The Effect of Rice Bran on Triglyceride Levels and Histopatologic Aorta in Rat (Rattus norvegicus) of High Cholesterol Dietary Model

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Abstract. Cholesterol is a steroid compound found in animals and humans. Total cholesterol normal levels in rat is 10-54 mg/dL and triglyceride 26-145 mg/dL. Rice bran has a crude fiber content and antioxidants that can be used to resolve these conditions. This study aims to determine the effect of rice bran as therapy in the rats (Rattus norvegicus) high-cholesterol dietary model towards triglyceride levels and histopathologic of the aorta. In this research, rats (Rattus norvegicus) were divided into 5 groups with 4 repetitions. Group A as a negative control, group B was rat which fed with high cholesterol, group C was high-cholesterol diet model of rat with the 16% rice bran therapy/rat/day, group D was high-cholesterol diet model of rat with the 38% rice bran therapy/rat/day, and group E was high cholesterol diet model of rat with the 57% rice bran therapy/rat/day. Rice bran therapy performed for 21 days. Triglyceride levels were calculated by the GPO-PAP method and aortic histopathologic features were observed with HE staining (Hematoxylin-Eosin). The effect of rice bran on blood triglyceride levels was analyzed by one way ANOVA (Analysis of Variance) with α=5% and aortic histopathologic analysis was qualitatively descriptive. The results showed that rice bran therapy could significantly reduce triglyceride levels (p < 0.01) with a dose of 57%/rat/day to decrease triglyceride levels. Rice bran therapy also repaired aortic tissue histopathology of rat with high cholesterol diet model.

1. Introduction

Feeding high fat and cholesterol often occur in animals such as pet dogs and cats. One example is the granting of pet food in the form of meat and offal that can increase blood cholesterol levels in dogs and cats [1].

Cholesterol is a steroid compound that is found in animals and humans [2]. Normally, the levels of total cholesterol in the rat are 10-54 mg/dL [3]. Hypercholesterolemia is a situation where an increase in cholesterol levels in the blood is beyond normal level. The level of hypercholesterolemia on cats and dogs measured as much as 13% [4], whereas the dogs in Western countries stated as hypercholesterolemia when the level of their cholesterol measured 25% to 44% [5].

High cholesterol levels in the blood will turn into bile acids. The more bile acids will lead to the higher production of free radicals. This causes internal antioxidants are not being able to handle it [6]. High free radicals in the body will produce oxidative stress thus causing damage to the cells, tissues, or organs [7]. One of the damaged organs is the aorta. Free radicals also led to a high concentration of
lipids so that the levels of triglycerides will increase. Normally, the levels of triglycerides in rats are 26-145 mg/dL [8].

The use of drugs in hypercholesterolemia animals is successfully controlling and lowering the cholesterol levels in the blood, but causing side effects in the long term [3]. Therefore, this research uses rice bran as a therapy for reducing cholesterol levels in the blood. Bran is the outer layer of rice that contains protein, fiber, fat, and vitamin E [9]. Rice brans contain some antioxidants, including tocopherol, tocochromanol, and oryzanol [10]. Types of rice bran used are white rice the rice bran. The crude fiber of rice bran in white (15.06 ± 2.09%) higher than the red rice bran (13.44 ± 0.40%) [9].

Rough fibers can accelerate the rate of feed in bind bile acids absorption of fat-free so hampered and excretion of fats including cholesterol through the feces increased. Fibers will decrease bile acids in the body, thus the body will establish a new bile acid from cholesterol in the blood, as a result, the cholesterol level in the blood will decrease [11]. Antioxidants in bran can reduce free radicals on the body [10], the levels of triglycerides in the body will decrease, and cells, tissues, or organs damaging of the aorta will be reduced. Based on those reasons, the purpose of this research is to investigate the effect of rice bran as a therapy in high cholesterol rats (Rattus norvegicus) diet model against the levels of triglycerides and to determine its effect and its effect on the level of cell damage in the aorta through the description of histopathology.

2. Material and Methods

2.1. Tools and Materials

The tools used in this study, among others, spectrophotometer, microscopes, glass object, hot plate, Buchner funnel, Erlenmeyer flask, compressors, porcelain bowls, scales, filter paper, oven, electric furnace.

