Table 3).

Table 3 shows that blood pressure, hemogram profile, random blood sugar (RBS), cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, and creatinine in pre-and post-treatment groups were normal, except for urea levels. Consumption of BCSO for 30 days did not affect blood pressure, hemogram profile, cholesterol, triglyceride, creatinine, and urea levels but did affect RBS levels. Consumption of 3x1 capsules for 30 days can increase RBS levels from 115.22 ± 14.99 mg/dL to 143.56 ± 56.07 mg/dL ($p < 0.05$), but there is no difference in RBS levels between treatment groups with placebo ($p > 0.05$) and the increase in RBS is still within the normal range of values. BCSO consumption decreased platelet count (Table 3).

Platelet count in the BCSO group was lower than the placebo group ($p < 0.05$), but there was no difference in pre-post platelet count in the BCSO group ($p > 0.05$), and the platelet count was within normal limits. In the intervention group, urea levels after BCSO administration were higher than before but not statistically significant ($p > 0.05$). After treatment, urea levels in the placebo group were higher than before treatment and were statistically significant ($p < 0.05$). There was no difference in urea levels in the placebo and BCSO groups, both pre-and post-intervention. Pre-post treatment changes in urea levels seemed influenced by the intervention and other factors, namely lack of drinking fluids.

DISCUSSION

Based on the research findings, it can be concluded that taking BCSO 3x1 capsules for 30 days does not affect homeostasis. Blood pressure, hemogram profile, RBS, cholesterol, triglycerides, creatinine, SGOT, and SGPT were within normal limits. There was a change in urea levels in the placebo group, namely an increase in urea levels after the intervention. Changes in urea levels in the placebo group indicate that urea levels are most likely to lack fluid

Table 3. Average leukocyte count after 30 days of placebo and BCSO administration in healthy-smoker volunteers.

Parameter	Group						P2
	Placebo (n=6)			Treatment/BCSO (n=9)			
	pre	post	p1	pre	post	p1	
WBC	8.81±2.32	9.86±1.27	0.21	8.73±1.88	8.28±1.56	0.78	0.09
RBC	5.05±0.45	4.98±0.48	0.89	5.01±0.50	4.91±0.47	0.71	0.76
Hb	14.85±0.81	14.56±0.91	0.80	14.62±1.28	14.33±1.13	0.81	0.80
Hct	42.98±2.18	42.25±2.14	0.82	42.12±3.27	41.14±2.95	0.79	0.46
MCV	85.36±3.97	85.11±4.64	0.84	84.30±3.21	83.88±3.37	0.78	0.28
MCH	29.48±1.68	29.36±1.86	0.88	29.24±0.99	29.20±1.01	0.82	0.81
MCHC	34.55±0.79	34.48±1.13	0.86	34.13±103.41	34.81±0.60	0.81	0.80
Platelet	255.50±69.99	261.67±32.62	0.11	215.44±49.92	213.33±31.18	0.80	0.02*
Lymphocyte	34.83±9.20	34.88±7.09	0.81	35.82±5.62	38.84±9.67	0.75	0.27
MXD	8.01±3.30	9.01±2.13	0.35	9.33±2.89	8.55±2.46	0.65	0.47
Neutrophile	48.13±17.55	56.10±8.39	0.13	54.84±5.42	52.60±10.21	0.78	0.25
PLR	7.54±6.00	11.44±4.90	0.65	7.43±6.71	8.13±4.19	0.76	0.17
NLR	1.76±0.77	2.35±0.88	0.55	1.74±1.07	2.25±1.83	0.65	0.79
SGOT	28.90±12.09	31.18±7.67	0.55	21.52±4.71	25.00±7.40	0.55	0.12
SGPT	32.80±29.33	45.65±41.68	0.45	19.00±10.37	24.22±15.29	0.39	0.17
Urea	22.71±3.03	33.30±11.63	0.01*	24.84±5.10	33.94±6.84	0.53	0.68
Creatinine	1.11±0.28	1.16±0.16	0.75	1.01±0.16	1.08±0.10	0.85	0.78
RBS	130.00±28.60	130.50±16.50	0.85	115.22±14.99	143.56±56.07	0.01*	0.59
Cholesterol	159.50±52.56	161.33±48.01	0.69	146.44±24.32	151.22±30.81	0.35	0.62
Triglyceride	229.0±157.36	193.50±73.14	0.12	143.56±55.60	143.89±78.22	0.80	0.23
SBP	131.83±16.24	135.00±10.31	0.78	130.88±12.47	139.71±14.59	0.35	0.65
DBP	85.00±6.78	90.40±9.44	0.67	83.88±12.99	91.57±11.34	0.75	0.74

