

## **Dietary Rice Bran Plays A Significant Role in the Hepatoprotective Effect in Hypercholesterolemic Rats**

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### **Abstract**

Cholesterol is obtained through biosynthesis and diet. When a level of cholesterol is above the normal level, this condition caused hypercholesterolemia. Long-term administration of synthetic chemical drugs can cause liver damage. Therefore, alternative natural medication is needed. One of the alternatives that can be used is the rice bran (RB), which contains antioxidant and crude fiber. This study is aimed at finding out the potential utilization of RB on total cholesterol level, liver enzyme as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), malondialdehyde (MDA), and the changes in liver tissue. This study uses five groups of rats: the negative control group, positive control group, and three therapy groups with the dosage of 270 mg kg<sup>-1</sup>, 540 mg kg<sup>-1</sup>, and 810 mg kg<sup>-1</sup> of body weight. This study shows that therapy using RB can significantly decrease the cholesterol level, AST, ALT, and MDA (p<0.01). The total cholesterol level is 21%, AST and ALT activities can be reduced to 54% and 64%, the level of MDA reduced to 79% and can repair the liver tissue. This study shows that RB can be effectively used as hepatoprotective in rats with hypercholesterolemia.

**Keywords:** AST and ALT, Hypercholesterolemia, Rice Bran, Total Cholesterol Level.

### **INTRODUCTION**

Hypercholesterolemia is influenced by age, sex, alcohol, obesity, stress, diabetes, and food intake [1,2,3]. The excessive consumption of fatty food can increase the level of cholesterol in the blood. Hypercholesterolemia can become the risk for atherosclerosis, pancreatitis, coronary heart, thyroid disorder, kidney failure, and liver damage [4].

Hypercholesterolemia indicates the existence of free radical within the body due to the cholesterol metabolic disorder. The increase of free radical can stimulate the peroxidation of lipid, hence, cause oxidative stress which can be measured using the MDA parameter [5]. The increase of free radical can reduce the activity lipoprotein lipase enzyme (LPL). This can cause a disturbance in the changes of very low-density lipoprotein (VLDL) into intermediate density lipoprotein (IDL). Therefore, VLDL will form a sediment in the liver and cause fatty in the liver, such as infiltration of fat into the surrounding of hepatocyte cells and sinusoid [6].

The cells damage within the liver can be clinically known at advanced stage. However,

ongoing liver damage can be known by measuring the parameter of liver function [7]. The indicator of the damage of liver cells is the increase of liver enzymes within the serum, such as AST and ALT enzymes [8].

Nowadays, several medicines have been reported to have cure Hypercholesterolemia which consists of statin, niacin, fibrate acid, nicotinate acid, and resin [9,10,11]. However, long-term medication of hypercholesterolemia using synthetic chemical medicine can cause liver damage and kidney functions disorder [12]. Thus, alternative medication from nature is needed. One of the alternatives for this medication of hypercholesterolemia is the utilization of RB.

RB has nutrition such as carbohydrate, protein, mineral, fat, and crude fiber [13,14,15]. In addition, RB also contains bioactive components such as phenolic compounds, oryzanol, vitamin E (Tocopherol and Tocotrienol) which serve as an antioxidant [15,16,17]. Several types of research have reported that rice bran can reduce the total cholesterol level, triglyceride, LDL, and increases the concentration of HDL [18,19]. In addition to finding out the influence of RB toward the

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cholesterol level, this study is also carried out to find the role of RB as hepatoprotective in rats with hypercholesterolemia, by using the liver function parameters of activity of the AST and ALT enzymes, MDA in liver organ, and supported by the observation of the liver tissue.

Moreover, RB also contains antioxidants that can prevent oxidative stress and inhibit the process of fat oxidation, and also contains crude fiber which can bind cholesterol [9]. This study will examine the effect of RB on total cholesterol levels, liver enzymes activity (AST and ALT), and MDA levels.

## **MATERIAL AND METHOD**

### **Material and Animals**

This study uses RB from white rice (*Oryza sativa*) from Anugrah Brand. The animal used in this study is 20 male Rats (*Rattus norvegicus*) age 2 months old, with the average weight of 200 gram. All the procedure for this animal usage has been approved acceptance of Brawijaya University's Research Commission number 941-KEP-UB.