Materials needed in the study were the acid cholate, pork, quail egg yolk, eosin, hematoxylen, distilled water, 1.5 N NaOH 0.3 N H$_2$SO$_4$, NaCl physiological, the white male rats (Rattus norvegicus), Wistar strain, 8 weeks with weight ± 100 g, BR1, feed and drinking water.

2.2. Preparation of Experimental Animals

The experimental animals used in the study were 20 male white rats (Rattus norvegicus) of Wistar strain aged 8 weeks with an average body weight of 100 g. They had been adapted for 7 days and feed with standard feed once a day and were drink by BR1 ad libitum.

2.3. Analysis of The Proximate Content of Crude Bran Fiber

Samples weighed ± 1 gram (A), then put in the Erlenmeyer flask. After that, 50 mL of H$_2$SO$_4$ 0.3 N was added, mixed for 30 minutes, then 25 mL of 1.5 N NaOH was added to the mixture, and the mixture was boiled. The filter paper was placed on the Buchner funnel which has been known to weigh in (B). The solution was filtered by using a Buchner funnel and rinsed with 50 mL of hot water, and then solution was filtered.

Hydrochloric acid (HCl) 0.3 N by as much as 50 mL was placed into a Buchner funnel and left on for 1 minute with the compressor. Residue in a Buchner funnels was rinsed 5 timed with hot water, then 5 mL acetone was poured into the mixture, and incubated for 1 minute. Porcelain cup was heated for 1 hour in the 105 °C preheated oven and then cooled in a desiccator for 10-15 min, and weighed out (C). Filter paper containing the residue was placed in the porcelain bowls and then dried in an oven for 1.5 hours at 105 °C and cooled in the desiccator for CA for 30 minutes, and it was weighed out (D). Porcelain bowls were put into the 550 °C electric furnace for 2 hours. The electric furnace was turned off and left until the temperature is 0 °C. After that, the porcelain bowls were removed and placed in desiccator for 15 min and weighed out (E). The calculation formula is [12]:

\[
\text{Crude Fiber} = \frac{D-E-B}{A} \times 100\% \tag{1}
\]
2.4. Acclimatization of Experimental Animals
Rats in Group of B, C, D, and E were given high cholesterol feeding. Feeding a high cholesterol diet was conducted using a method that was reported by Larasathi and co-workers [13], as much as 2 grams of oil, 0.02 grams of acid cholate, 1 gram of quail egg yolk that has been heated at a temperature of 100 °C, and 2 mL of water. High cholesterol diet was given starting at 8th day for 35 days as much as 3.02 grams/2 mL.

2.5. Induction of Rice Bran Therapy
Bran therapy was given in Group C, D and E. The dose of each group was: the 16% rice bran therapy/rat/day for group C, 38% rice bran therapy/rat/day for group D, and 57% rice bran therapy/rat/day for group E.

2.6. Isolation of Blood Serum
Blood was taken at each group on the 22nd and 44th day. Blood sampling on the 22nd day was performed in sinus orbitalis, and blood sampling in 44th day were done by sacrificing them and collecting their blood from the heart. After the blood was collected, blood was centrifuged at 3000 rpm for 15 min, in order to obtain blood serum[3].

2.7. Measurement of Levels Of Triglycerides
Determination of the triglyceride levels using the method of GPO-PAP (Gliserolphosphat Oxidase-Aminoantipyrine Peroxidase). 500 µl of serum drawn using micropipette and inserted into the sample cup and the examination was being conducted. Absorbance reading was performed using Spectrophotometer Biosystem A15 at wavelength of 500 nm [8].

2.8. Preparation and Observation of Aorta Histopathology
At the end of study, the aorta of the rats was taken after the blood was collected. After that, the aortic samples were washed using a 0.7% NaCl [3]. Steps in making preparations according to the Putra [14] and Rahmawati [15] i.e. the fixation, dehydration, clearing, impregnation, embedding and blocking, cutting and shaping object-glass, hematoxylin and eosin staining, and mounting. The observation was carried out using a Olympus microscope with magnification of 400×.