Note: p1*=p<0.05, pre-post test in one group; p2*=p<0.05 post to post-test between-group

intake. Most of the participants have jobs as builders.

According to the results of previous studies, the research data also shows that the side effects of consuming BCSO are relatively mild. The health monitoring results that we did during the provision of BCSO did not find any complaints or health problems from the volunteers. Three volunteers complained of feeling sleepy more often after consuming BCSO. At the initial consumption, there were complaints of nausea in two volunteers, more often belching in two volunteers, and one volunteer complained of diarrhea; however, it was not severe and could be overcome immediately. Most of the volunteers stated that their bodies felt better, increased their appetite, and reduced stomach complaints.

Data from several researchers show that smoking increases leukocytes and hematological levels.^{13,20} Nicotine causes leukocytosis by increasing hormones, such as epinephrine and cortisol.¹⁸ In addition, cigarettes can stimulate hormone secretion, accumulate of blood cells, and platelet aggregation.²¹ Leukocytes are a type of white blood cell involved in the body's defense system.²² Normal leukocyte counts of aiding in preventing and treating many infections; the usual percentage of lymphocytes in the blood is between 20% and 35%. Several factors, however, can influence leukocyte counts, including physical activity, bacterial and fungal infections, malnutrition, severe stress, and congenital disorders.²³

As shown in Table 3, BCSO administration did not generally affect

platelet counts since they remained normal; however, BCSO administration at a dose of 3x1 capsules/day resulted in a lower platelet count than the placebo group. However, there was no difference in platelet count between the BCSO and non-BCSO groups (p>0.05). Therefore, based on the data in Table 2, it can be concluded that the administration of BCSO for 30 days in healthy-smoker volunteers does not affect the platelet count.

Thrombocytes or platelets are small, unnuclated fragments or pieces of the megakaryocytic cytoplasm. Platelets are formed in the bone marrow. Platelets are the most critical component in the hemostatic response, protecting against possible bleeding or blood loss. Platelets play an essential role in controlling bleeding.²⁴ Smokers tend to experience

hypoxia due to part of their hemoglobin binding to carbon monoxide or carbon dioxide, thereby stimulating a tolerance mechanism in the form of an increase in erythropoiesis, including the platelet count.²⁵

CONCLUSIONS

It was concluded that the consumption of BCSO 3x1 capsules/day for 30 days did not affect the number of platelets and leukocytes in healthy smoker volunteers. Consumption of BCSO 3x1 capsules/day for 30 days did not affect the subject's homeostasis. The issue can maintain a stable condition so that his body can function normally in a balanced manner.

CONFLICT OF INTEREST

There is no potential conflict of interest in this research.

ETHICAL CLEARANCE

The study protocol and interview guidelines have been reviewed and approved by the Research Ethics Committee, the Universitas Muhammadiyah Yogyakarta (078/EP-FKIK-UMY/III/2021).

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AUTHOR CONTRIBUTION

Literatures search data acquisition, and analysis, TT, ED, SS; clinical studies, TT, II, SS; experimental studies, TT, ED. All authors contributed to conceptualization, design, the definition of intellectual content, statistical analysis, preparation, editing, and manuscript review. The first author serves as a guarantor for the current study.

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