### **Analysis of crude fiber content in rice bran**

One gram of RB is inserted into 300 mL Erlenmeyer tube. Then, the RB is added with 0.3 N H<sub>2</sub>SO<sub>4</sub> under the cooling back. The sample is boiled for 30 minutes and often shook. The sample is filtered using the filter paper. The residue is washed using the boiling water, hence, no longer acidic (tested using the Litmus paper). The residue then is inserted into the Erlenmeyer tube. The residue left in the paper then washed using 200 mL of boiling NaOH hence all the residue entered the Erlenmeyer tube. The sample is boiled for another 30 minutes. Then the sample is washed with 105 K<sub>2</sub>SO<sub>4</sub> solutions. The residue is then washed using 15 mL of 95% alcohol, and then the filter paper is dried in the temperature of 110°C hence, the weight is constant then the sample is weighed.

$$\text{The crude fiber level (\%)} = (A-B)/C \times 100\%$$

#### **Description:**

A : the weight of the filter paper+residue)  
B : the weight of empty filter paper  
C : sample weight

### **Determining the level of Antioxidant using the DPPH test**

The antioxidant activity is determined by using the DPPH test which through the procedure adapted from Dudonne [20]. 1 mL of DPPH solution (0.05 mM) is mixed with 2.5 rice bran extract. Then the mixture is incubated in the

temperature of 37°C for 20 minutes. The absorbance is measured using spectrophotometer UV-Vis in the length of wave 515 nm. Then, 2.5 mL of methanol is mixed with 1 mL of DPPH solution, and this mixture is used as a blank solution. The percentage of antioxidant activity to prevent the free radical (IC<sub>50</sub>) can be calculated using the formula:

$$\% \text{ inhibition} = (A \text{ Blank} - A \text{ Sample})/A \text{ Blank} \times 100$$

### **Induction of High Cholesterol Diet and Treatment**

The rats is distributed into 5 groups, each group with 4 rats: (1) negative control group, (2) positive control group, (3) RB therapy dosage is 270 mg kg<sup>-1</sup> bw, (4) RB therapy dosage is 540 mg kg<sup>-1</sup> bw, and (5) RB therapy dosage is 850 mg kg<sup>-1</sup> bw. Each mouse is fed with a high cholesterol meal, except for the negative control group. The food is administered for 8 weeks. The composition of high cholesterol meal consists of 32 g of goat fat, 0.32 g of folate acid, 16 g of cooked quail yolk eggs, and 4 mL of used cooking oil. The cholesterol level is tested every 14 days. The rats with a cholesterol level of more than 200 mg dL<sup>-1</sup> will be treated with rice bran with the previously mentioned dosage. On the 15<sup>th</sup> day, the rats will be operated to take the blood and liver organ samples. The serum is taken after it is being separated from blood.

### **Measurement of total cholesterol level in serum**

Total cholesterol level is tested using the CHOD-PAP reagent cholesterol method. The total cholesterol level is measured by adding 10 µL serum with 1000 µL reagent. After incubated, the absorbance is read using the spectrophotometer UV-Vis in the length of wave 520 nm.

### **Measurement of Aspartate and Alanine Aminotransferase Activities (AST and ALT)**

The AST and ALT level is tested using the IFFC method. The activity of AST enzyme is measured by adding 500 µL reagent AST in 50 µL serum. Whereas, the measurement of ALT enzyme is measured by adding 500 µL reagent ALT in 50 µL serum. After the mixture is homogeneous, each absorbance is read using the spectrophotometer UV-Vis in the length of wave 340 nm.

### **Measurement of Malondialdehyde (MDA)**

Supernatant formed from the isolation of protein liver organ is taken by the amount of 100 µL. The supernatant then added with 550 µL distilled water and 100 µL trichloro ethanoic acid, then the sample is centrifuged. The result is added with 250 µL HCl 1 N and centrifuged. The

supernatant then added with 100  $\mu$ L of 1% Na-Thio and the result is made homogeneous. After that, the supernatant is heated in the temperature of 100<sup>o</sup>c for 30 minutes. This supernatant the centrifuged in 500 rpm for 15 minutes. Following the incubation, the sample is read using the spectrophotometer UV-Vis in the length of wave 533 nm.