2.9. Data Analysis
The analysis of the data used in this research in the form of quantitative data in order to determine the effect of the grant of triglyceride levels against the bran is analyzed using one way ANOVA (Analysis of Variance). If there is a significant difference, then followed by the Tukey test with α = 5%. Analysis of histopathology data from cell damage of the aorta was descriptive qualitative.

3. Results and Discussion

3.1. Effects of Bran Rice to Triglyceride Levels of Rats (Rattus norvegicus) High Cholesterol Diet Models
The measurement method using triglyceride levels GPO-PAP (Gliserolphosphate Oxidase-Aminoantipyrine Peroxidase) aims to find out the effects of the rice bran in lowering levels of triglycerides in white rats (Rattus norvegicus) high cholesterol model. The results of level measurements of triglycerides that have been obtained are statistically analyzed using one way ANOVA test followed by Tukey test with α = 5%. Triglycerides levels test were carried out twice, pre-therapy and post-therapy analysis. Post-therapy data were analyzed statistically which can be seen in Table 1.
Antioxidant compounds found in rice bran is γ-IC50% of oxidation. Very strong antioxidant activity when the values of IC50 value when 100-150 µg/mL, and weak in IC50 values 150-200 µg/mL [21]. Antioxidant compounds found in rice bran is γ-oryzanol.

### Table 1. Triglyceride levels in white rats (Rattus norvegicus) high cholesterol diet model

| Treatment groups | Average of triglyceride levels (mg/dL) |
|------------------|----------------------------------------|
| Group A (negative control) | 89.00 ± 10.39bc |
| Group B (positive control) | 186.00 ± 3.92d |
| Group C (treatment 1) | 100.75 ± 29.34c |
| Group D (treatment 2) | 60.75 ± 6.65ab |
| Group E (treatment 3) | 33.75 ± 2.63a |

*Notes: Different notations show real differences between treatments

The statistical analysis results from triglycerides levels post-therapy with one way ANOVA test shows that the treatment of the rice bran in white rats (Rattus norvegicus) was able for lowering levels of triglycerides (p < 0.01), and it is indicated by the presence of 4 types of notation on the Tukey test Advanced (Table 5.1). Triglyceride levels in Group A (89.00 ± 10.39mg/dL) as negative control was highly significant differences with Group B and E, but no significant difference in groups C and D. While in Group B (186.00 ± 3.92mg/dL) as positive control, triglyceride levels differ markedly with those in groups A, C, D, and E. Group C (100.75 ± 29.34mg/dL) triglyceride levels had significant differences with those B, D, and E groups, but there is no significant difference with that in group A. Group D (60.75 ± 6.65mg/dL) triglyceride levels resulted in differences with those in groups B and C, but differs markedly with those in Groups A and E. Group E triglyceride levels (33.75 ± 2.63mg/dL) had significant differences with those in groups A, B, and C, but there is no significant differences with Group D.

Normal triglyceride levels in the rat were 26-145 mg/dL [16]. Oil pork contains 38-43% of saturated fatty acids and cholesterol [17]. Quail egg yolk has a very high content of cholesterol that is 2,139.17 mg/100 gr [18]. Acid cholate accelerates the increasing of cholesterol in the blood [19]. An increase in cholesterol in the blood will increase free radicals in the body [1], thereby causing an increase in triglyceride levels in the blood [8].

Group C was high-cholesterol diet model of rat with the 16% rice bran therapy/rat/day, group D was high-cholesterol diet model of rat with the 38% rice bran therapy/rat/day, and group E was high cholesterol diet model of rat with the 57% rice bran therapy/rat/day has decreased levels of triglycerides. Calculation based on average test results triglyceride levels pre-and-post therapy-therapy on each of these treatment groups. Notes that group C decreased by 93 mg/dL, from 193.75 to 100.75 mg/dL. Group D decreased by 107.25 mg/dL, from 168 became 60.75 mg/dL. Group E decreased by 131.75 mg/dL, from 165.5 to 33.75 mg/dL. Group C, D, and E have triglycerides levels that classified as normal, but group E had the highest cholesterol reduction, therefore, the dose given to group E was the most effective dose in reducing triglyceride levels.

