HASIL CEK_Santoso, Hidayati, Akrom, Nurani_Active smokers, ALT, BCSO, folic acid, randomized controlled trial, vitamin C.

by Santoso, Hidayati, Akrom, Nurani The Effect Of Black Cumin Seed Oil On Alanine Amin

The Effect of Black Cumin Seed Oil on Alanine Aminotransferase Levels which are Influenced by Nutritional Status in Active Smokers

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ABSTRACT

Black cumin seed oil (BCSO) has the main compound thymoquinone which is thought to have hepatoprotective activity through an antioxidant mechanism. Exposure to cigarette smoke can cause cellular oxidative stress which is the key to the inflammatory process of liver cells and an increase in the activity of the *Alanine aminotransferase* (ALT) enzyme. Vitamin C and folic acid are nutrients that affect ALT levels through antioxidant activity. The main objective of this study was to see the effect of BCSO administration on ALT levels which were influenced by the intake of vitamin C and folic acid in healthy respondents who were active smokers for 30 days. This study used a single-blind Randomized Controlled Trial (RCT) method, with a total of 39 test subjects divided into four groups. Group 1 received 3x1 placebo, group 2 received BCSO 3x1, group 3 received BCSO 3x2, group 4 received BCSO 3x3. Investigation based on the ANOVA test, it turned out that there was no significant difference in the mean ALT levels in both the placebo group and the treatment group with p value = 0.452. For the p value of the intake of vitamin C and folic acid were 0.359 and 0.669, respectively. Based on the results of the study showed that the administration of BCSO for 30 days had no significant effect on the average activity of ALT in healthy active smokers and there was no significant difference in the intake of vitamin C and folic acid.

Keywords: active smokers, ALT, BCSO, folic acid, randomized controlled trial, vitamin C.

Introduction

Smoking in modern times is one of the most common addictions. Smoking is an etiological agent for a variety of chronic diseases including various infections, cancer, heart disease and respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD), which has a disruption in the balance between cell death and cell growth which together are the leading causes of morbidity and mortality in society now. Cigarette smoke contains more than 4000 compounds, including 200 toxic compounds, 80 are suspected carcinogens and the rest are oxidants and free radicals that induce oxidative stress, inflammation in the liver and apoptosis (Zhong et al., 2008).

Oxidative stress can affect a wide variety of organs. One of the organs that is prone to damage due to oxidative stress is the liver. The liver is the largest organ in the body that has various functions to maintain the survival of most of the organs in the body. The liver is the gateway to all materials that enter the body through the digestive tract, making it very susceptible to metabolic, toxic and microbial disorders (Baratawidjaja, 2014). One of the liver organs that is important in the inflammatory process is hepatocytes. Hepatocytes are the main parenchymal cells in the liver that play a role in many metabolic pathways, weighing about 80% of the liver mass, and the nucleus of both single and multiple cells. Hepatocytes are very active in synthesizing protein and lipids to be secreted as a sign of inflammation (Kresno, 2013). Liver dysfunction is indicated by increased ALT and SGOT enzyme activity. ALT can be produced the most in the liver, while in the heart and skeletal muscle is slightly reduced when compared to SGOT. Activity in serum is increased especially in liver damage, when compared to AST (Tanoesian et al., 2016). Excretion through bile triggers xenobiotics in the liver so that it can cause hepatotoxic effects. ALT enzyme measurements can be used to identify the safety of a substance that is entered and metabolized by the liver (Setiawati et al., 2007).

Vitamin C (L - ascorbic acid) is a natural compound that has strong antioxidant properties and free radical scavengers but is not enzymatic. One of the importance of this compound for humans is based on its ability to bind superoxide radicals and hydroxyl radicals which also react directly to hydrogen peroxide, therefore vitamin C can prevent toxic free radicals that cause pollution (Fauzi, 2008). Many studies have shown that vitamin C is very beneficial for the prevention and treatment of diseases, including: lowering blood pressure and cholesterol, working as an antioxidant that can restore the effects of hepatocyte damage caused by free radicals, and increase the immune system (Sulistyowati, 2006).

Folic acid is a water-soluble B vitamin and has been shown to influence the metabolism of oxidative stress. Folic

acid has also shown function as an antioxidant, being associated with its ability to capture Reactive Oxygen Species (ROS), inhibiting ROS-producing activity. Folic acid can also reduce circulating levels of proinflammatory mediators in overweight individuals and in patients with hyperhomocysteinemia (Sarna et al., 2012). Folic acid is also known to have anti-inflammatory effects through inhibition of Il-6-induced proinflammatory cytokine expression in the liver. The ability of folic acid to suppress the inflammatory response of the liver suggests that this micronutrient has therapeutic potential for fatty liver management (Sid et al., 2015).