#### **Histopathological Examination**

The liver organ is taken from the operated mice then washed using the 0.9% of NaCl physiology. The preparation stage includes fixation, dehydration, cleaning, soaking, splitting, and attachment to the glass object. The rat liver is prepared for hematoxylin and eosin (HE) coloring. The rat liver is observed its histopathology description by using the BX51 Olympus microscope in the magnification of 100x and 400x.

#### **Statistical Analysis**

All the value is stated in the means  $\pm$ SD. The data are analyzed using the ANOVA and post-hoc LSD test, and the highly significant result in  $p < 0.01$ .

### **RESULT AND DISCUSSION**

#### **The influence of rice bran toward the total cholesterol of rats with hypercholesterolemia**

Based on Tabel 1 below, the RB in the dosage of 270 mg  $\text{kg}^{-1}$  bw, 540 mg  $\text{kg}^{-1}$  bw, and 850 mg  $\text{kg}^{-1}$  bw can significantly decrease the total cholesterol level in mice with hypercholesterolemia with the percentage of 7%, 17%, and 21% respectively compared to the positive control group. According to several previous studies, RB can inhibit the increase of cholesterol, and decrease the cholesterol level and lipid profile.

RB has the characteristic of reducing cholesterol called cholesterolemic effect. RB has 10.89% crude fiber content. This fiber is able to absorb lipid from the digestive channel. This fiber is also able to bind the bile acid and cholesterol that can be disposed with feces. Therefore, the radical bile acid will decrease and new bile acid will be needed. The more the new bile acid produced from the absorption of cholesterol in the blood, the more cholesterol will be excreted. This becomes the basis for the decrease in cholesterol level [14,21,22]. Another mechanism is that fiber can also inhibit the reduction of HMG-CoA enzyme, thus, decrease the synthesis of cholesterol within the liver [21].

#### **The influence of rice bran as hepatoprotective in Rats with hypercholesterolemia.**

An activity of hepatoprotective of RB in rats with hypercholesterolemia can be seen based on several biochemical parameters such as, the activity of the AST and ALT enzymes which indicates the damage in the liver cells. The MDA level to see the possibility of lipid peroxidation in liver organ. Hepatoprotective activity can also be supported by the histopathology observation of the liver tissue.

These enzymes are very sensitive indicators of damage or destruction happening in the liver cells. When the liver cells are damaged such as fatty, inflammation, up to cells damage such as apoptosis or necrosis, these AST and ALT enzymes will be leaked into blood circulation due to the increase of membrane permeability. Therefore, within the blood, the level of these enzymes increases [23,24].

In Table 2, hypercholesterolemia therapy using RB with the variation of dosages of 270 mg  $\text{kg}^{-1}$  bw, 540 mg  $\text{kg}^{-1}$  bw, and 850 mg  $\text{kg}^{-1}$  bw influences the significant decrease of AST and ALT enzymes activities ( $p < 0.01$ ). Compared to the negative control group, the AST activity decreases by 29%, 40%, and 54%, while the activity of ALT enzyme decreases by 31%, 47%, and 64.88%. This decrease is due to the antioxidant effect of RB which serves in preventing the free radical, prevent oxidative stress and inhibit the fat oxidation [24].

The MDA level measurement in liver organ shown in Table 3, where administration of RB with the variation of dosages mentioned above has experienced significant reduction ( $p < 0.01$ ) compared to the positive control group with the reduction of MDA level by 46%, 63%, to 79% sequentially. RB is proven to be able to reduce MDA level due to its antioxidant content which able to capture the free radical and inhibit lipid peroxidation, hence, can reduce the free radical due to induction of high cholesterol diet.

