

Black Cumin Seed Oil as Hepatoprotector in Decreasing SGPT and SGOT Activity and Increasing p53 Gene Expression in Sprague Dawley Rats Induced by Alloxan

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ABSTRACT

The objective of this research was to determine the effect of BCSO on SGPT/SGOT levels and p53 expression in rats induced by alloxan. This study applied a post test only control group design. A total of 42 SD rats were divided into 6 groups. Group I was normal rats. Group II (negative control) was given alloxan. Group III and IV (treatment groups) were given BCSO with equivalent doses of 6.8 mg/kg and 68 mg/kg thymoquinone, respectively (BCSO 6.8 and BCSO68 groups). Group V (positive control) was given vitamin C. Group VI (control media) were given DMSO. BCSO and Vitamin C are given for 1 week. Alloxan was given on 7th day, i.p. On the 8th day did blood sampling for measurement of SGPT and SGOT levels. Liver retrieval was performed on the 9th day continued immunohistochemistry staining. The average levels of SGPT/SGOT were then analyzed with ANOVA test. p53 expression was analyzed by Kolmogorov Semirnov and Levene then followed by Kruskal Wallis and Mann-Whitney. Statistical tests performed on 95% confidence level. The results showed that the induction of alloxan increased levels of SGPT/SGOT. BCSO68 reduced SGPT /SGOT levels in alloxan-induced rats ($p < 0.05$). Both BCSO 6.8 and BCSO68 increased the expression of p53 in liver tissue of alloxan – induced rats. Average percentage of p53 expression in group I to group VI were 11.12 ± 0.37 ; 19.24 ± 4.47 ; 30.31 ± 1.59 ; 40.43 ± 1.74 ; 29.67 ± 0.47 ; 12.02 ± 1.01 , respectively. The result of statistical analysis showed the value of $p < 0.05$ in all groups except in group III. It can be concluded that BCSO may have hepatoprotective effect and increase the expression of p53 in the liver of alloxan-induced rats.

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1. INTRODUCTION

Oxidative stress can affect various organs. Instead of pancreas, one of organ which is vulnerable to damage due to oxidative stress is liver. Liver is the largest organ in the body which has a variety of functions to sustain the survival of most organs in the body. Hepatic dysfunction demonstrated by the increased activity of the SGPT and SGOT enzyme [1],[2].

The prevention of degenerative diseases due to oxidative stress can be done by consuming antioxidants [3],[4]. Black cumin seed oil (BCSO) has the main compound of thymoquinone allegedly having hepatoprotective activity through antioxidative mechanism [5],[6]. Previous research reported

thymoquinone has an antioxidant, anti-infective, and antihistamine effects [7]-[9]. But the mechanism of BCSO protects the liver function and p53 expression in rat was unknown. This study was aimed to determine the effect of BCSO on levels of SGOT/SGPT and p53 expression in liver rats induced by alloxan.

2. RESEARCH METHOD

2.1. Materials

Black cumin seeds were obtained from the distributor of herbal ingredients in Semarang. The authenticity of black cumin seeds carried out at the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta. Other materials are white male rats (*Rattus norvegicus*) Sprague Dawley strain obtained from BPOM Jakarta, distilled water, alloxan, dehydration solution (alcohol 70%, 90%, 95%, and absolute), clearing solution (xylol), PBS, normal serum, H₂O₂, methanol, anti-p53 monoclonal antibody, paraffin, 0.9% NaCl physiological, xylene, peroxidase blocking solution, chromogen DAB (Diaminobenzinidine) and hematoxylin eosin.

2.2. Methods

2.2.1. Preparation of BCSO

BCSO was obtained by pressing black cumin seeds. Black cumin seeds 400g pressed with engine power of 600 tons. The oil was collected in a petri dish steril to be stored at room temperature.

2.2.2. The hepatoprotective activity test of BCSO induced by alloxan

Forty two rats were divided into six groups, each consisting of 7 animals. Group I (normal), Group II (ill), Group III, IV were treated by BCSO at dose 6.8 mg/kg and 68 mg/kg BW. Group V as the positive control group were given vitamin C. Group VI was a control solvent given DMSO. BCSO and Vitamin C were given during one week, then alloxan was given on day 7.