Black Cumin Seed Oil (BCSO) is a traditional medicine that has proven efficacy and safety. BCSO contains a lot of unsaturated fatty acids, namely linoleic and linolenic acids and essential oils with active substances thymoquinone, nigellone, and negelin (Akrom, 2013). BCSO is a medicinal plant known in Indonesia by the name of black cumin. Black cumin (*Nigella sativa*) seeds and essential oil have been widely used in traditional medicine (El-Dakhakhny et al., 2002). Many studies have proven the effects of black cumin seed oil extract. In various studies, BCSO has shown properties as anticancer, free anti-radical, immunomodulatory, anti-inflammatory, and hepatoprotective (Ahmad et al., 2013).

Research on the benefits of BCSO administration with ALT level parameters which are influenced by nutritional status (vitamin C and folic

acid) in active smokers has not been widely conducted. This study was conducted to determine how the effect of BCSO administration for 30 days with various doses on ALT levels which are influenced by nutritional intake of vitamin C and folic acid in healthy volunteers who are active smokers.

Research Method

Type and Design of the Study

This research is an experimental study with a single-blind Randomized Controlled Trial (RCT) design. The sampling technique used is purposive sampling, where the researcher determines the sample based on predetermined criteria. The independent variable in this study was BCSO, while the dependent variable was the ALT level, vitamin, and folic acid. Eligible subjects were divided into four groups by simple random sampling. Subjects received either three-dose BCSO capsules or a placebo 3x1 for 30 days. Reviewing health conditions and side effects is carried out daily through smartphones. The researchers applied good clinical practice principles for human testing, according to the Helsinki declaration.

Instruments and Materials

Tools and materials used in this study included primary and secondary data collection sheets using a case report form (CRF), blood collection equipment, and a set of tools for blood analysis. The CRF was used to collect demographic and lifestyle data. ALT

levels were analyzed using a Hematology Analyzer. Measurement of vital signs, filling out CRF, and nutritional status and blood collection were carried out at residents' homes. Data collection in this study was conducted by means of interviews, observation, and direct measurement. Complete blood count was carried out at Nur Hidayah Hospital.

Research Subject and Intervention

The target population in this study were healthy adult men and active smokers. The affordable population includes healthy participants, active smokers and residing in the working area around the Jetis 1 Primary Health Center (PHC) in Bantul, Yogyakarta. The inclusion criteria in this study were healthy adult men, aged > 18 years, active smokers and willing to participate in the study by filling out and signing an informed consent. Prospective subjects who have been selected through the inclusion criteria will be excluded from the study subject if the research subject has a history of chronic disease, has an allergic reaction after the administration of BCSO, the test subject does not participate until the end of the study and is also not willing to attend on the 31st day for the blood collection process.

Data Analysis

The analysis used to compare the average ALT levels between treatment groups and folic acid consumption between treatment groups was one-way ANOVA. Meanwhile, the consumption of vitamin C between each treatment group used the Kruskal Wallis method (non-parametric).

Research Ethics

This research involves human participants, it is very important to explain to all potential respondents about the intent, purpose, benefits, and usefulness of this research as expected and also the consequences of the respondent (informed consent). In addition, the management of ethical licenses (166/EP-FKIK-UMY/V/2019).

Results and Discussion

General Description of Participants

The subjects of this study consisted of 39 active smoker participants who were divided into 4 treatment groups located in the working area of the Jetis 1 Public Health Center in Bantul. Demographic conditions of research subjects according to gender, education, status, occupation, smoking status, age, blood plessure, blood glucose, length of smoking (years) and number of cigarette consumption (sticks/day).

As shown in Table 1, it can be seen that the characteristics of respondents as subjects to various treatment groups, consisting of placebo 3x1 capsules/day, BCSO 3x1 capsules/day, BCSO 3x2 capsules/day and BCSO 3x3 capsules/day. Based on the table above shows that the gender of the subjects are all male (100%), the average level of education of high school graduates (SHS) (61.5%), most are married (66.7%), and work they

work as laborers on average (46.2%). The mean age of the subjects was 39 years, smoking duration was approximately 18 years with an average number of cigarettes consumed was 10 cigarettes/day. Based on the table above, both the placebo and treatment groups have a significance value of p> 0.05, this shows that there is no significant difference in the characteristics of respondents between groups.

The characteristics of participants as subjects. The subject is male and smokers (100%), this is in accordance with research in Southeast Asia, it is estimated that the prevalence of smokers who are male is around 45% and only 4% is female (Gowing et al., 2015). Smoking in pregnancy causes fetal underdevelopment and increases the risk of miscarriage, neonatal death, respiratory disease in the offspring, and is a major cause of mental health problems in the offspring. This is one of the reasons why the prevalence of women is smaller than men (West, 2017).