Rice bran therapy can lower triglyceride levels shown through changes in the levels of triglycerides from pre-to post-therapy therapy. Changes in the levels of triglycerides because bran contains rough fiber and antioxidants that can help lower triglyceride levels. Crude fibers in the bran that is used for the therapy contains 21.20% and strong antioxidants activity with the IC50 of 51.79 µg/mL. The IC50 value is a parameter used to determine the concentrations of the antioxidant compounds in inhibiting 50% of oxidation [20]. Very strong antioxidant activity when the values of IC50 < µg/mL, 50 strong IC50 value when 50-100 µg/mL, while IC50 value when 100-150 µg/mL, and weak in IC50 values 150-200 µg/mL [21]. Antioxidant compounds found in rice bran is γ-oryzanol.
Crude fiber has the ability to bind bile acids and therefore cannot be absorbed and circulated back. Crude fibers that have binding bile acids will then enter into the colon to degrade and excreted. Fibers degradation in the intestinal microorganisms capable of producing propionic acid or other short-chain fat can inhibit the synthesis of fatty acids and cholesterol [22]. Therefore, this will decrease cholesterol and triglycerides will also cause decreased in number.

γ-oryzanol has the ability to lower triglycerides and cholesterol [23]. γ-oryzanol is nonpolar antioxidants that functions inhibit the concentration of lipids and preventing oxidative stress. The higher the antioxidant levels will cause a higher rate of inhibition of lipid concentration by free radicals [24].

3.2. Effects of Bran Rice to Aorta Histopathological Profiles of Rats (Rattus norvegicus) High Cholesterol Diet Models

Observations were performed using microscopes Olympus 400x zoom in. The change that occurs in the aorta of each group can be seen in the description of the histopathology following aortic (Figure 5.2).

An overview of histology aorta Group A (negative control) (Figure 5.2A) is profile of the aorta in the healthy rats. Tunica adventitia of the aorta in Group A shows no fat cells and no inflammation. In addition, in the tunica media shows smooth muscle cell proliferation. The profiles of histopathology aorta in Group B (positive control) (Figure 5.2B) indicates the presence of inflammatory cells and fat cells in tunica adventitia. Moreover, there are some foam cells formation in tunica media. In tunica intima looks the existence of smooth muscle cell proliferation. Fat cells formation are indicated by yellow arrows, inflammatory cells are indicated by a red arrow, foam cells are indicated by blue arrow, and the proliferation of smooth muscle cells is indicated by green arrow.

The description of the aorta histopathology in Group C (treatment I) (Figure 5.2C) showed fat cells without the presence of inflammatory cells followed in tunica adventitia, indicated by a yellow arrow, on the media there is a foam cell tunica, indicated by blue arrow, and in the presence of visible tunica intima proliferation of smooth muscle cells, indicated by green arrow. The histopathological description of Group D (treatment II) (Figure 5.2D) shows that there are some fat cells formation and there are no cells inflammation in tunica adventitia. This figure shows that there are no tunica foam cells, but it can be seen that there are tunica intima proliferation of aortic smooth muscle cells.
The description of the aorta histopathology of Group E (treatment 3) (Figure 5.2E) indicates the absence of inflammatory cells in fatty and tunica adventititia; furthermore, there is no foam cells in tunica media. But the visible presence of the proliferation of smooth muscle cells on intima tunica. Presence of the smooth muscle cell proliferation likely due to still existing of macrophages in it or the presence of staining his cell so it looks less than perfect conduct proliferates. The fat cells in the cell will be carried by HDL to the liver for inflammatory cells metabolize and the numbers are dwindling because of the inflammation is reduced, while the foam cells will have emigrated to the lymphatic system so that the amount will be reduced. An overview of the histopathology cohort in group E approaching the normal aorta picture that shown by Group A as a negative control.

