The Rice Bran as Therapy Agent to Decrease the SGOT/SGPT activities and Improve the Histopathology of Liver in White Rat (Rattus norvegicus) Induced by High Cholesterol Diet

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Abstract. Hypercholesterolemia is a metabolic disorder on increase of total cholesterol levels in blood exceeding normal values. The high cholesterol levels in the blood will be balance by converting to bile acids. The synthesis of bile acids will produce the excessive free radicals leading to oxidative stress. The oxidative stress cause lipid peroxidation of hepatic cell membranes that will increase SGOT and SGPT activities in the blood and changes in the histology of the liver. The content of crude fiber and antioxidants in rice bran are able to overcome the hypercholesterolemia. This study aimed to determine the effect of rice bran as a therapy in white rats (Rattusnorvegicus) high-cholesterol diet model on SGOT and SGPT activities and histopathologic images of liver. Rats were divided into 5 groups, namely: negative control group, positive control group, rice bran therapy group with dose 16% /rat/day,38% /rat/day and 57% / rat/day. Rice bran therapy performed for 21 days. SGOT and SGPT activities were determined by spectrophotometry and hepatic histopathologic images were observed with HE (Hematoxylin-Eosin) staining. The levels of SGOT and SGPT were analyzed by one-way ANOVA with α = 5% and histopathology of liver was analyzed descriptively. The results showed that rice bran with dose of 57%/rat/day significantly (p <0.05) can decrease SGOT and SGPT activities. Histopathologic observations showed that antioxidants in rice bran could reduce fatty acids on hepatic histopathology. The dosage of 57%/rat/day showed to decrease SGOT, SGPT activities and can reduce fatty acids on hepatic. It can be concluded that the administration of rice bran can reduce the activities of SGOT, SGPT and improve the histopathology of liver rat induced by high cholesterol diet.

1. Introduction
Hypercholesterolemia is metabolic disorder which indicated by the increase the total cholesterol level exceeds the normal level in blood circulation. This condition caused by the unhealthy lifestyle, less physical activities and imbalance meal pattern such as consumption of high fat diet, carbohydrate and less fibre. Hypercholesterolemia prevalent globally is 40-70% to all human ages and genders [1]. Hypercholesterolemia also occurred in pet animal like dog 32.8% of 192 American dog [2].

The increase of cholesterol level will be compensated by liver to produce bile acid which release reactive oxygen species (ROS) as by product. In increasing ROS can cause an increase of lipid.
peroxidation which occurred between ROS and poly unsaturated fatty acid (PUFA) of phospholipid membrane and causes wide spectrum cell damage including enzyme inactivation, abnormalities in intracellular oxidation-reduction function, DNA modification, and chromosomal abnormalities [3] All of these cytotoxic effects will lead centrilobular necrosis with increased hepatic enzyme plasma activity such as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloalxate transaminase(SGOT). High activity of SGPT is specific associated with liver cell damage, since SGPT is a cytoplasmic enzyme and present in high level only in liver cells. Therefore, SGPT is released from damaged cells associated with the increase cell membrane permeability or necrosis cells. The damage of hepatocyte cell can decrease lipoprotein lipase(LPL) that inhibit the conversion of very low density lipoprotein (VLDL) to intermediate density lipoprotein(IDL), consequently, VLDL is accumulated in liver lead to occur fat vacuole in hepatic cell.

Treatment of hypercholesterolemia can be conducted with statin, however in the long term usage can produce adverse effect to liver. The effort to decrease the blood cholesterol level is used natural product such as rice bran. The rice bran is an important product from rice milling processing industry. The rice bran contains carbohydrate 61.13%, ash 10.32%, crude fibre 15.06%, water 8.31% [4] and 12-18% oil which can act as antioxidant such as tocopherols, tocotrienols, phytosterols, squalene, Υ-oryzanol and phospholipid [5]. The rice bran has been widely utilized to overcome hypercholesterolemia. Hernawati et al studied the effect of rice bran on hypercholesterolemia Mus musculus, that rice bran at dose of 57% can decrease 10.31% of body weight, 17.28% of total cholesterol, 79.35% of LDL and increase 24.41%of HDL level [6]. However, the rice bran has not been reported to improve the liver with hypercholesterolemia. Based on the description above, this study has been conducted to determine the effect of rice bran to decrease activities of SGOT, SGPT and improve the histopathology of liver rat induced high cholesterol diet.