2.2.3. Measurement of SGPT and SGPT level

Blood samples were inserted into ependroff containing heparin. The blood centrifuged at a speed of 4000 rpm. Once centrifuged, the samples were separated by using pipet. SGPT/SGOT levels of samples were determined using 200 mL sample which was added in 1000 µlmonoreagen. Each incubated at a temperature of 250C for 60 seconds and then read at a wavelength of 344 nm (A1). Wait 60 seconds and then re-read the absorbance (A2), wait 60 seconds, and read again (A3). Absorbance used to measure levels of SGPT and SGOT using the equation:

$$\text{Levels of SGOT or SGPT (U / I)} = \Delta A / \text{min} \times F$$

Formula:

$$\Delta A / \text{min} = ((A1-A2) + (A2-A3))/2$$

F = Factor (971)

2.2.4. p53 expression examination

Liver retrieval is performed on 9th day by means of cervical dislocasio. Liver was cut using a microtome. In the dehydration process of tissue sections was added to a solution of the alcohol concentration of multi-storey. The tissue was then put in a solution of pure xylol alcohol during 2x2 hour. The next process is the impregnation. This process is included in the liquid paraffin tissue for 2x2 hour. The last step is embedding. In this process the tissue embedded in paraffin solid having a melting point of 560C-580C until the paraffin solidifies [10]. The hepatic sample was finished preparations made Immunohistochemistry staining. p53 expression (brown) was observed in cells using a light microscope with a magnification of 40 times.

2.2.5. Data Analysis

Percentage of p53 expression was calculated by (number of cells that expressed / total cell number) x 100%. Analysis applied the Kolmogorov-Smirnov and Levene statistical tests followed by Kruskal Wallis and Mann-Whitney test on 95% confidence level.

3. RESULTS AND ANALYSIS

3.1. Activity SGOT

The result of SGOT are presented in Table 1. The results showed that administration at equivalent doses BCSO 6.8 and 68 mg / kg thymoquinone for 7 days did not affect the activity of SGOT. SGOT before induced by alloxan in rats given BCSO and vitamin C are not different from the normal group. The treatment given to the test animals did not affect the levels of SGOT and does not change the normal conditions. SGOT levels are normal in this study is different from that expressed by Giknis and Clifford (2008) [11] in which the average level of SGOT normal SD rats was 23.35 ± 1.11 U / l. Solvent DMSO group had higher levels compared to the normal group ($p < 0.05$). Himawan (2008) [12] states that the factors affecting the levels of SGOT, among others, the psychological condition of rats, liver disease, or immune from each of the test animals. Moreover, according to Walter (2008) [13] that DMSO can cause side effects that damage the liver and kidneys that can cause increased SGOT.

Table 1. The SGOT level of rats pre- and post- induced by alloxan with treatment of BCSO or vitamin C

Groups	SGOT level	
	Pre(mean \pm SD) U/I	Post (mean \pm SD) U/I
Normal	23.35 \pm 1.107	23.71 \pm 0.542 ^a
Negative control (alloxan)	23.35 \pm 1.107	33.62 \pm 0.469*
BCSO 6.8	23.44 \pm 0.464	34.88 \pm 0.600*&**
BCSO 68	23.58 \pm 0.619	30.24 \pm 0.539 ^a
Vitamin C	22.42 \pm 0.720	27.86 \pm 0.552 ^a
DMSO	25.38 \pm 0.461*	

Ext: * = $p < 0.05$ (significant difference to normal group); ** = $p > 0.05$ (not significant difference to alloxan group); ^a= $p < 0.05$ (significant difference to alloxan group)

Alloxan induction on day 7 shown to increase the activity of SGOT. Negative group, have a higher activity than normal SGOT ($p < 0.05$), indicating that the administration of alloxan affect liver function and increasing the activity of SGOT. According to research Mardiasuti (2002) observed microscopically on alloxan-induced liver 125 mg / kg showed the presence of liver damage in the form of degeneration to necrose. BCSO at dose of 68 mg/kg showed a significant difference with the negative control ($p < 0.05$). BCSO at a dose of 68 mg / kg have been able to prevent the increase in SGOT levels rats but not yet reached normal. Another study conducted by Juwita (2011) [14] proved that the administration of BCSO can lower elevated levels of SGPT and SGOT in rats induced by ethanol. Thymoquinone, the main active ingredient of *Nigella sativa*, has been reported to prevent liver damage in rats through antioxidative and anti-inflammatory mechanisms [15],[16]. Thymoquinone have antioxidant activity through the donation of electrons to free radicals [17],[18]. BCSO group of 68 mg / kg when compared with positive group that vitamin C had a significant difference, in which simvastatin group had higher levels closer to normal.

3.2. Activity SGPT

The result of SGPT are presented in Table 2. The results showed that administration at equivalent doses BCSO 6.8 and 68 mg / kg thymoquinone for 7 days did not affect the activity of SGPT. SGPT before induced by alloxan in rats given BCSO and vitamin C are not different from the normal group ($p > 0.05$). The treatment given to the test animals did not affect the levels of SGOT and does not change the normal conditions but DMSO administration increases SGPT levels ($p < 0.05$).