The average age of participants in this study between each treatment group is 39 years, this is in accordance with research that the prevalence of smoking increases with age and is the highest among the ages of 25-44 years (Aji et al., 2015). From the results of the analysis in Table 1. the average blood pressure measurement of participants was 139/87 mmHg with a value (p>

0.05), this indicates that there is no significant difference between the control group and the treatment group on blood pressure after treatment with BCSO for 30 days. Smoking one cigarette lasting 15 minutes or more can cause an increase in blood pressure. Smoking habits show an increase in blood pressure during daytime activities (Takami and Saito, 2011).

Based on Table 1, from the analysis, the participants' average blood glucose level was 137 mg/dl. There was a temporary difference in blood glucose levels between each treatment group but the difference was not significant as evidenced by the p>0.05. Where the average instantaneous blood glucose level obtained by 39 participants was 137 mg/dl < 200 mg/dl (ADA, 2019).

Based on Table 2, it can be seen that the differences in the characteristics of folic acid consumption in this study were not significantly different between each treatment group, namely the average was 123.72 µg. Based on Restutiwati's research in 2019 that in active smokers, the level of folic acid sufficiency tends to be low because it is influenced by nicotine levels in cigarettes which cause reduced appetite. Low folate levels have been shown to be associated with higher death rates from all causes and cardiovascular disease (Loria *et al*., 2000).

Table 1. Demographic characteristics of participants at Jetis 1 Health Center Bantul

Participants characteristics		Treatment				Percentage	Sig (2-tailed)
		Placebo 3x1 capsules/days	BCSO 3x1 capsules/days	BCSO 3x2 capsules/days	BCSO 3x3 capsules/days	Total	
Type gender	Male	9 (23.1%)	11 (28.2%)	10 (25.6%)	9 (23.1%)	100%	0.388
Education	Elementary		3 (50%)	1 (16.7%)	2 (33.3%)	15.4%	
	JHS	2 (40%)	2 (40%)		1 (20%)	12.8 %	
	SHS	7 (29.2%)	5 (20.8%)	7 (29.2%)	5 (20.8%)	61.5%	
	S1/S2/S3		1 (25%)	2 (50%)	1 (25%)	10.3%	
Marital Status	Married	8 (30.8%)	5 (19.2%)	6 (23.1%)	7 (26.9%)	66.7%	0.188
	Unmarried	1 (7.7%)	6 (46.2%)	4 (30.8%)	2 (15.4%)	33.3%	
Job	*Private*	4 (23.5%)	6 (35.3%)	5 (29.4%)	2 (11.8%)	43.6%	0.631
	Teacher			1 (100%)		2.6%	
	Farmers				1 (100%)	2.6%	
	Laborers	5 (27.8%)	5 (27.8%)	3 (16.7%)	5 (27.8%)	46.2 %	
	retirees				1 (100%)	2.6%	
Age		39.44±6.74	41.55±12.32	36.4±15.23	41.11±14.63	39.64±12.40	0.798
Blood presure (mmHg)		141/92±23/16	138/87±18/9	143/91±30/15	135/80±10/7	139/87±21/13	0.524
Blood glucose levels (mg/dL)		137,78±40,47	163.36±94.33	115.70±14.21	126.44±23.65	136.72±56.56	0.632

Table 2. Characteristics of smoking behavior and nutritional intake of healthy participants in the work area of Jetis 1 Health Center Bantul

Participants characteristics	Treatment			Percentage		Sig (2-tailed)
	Placebo 3x1 capsules/days	BCSO 3x1 capsules/days	Placebo 3x1 capsules/days	BCSO 3x1 capsules/days	Placebo 3x1 capsules/days	
Length of smoking (years)	15.89±8.84	20.64±13.40	14.80±11.97	20.56±15.33	18.03±12.43	0.632
Total consumption of cigarette (cigarettes/day)	9.89±10.45	10.55±5.26	7.6±4.25	11.56±7.72	9.87±7.02	0.661
Vitamin C intake (mg)	29.10±36.54	21.18±14.67	30.65±36.32	28.35±24.85	27.09±28.19	0.699
Folic acid intake (µg)	102.02±48.31	111.78±43.77	138.03±74.67	144.10±65.25	123.72±59.31	0.359

Bivariate analysis using Kruskal Wallis, if p<0.05 there is a significant difference

Folic acid is known to have anti-inflammatory effects through inhibition of NF-κB-induced proinflammatory cytokine expression in the liver. From the results of the same study, it shows that folic acid is able to suppress oxidative stress that occurs due to exposure to xenobiotic substances, but the results cannot approach the normal group even though it is significantly different when compared to the P1 group of xenobiotic induction (Sid et al., 2018).In a 2013 study by Guan and He, he said that folic acid would be good if the levels were right. If the levels are not enough, folic acid does not provide sufficient cell repair effect, while if the levels are too large it can also cause damage to liver tissue.