In tunica adventitia, there are fat cells formation and inflammations signs. Lipids accumulation is characterized by the presence of fat cells. Fat cells have large and flat-bottom vacuole line, and located in the peripheral [26]. Cell inflammation is characteristic of the existence of inflammation in the area. Lipids accumulations and inflammation in tunica adventitia aorta caused by the granting of a diet high in cholesterol. One of the materials used as a diet high in cholesterol is oil derived from pork lard. Fat pork contains saturated fatty acids. Saturated fatty acids that cause high cholesterol levels increase [27]. High cholesterol levels in the blood cause cholesterol plaque. The cholesterol plaque attached to the walls of the blood vessels causing the onset of inflammatory [28].

In tunica media, cell foam is formed. Foam cells are formed from macrophages that to phagocyte LDL (Low-Density Lipoprotein) oxidized [29]. The process of the formation of foam cells begins with endothelial function disturbance due to the existence of the increased formation of free radicals of oxygen that disable the nitric oxide. Fats in the blood vessels will undergo chemical changes due to the free radicals resulting in oxidized LDL. Oxidized LDL will be captured by macrophages via scavenger receptor macrophage continuously and will be transformed into foam cells [27].

In tunica intima the existence of smooth muscle cell proliferation was shown. The proliferation of smooth muscle cells is increasing the number of smooth muscle cells derived from cleavage of one cell into two cells. The proliferation occurs caused by macrophages which secrete growth factors [29]. Damage to endothelial dysfunction led to endothelial adhesion molecules, thus, stimulating that cause monocytes to endothelial bonding. Monocytes penetration to tunica intima through fissures and activated endothelial become macrophages. Macrophages release growth factors that will trigger the proliferation of smooth muscle cells [30].
High cholesterol in the blood will cause the production of bile acids increase. Increasing bile acids will increase the amount of free radicals production. Free radicals generated from the reduction of oxygen on the reaction of 7-α-hydroxylation from the process of the bile acids formation [20]. Free radicals may lead to oxidative stress formation. Oxidative stress resulting in cell damage [7]. Damage to these cells led to cuts in the endothelial cells. The wound on the endothelial cells resulting in inflammatory reaction and lead to the occurrence of vasodilator which increases the permeability of the endothelial cells against a variety of materials in the plasma. As a consequence, this will form cavities between cells, and inflammatory cells and fatty infiltration in tunica adventitia [3].

Group C, D, and E that received rice bran treatment with the dose of 16%/rat/day, 38%/rat/day, and 57%/rat/day, respectively, showed improvements in tunica adventitia and tunica media. It is indicated by a devastating drop in fat cells and inflammatory in tunica adventitia, as well as reduced cell foam in tunica media. Foam cells are in tunica media due to impaired endothelial function due to free radicals. Fat cells and inflammation are in tunica adventitia due to the presence of sores on endothelial cells caused by damage to the cell damage of cells due to increased oxidative stress in the body. Increased oxidative stress is caused by an imbalance between free radicals and antioxidants in the body, therefore, the body needs extra antioxidants from outside in order to balance the number of free radicals.

Rice bran contains rough fiber and antioxidants that are able to repair damage to endothelial cells. It repairs mechanism that is to speed up the process of disposal of bile acids out of the body and also adds antioxidant in the body to prevent the occurrence of oxidative stress. The content of antioxidants in the form of γ-oryzanol bran increases the metabolism of cholesterol [31]. γ-oryzanol can lower cholesterol levels and may inhibit the oxidation of cholesterol [32]. Decreased cholesterol levels will cause the synthesis of bile acids is declining. A decrease in the synthesis of bile acids will reduce the number of free radicals. The result of the decrease in free radicals is a decrease in oxidative stress. A decrease in oxidative stress will repair damaged cells so inflammatory, fatty, and foam cells decrease.

4. Conclusion
The current study concludes that rice bran is significantly decreased the levels of triglycerides and repair the damage of aorta cell in hypercholesterolemia rats (Rattus norvegicus) that were fed with high cholesterol diets, with the effective dose was 57% of rice bran/rat/day. However, this study requires further studies using other rice bran varieties in reducing triglyceride levels and repairing aortic cells due to a high cholesterol diet. Results of this study proves that rice bran fiber is a promising alternative treatment for hypercholesterolemia.

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