The antioxidant content in rice bran seen in antioxidant test  $\text{IC}_{50}$  is 75.18  $\mu\text{g mL}^{-1}$ .  $\text{IC}_{50}$  is a number which shows the concentration of extract ( $\mu\text{g mL}^{-1}$  or ppm) which can inhibit 50% of oxidation. Based on table 4 the antioxidant within the rice bran is classified as a strong antioxidant [20]. Several previous studies have shown that one of the antioxidants within the RB is Vitamin E (tocopherol/tocotrienol) [25,26].

**Table 1.** Total cholesterol levels in serum of hypercholesterolemic rats

Treatment Group	Total cholesterol levels (mg/dL)	Total cholesterol levels (%)	
		Increasing	Decreasing
Negative control	173.5 ± 3.69	-	-
Positive control	247.5 ± 2.88	42	-
RB (270 g/kg <sup>-1</sup> bw)	229.75 ± 2.5**	-	7
RB (540 g/kg <sup>-1</sup> bw)	204.75 ± 5.9**	-	17
RB (810 g kg <sup>-1</sup> bw)	193.75 ± 4.57**	-	21

The data showed the mean ± SD (n=4). The analysis shows that rice bran significantly decreases the total cholesterol level (p-value = 0.000). Note: \*\* showed the value of p<0.01 toward the positive control group.

**Table 2.** Effect of rice bran on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in hypercholesterolemic rats

Treatment Group	AST (U L <sup>-1</sup> )	ALT (U L <sup>-1</sup> )
Negative control	56.25 ± 3.5	26.00 ± 1.82
Positive control	136.25 ± 3.3	89.00 ± 2.16
RB (270 g/kg <sup>-1</sup> bw)	96.75 ± 2.21**	61.00 ± 2.94**
RB (540 g/kg <sup>-1</sup> bw)	81.75 ± 2.75**	47.00 ± 2.16**
RB (810 g kg <sup>-1</sup> bw)	61.5 ± 2.38**	31.25 ± 2.21**

Data shows mean ± SD (n=4) the result showed that rice bran significantly decreases the AST and ALT level (p value=0.000) note: \*\* shows the value of p<0.01 toward the positive control group.

**Table 3.** MDA levels in liver organ of hypercholesterolemic rats

Treatment Group	MDA level (µg/mL)	MDA Level (%)	
		Increasing	Decreasing
Negative control	0.67 ± 0.125	-	-
Positive control	4.7 ± 0.141	596	-
RB (270 g/kg <sup>-1</sup> bw)	2.5 ± 0.216**	-	46
RB (540 g/kg <sup>-1</sup> bw)	1.7 ± 0.081**	-	63
RB (810 g kg <sup>-1</sup> bw)	0.95 ± 0.208**	-	79

Data shows mean ± SD (n=4) the result showed that rice bran significantly decreases the MDA level (p value=0.000) note: \*\* shows the value of p<0.01 toward the positive control group.

Vitamin E plays a role in neutralizing the free radical by contributing to the atom hydrogen from the hydroxyl group (OH) which available in its ring structure to the free radical [27]. The existence of this antioxidant mechanism in RB helps protect the cell structure from the free radical effect, hence reduce the level of ROS which is signified by the reduction of MDA level and reduction of AST and ALT enzymes activities. This is what made the RB potential as hepatoprotective.