2. Materials and Methods

2.1. Chemicals and Instrumentations

The material used in this research were rice bran which purchased from Rice Milling kelompok Tani Makmur, Trenggalek. The determination of rice bran (oryzasativa L) was carried out by PT. Materia Medica, Batu City, East Java. Other materials used were SGPT reagent (Buffer TRIS pH 7.8, L-alanine, lactate dehydrogenase (LDH), 0.18 mmol/L NADH, 2-oxoglutarate) and SGOT reagent (buffer trisPH 7.8, L-aspartate, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), NADH2, 2-oxoglutarate), ethanol, xylol, paraffin, hematoxyline-eosin (HE), and 10% paraformaldehyde (PFA).

2.2. Experimental Animals and Design

The animal model of male Wistar white rats were purchased from Institute of Biosains, University of Brawijaya, Malang. The protocols for animals’ study were approved by a certificate of ethics from Research Committee of Brawijaya University (No:861-KEP-UB-2018). Ten to twelve weeks old, male wistar rat weighing about 130-180 g were allowed to acclimatize for 7 days prior to initiation of study. The rats were housed in completely controlled environment (room temperature 25±2 °C and 12 h light and dark cycle) with free access to water and food. Twenty rats were divided into five groups. Each group consist of 4 rats. Group I was designated as the negative control group received normal diet, while group II was the positive control received high cholesterol diet. Group III, IV and V were designated as experimental groups which received high cholesterol diet and rice bran therapy with dose 16%/rat/day, 38%/rat/day and 57%/rat/day for 21 days. At the end of the assay, all rats were sacrificed, hepatic organ and serum were collected for further analysis.

2.2.1. Measurement of Serum Glutamate Pyruvate Transaminase (SGPT) activities. Measurement of SGPT/SGOT activities were conducted by spectrophotometry method which recommended by IFCC (International federation of clinical chemistry) [7].
2.2.2. Liver histopathological observation. Histopathologic preparations of the rats’ liver were made using the HE staining method. Histopathologic image of the liver was visualized using the Olympus BX51 microscope with 400× magnification to observe the degeneration of lipids and damage to hepatocyte cells. Hepatic histopathology images were captured using a digital camera.

2.2.3. Statistical Analysis. The data of SGOT and SGPT activities were analyzed statistically using One-way Analysis of variance (ANOVA), followed by Tukey test multiple comparation tests. The level for statistical significance was set at a P value <0.05. The analysis used SPSS 23.0 for windows, while the liver histopathology image was descriptively analyzed.

3. Results and Discussion
Hypercholesterolemia rats have been successfully prepared by force feeding high cholesterol diet. All hypercholesterolemia rats of groups II, III, IV, V showed total cholesterol level in blood above 54 mg/dL that was 85.75 mg/dL, while the healthy rats were 43.25 mg/dL (normal standard of total cholesterol level in rats blood is 10-54 mg/dL) [8]. The high total cholesterol level is associated with producing free radical such as ROS. The imbalance of ROS and endogenous antioxidant causes oxidative stress lead to occur lipid peroxidation in hepatocyte cell membrane. The damage of hepatocyte cell membrane will enhance releasing of the hepatic enzyme plasma including SGOT and SGPT to blood circulation. Therefore, the SGOT and SGPT can be used as indicator of damaged liver.

The groups III, IV, V were then treated with rice bran and the results of SGOT and SGPT activities of hypercholesterolemia rats are displayed at Table 1.

Table 1. Average SGOT and SGPT activities in Group I, II, III, IV, V after 21 days of therapy with rice bran

| Group                      | SGOT activity (U/L)* | SGPT activity (U/L)* |
|----------------------------|----------------------|----------------------|
| I. Negative control (healthy group) | 64.75 ± 4.50^a       | 24.00 ± 4.55^a       |
| II. Positive control (Hypercholesterolemia Group) | 140.25 ± 17.37^c     | 74.50 ± 6.45^d       |
| III. Therapy 16%/rat/day | 97.00 ± 7.83^b       | 40.00 ± 5.71^c       |
| IV. Therapy 38%/rat/day  | 89.25 ± 4.78^b       | 37.50 ± 3.51^bc      |
| V. Therapy 57%/rat/day   | 68.50 ± 4.65^a       | 28.25 ± 5.85^ab      |