Table 2. Level of SGPT liver rats pre- and post-induced by alloxan and treated by BCSO or vitamin C

Groups	SGPT level	
	Pre (mean \pm SD) U/I	Post(mean \pm SD) U/I
Normal	18.73 \pm 0.638	19.28 \pm 0.542 ^a
Negative control (alloxan)	18.15 \pm 0.268	27.31 \pm 0.612*
BCSO 6.8	18.44 \pm 0.396	26.78 \pm 0.00*&**
BCSO 68	18.98 \pm 0.554	24.97 \pm 0.473 ^a
Vitamin C	17.96 \pm 0.592	23.01 \pm 0.554 ^a
DMSO	23.28 \pm 2.288*	

Ext: * = $p < 0.05$ (significant difference to normal group); * = $p > 0.05$ (not significant difference to alloxan group); ^a= $p < 0.05$ (significant difference to alloxan group)

After induction of alloxan day 9 SGPT levels were normal group has significant difference compared to other treatment SGPT levels. SGPT levels of negative group compared with the normal group had significant differences. Giving alloxan affect SGPT levels are characterized by increased levels of SGPT mice was higher than normal control group [19]. According to research Mardiasuti (2002) [19],[20] observed microscopically on alloxan-induced liver 125 mg / kg showed the presence of liver damage in the form of degeneration to nekrose. BCSO group SGPT levels 6.8 mg / kg when compared with the negative group had no significant difference, indicating that the BCSO dose of 6.8 mg / kg have not been able to prevent the increase in SGPT levels alloxan-induced mice ($p > 0.05$). BCSO equivalent dose of 68 mg / kg thymoquinone SGPT levels lower than negative group ($p < 0.05$), indicating that BCSO equivalent dose of 68 mg / kg thymoquinone can prevent and reduce levels of SGPT SD rats induced by alloxan. These results are supported by research conducted by Juwita [11],[21] that the provision of black cumin oil may lower elevated levels of SGPT and SGOT on ethanol-induced rats.

3.3. p53 Expression

The result of p53 expressions are presented in Table 3. Alloxan administration decreased p53 expression ($p > 0.05$). The results showed that administration at equivalent doses BCSO 6.8 and 68 mg / kg thymoquinone for 7 days increased of p53 expression ($p < 0.05$).

Tabel 3. Percentage of mean p53 expression each group in rats induced by alloxan treated by BCSO or vitamin C

Groups	% mean p53 expression
Normal	11.12±0.37
Negative control (alloxan)	19.24±4.47*
BCSO 6.8	30.31±1.59* ^a
BCSO 68	40.43±1.74* ^a
Vitamin C	29.67±0.47* ^a
DMSO	12.02±1.01 ^a

Ext: *= $p < 0.05$ compare to normal group; ^a= $p < 0.05$ compare to negative (alloxan) control group

Table 3 shows that the BCSO have increased the expression of p53 activity when compared with the control group alloxan as sick. Expression of p53 in a dose of 68 BCSO group is the largest compared to the other groups in the amount of 40.43 ± 1.74 . In the healthy control group contained p53 expression because apoptosis is one way to keep a balance (homeostasis) in the body. Normal cells have low levels of p53 protein because they have a short life, and have a latent form. Level and p53 activity increases cellular damage such as responding to DNA damage by exposure alloxan. Generally, the activity of p53 has a pivotal role in the cell cycle or programmed death (apoptosis) [22]. P53 protein was used to detect DNA damage, DNA repair help through the termination of the G1 phase of the cell cycle and DNA repair genes trigger. Damaged cells and DNA can not be repaired, then directed by p53 to undergo apoptosis. In normal cell growth, survival time is very short p53 in minutes, whereas if there is damage to DNA can live a longer time, which in a matter of hours. With the increased expression of p53 means it will directly drive the process of apoptosis. When the injured cells, p53 in the nucleus of cells triggered a temporary halt to the border G1 / S by inducing CDK inhibitors (cyclin D kinase) and DNA repair system in advance to eliminate the injury before the cell enters the S phase in the absence of injured DNA [22],[23].

Thymoquinone is black cumin seed bioactive compounds that have antioxidant and immunomodulatory effects that can optimize the immune response against foreign substances or antigens [7],[8],[24]. Wirries et al. (2010) [25] stated that Thymoquinone, the main compound of black seed oil can induce pro-apoptotic signaling pathways. Treatment with cytostatic effect thymoquinone caused, mediated by up-regulation of p21 and down-regulation of cyclin E, which is associated with the arrest of S / G2 of the cell cycle. These findings indicate that Thymoquinone can inhibit cell proliferation dependent on p53 status by activating the cell cycle inhibitor p21. Thymoquinone also can cause non-specific cytotoxic reactions, for example, due to the formation of oxidative stress.

4. CONCLUSION

- BCSO at equivalent dose of 68 mg/kg thymoquinone have hepatoprotector effect by lowering SGPT and SGOT levels in rats induced by alloxan.
- BCSO may increase the expression of p53 in the liver of rats induced by alloxan.

- c) BCSO at equivalent dose of 6.8 mg/kgWB thymoquinone can increase p53 expression equivalent to the administration of vitamin C in the liver in rats induced by alloxan.

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