Consumption of vitamin C was analyzed using the Kruskall Wallis method with a significance value of p> 0.05, which means

there is no significant difference between the average level of vitamin C consumption in the placebo group and the treatment group. According to Hariyatmi (2004), vitamin C functions as a scavenger of superoxide and free radicals and is very effective as an antioxidant at high concentrations. Administration of high doses will reduce lipid peroxide. One of these compounds is its ability to bind superoxide radicals and hydroxyl radicals also react directly to hydrogen peroxide, therefore vitamin C can prevent toxic free radicals that cause pollution (Simanjuntak, 2010). The results of other studies showed that there was a significant protective effect of vitamin C (p <0.05) which reduced levels of the transaminase enzyme when compared to the group that was only given plumbum acetate (Syahrizal, 2008).

Analysis of ALT Activity between Treatment Groups

ALT works to catalyze the reversible transfer of an amino group between alanine and alpha-ketoglutaric acid which functions in the formation of amino acids needed to make protein in the liver. This enzyme can be found mostly in the liver, while the heart and skeletal muscles are slightly reduced. The increase in ALT activity occurred significantly with the number of cigarettes consumed or heavy smokers. ALT is the most commonly found enzyme in liver cells so it is very effective in diagnosing liver damage (Tanoeisan, 2016).

The average ALT levels after giving placebo and BCSO for 30 days were the placebo group of 36.967 U/l, the 3x1 BCSO group of 22.991 U/l, the 3x2 BCSO group of 23.560 U/l and the 3x3 BCSO group of 23.344 U/l. The normal value of ALT in men is 0-42 U/L, and women ranges from 0-32 U/L (Mahaboob, 2013).

From the results of the study showed that the average value of ALT activity of participants in both the control (placebo) and treatment groups was still within the normal range of values. This study is in accordance with previous research which stated that giving MBJH for 20 days there was no significant difference in the average difference in ALT activity in the measurement of the treatment group and control group (p> 0.05) (Octavianti, 2015).

The results of ALT measurements are presented in Table 3. It can be seen that there is no significant difference in the mean ALT activity in both the placebo and treatment groups with a p value> 0.05. So, the administration of BCSO for 30 days did not significantly affect ALT activity in healthy active smoking volunteers. There was no significant difference between the placebo and treatment groups because changes in ALT activity were still within the normal range. The results showed that the average value of ALT activity of participants in both the control (placebo) and treatment groups was still within the normal range. Research conducted on white mice showed that giving black cumin seed oil orally for 4 weeks did not adversely affect the levels of ALT and AST, alkaline phosphatase, serum bilirubin in normal albino rats.

The decrease in ALT activity in the BCSO treatment group showed that the administration of BCSO could protect liver function although the effect was not significant in healthy volunteers who were active smokers. This is supported by the research that BCSO containing timokuinone has been shown to increase the anti-oxidative enzyme Glutathione S-Transferase (GST) and increase the number of treg in SD rats induced by DMBA so that it can inhibit excessive inflammatory reactions (Akrom et al., 2015). GST as an antioxidant can protect liver cells from the influence of free radicals so that the ALT enzyme is in the normal range (Alsaif, 2007).

Table 3. Average ALT activity after giving placebo and BCSO for 30 days

Treatment group	N	Average ALT Activity (U/I)	Standard Deviasi	Sig – (2-tailed)
Placebo 3x1 capsules/day (control)	9	36.967	35.64	
BCSO 3x1 capsules/day	11	22.991	13.82	0.452
BCSO 3x2 capsules/day	10	23.560	14.56	
BCSO 3x3 capsules/day	9	23.344	18.94	
Total	39	26.444	21.85	

Bivariate analysis using one-way ANOVA, if p<0.05 there is a significant difference

The provision of BCSO as an antioxidant can prevent damage to the liver cell membranes due to oxidative stress so that the ALT enzyme does not come out of the blood, as a result the activity of the ALT enzyme in the blood will decrease and go to normal. Several other studies also stated that BCSO administration could reduce ALT activity in ethanol-induced mice (Juwita, 2011).

Conclusion

From the results of the analysis, it was concluded that there was no significant difference in the nutritional intake of the subjects as seen from the results of the SPSS analysis of vitamin C and folic acid. Administration of BCSO for 30 days did not significantly affect ALT activity in either placebo or treatment groups. There was a decrease in ALT activity in the BCSO treatment group, indicating that administration of BCSO as an antioxidant can protect liver function even though the effect is not significant in the treated subjects.

Acknowledgment

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