*different letters (a–d) show significant statistical different effect in each group p<0.05

As shown in Table 1, the SGOT and SGPT activities of hypercholesterolemia rats were significantly higher compared to healthy rat. The standard normal SGOT and SGPT activities of rat are 45.7-80.8 U/L and 17.5-30.2 U/L respectively [9,10]. The increase of SGOT and SGPT activities indicated the occurrence of oxidative stress which caused by feeding high cholesterol diet. The increase of blood total cholesterol level can be responded by body to produce bile acid. The synthesis of bile acid involves 7 α-hydroxylase, O₂, NADPH and cytochrome P₄₅₀ which produce ROS as by product.

The therapy of rice bran may reduce the elevated level of total cholesterol. The activities of SGOT and SGPT decreased with increasing dosage of rice bran. Statistical test results showed there were significantly difference (p<0.05) between activities of SGOT and SGPT of hypercholesterolemia and therapeutic hypercholesterolemia rats. The dose of rice bran 57%/rat/day is effective dose that SGOT and SGPT activities of Group V did not show different compared to Group I.
Group I
Group II
Group III
Group IV
Group V

Figure 1. comparison of Histopathologic of liver rats from group I, II, III, IV and V after 21 d treatments with rice bran with HE staining (100× and 400× magnification), H: hepatocyte cell, CV: central vein, S: sinusoid, D: fat degeneration.

Feeding high cholesterol diet and rice bran therapy affected to the histopathologic image of hypercholesterolemia rat liver. Changes in the histopathologic image of liver can be seen in Figure 1, particularly, the changes of hepatocyte cells. The hepatocyte cells condition of negative control group (Group I) shows that normal hepatocyte cells are arranged radials from central vein, the hepatocyte cells appear to have one or more nuclei (binuclei) that is clearly purple, the space between hepatocyte cells or sinusoid is clear in line with Utomo et al; finding \[11\]. The hepatocyte cells of hypercholesterolemia rats (Group II) indicates the presence of accumulation of fat vacuoles, the cell nucleus are damaged and black. In addition, there is irregular sinusoid structure and appear wide empty spaces. Improvement of histopathologic features of the liver can be seen in the therapy groups. In Group III shows a reduction of fat vacuoles accumulation in hepatocyte cell compared to Group II and sinusoid still looks irregular. In Group IV shows a more reduction of fat vacuoles accumulation in hepatocyte cells and sinusoid have been seen regular structure. In Group V shows there is very little fat vacuoles accumulation, hepatocyte cells nucleus is purple and sinusoid is clearly visible.

Decreases in SGOT and SGPT activities, improvements in hepatic histopathology of hypercholesterolemia rats treated with rice bran can be related to the content of crude fiber, vitamin E and \(\beta\)-oryzanol in the composition of rice bran. This suggests that crude fiber in rice bran binds the bile acid lead to cholesterol from diet cannot be emulsified or absorbed by intestine that increasing excretion of cholesterol and fat from diet through feces. In addition, \(\beta\)-oryzanol has ability to suppress lipogenesis in liver, anti-inflammatory activity and inhibit lipid peroxidation. The decrease of bile acid can attract the cholesterol in blood circulation to synthesize a new bile acid that bring to reduce.
blood cholesterol level. The decreased cholesterol level can reduce the bile acid synthesis resulting in the reduced free radicals (ROS). The γ-oryzanol and vitamin E can suppress the free radicals in order not to binds PUFA in hepatocyte cell membrane by donating H atom of hydroxyl group (-OH) to free radicals to form the stable radicals. Moreover, γ- oryzanol and vitamin E will balance the amount of oxidant and antioxidant in the body protecting the cells from free radicals, repairing hepatic damage and decreasing SGOT/SGPT activities.

4. Conclusion
This study demonstrated that rice bran at effective dose of 57%/rat/day can decrease SGOT and SGPT activities on Rattus norvegicus induced by high cholesterol diet. More over the rice bran can improve histopathology of its liver which showed by decreasing hepatic fat vacuoles, improving sinusoid and hepatocyte cell shape. Further study should be made in order to fully understand the molecular mechanism of γ-oryzanol acting as antihypercholesterolemia and the highest safe dosage of rice bran.

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