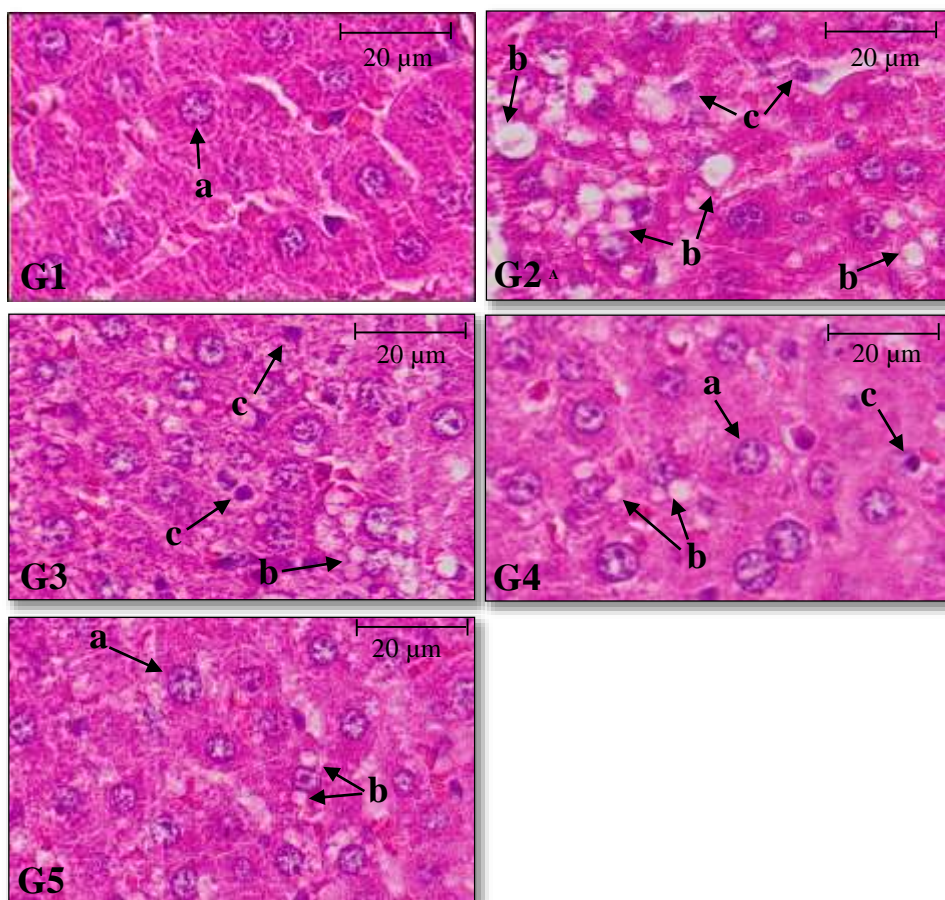
**Table 4.** The intensity value of IC<sub>50</sub>

Intensity	IC <sub>50</sub> value
Very Strong	< 50 mg mL <sup>-1</sup>
Strong	50 – 100 mg mL <sup>-1</sup>
Medium	100 – 150 mg mL <sup>-1</sup>
Weak	150 – 200 mg mL <sup>-1</sup>

Liver damage can be known from the increase of AST and ALT level in the positive control group which supported by the image of liver histopathology. This group shows the

accumulation of lipid around hepatocyte and plenty of necrotic hepatocytes (Figure 1.G2). Meanwhile, a significant decrease of AST and ALT level in each therapy group indicates an improvement of the hepatocyte. This is proven by the figure of liver histopathology on 270 mg kg<sup>-1</sup> BW therapy which shows a decrease of lipid accumulation, regardless that the necrotic hepatocytes are still exist (Figure 1.G3). In therapy group of RB with the dosage of 540 mg kg<sup>-1</sup> BW, it shows a more significant decrease of lipid accumulation, there are only a few necrotic hepatocytes left (Figure 1.G4). The histopathology image of therapy group of RB with a dosage of 810 mg kg<sup>-1</sup> BW shows much smaller lipid accumulation (Figure 1.G5).

The repairment of histopathology picture in liver organ due to the antioxidant from the RB, hence, can reduce the level of free radical by inhibiting the lipid peroxidation, thus, the ability of the crude fiber to reduce cholesterol level in blood [28].



**Figure 1.** Histopathology Overview liver of Hypercholesterolemic Rats Using Hemotoxylin-Eosin Staining (HE) (Magnification 400x). (G1) negative control group, (G2) positive control group, (G3) therapy 270 mg kg<sup>-1</sup> body weight, (G4) therapy 540 mg kg<sup>-1</sup> bw and (G5) therapy 810 mg kg<sup>-1</sup> bw. Hepatocyte (a), lipid accumulation (b), necrotic hepatocytes (c).

## CONCLUSION

The conclusion of this study is that rice bran in addition to reducing the cholesterol level is also very effective in protecting the liver from free radical due to induction of high cholesterol diet, which is signified by the reduction of MDA and activity of AST and ALT enzymes.

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