

Rice Bran's Effect on Total Cholesterol, HDL, and LDL/HDL Ratio on Hypercholesterolemia Rats

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Abstract:

Hypercholesterolemia is a metabolism disorder characterized by increased or decreased lipid fraction in plasma. The of this research was to prove the effect of Rice bran to decrease of total Cholesterol rate, ratio of LDL / HDL and increase rate of HDL in hypercholesterolemia male white rats (*Rattus norvegicus*). The Research of the study was pure experiment by using post test randomized control design. Research Sample divided into five groups. The first group as a negative control, the second group as a positive control, and another groups were given rice brand, with various dose: 0,54 ml/a/day, 1,08 ml/a/day, 1,62 ml/a/day. The results of this research is rice brand can decrease of total cholesterol rate ($r^2=0,964$), decreased of ratio of LDL / HDL ($r^2=0,836$), and increase of rate of HDL ($r^2=0,951$) (ANOVA $P=0,000$). Rice bran can decrease total cholesterol rate, ratio of LDL / HDL and increase rate of HDL blood.

Keywords: Rice Bran; Total cholesterol; ratio of LDL/HDL; HDL.

Introduction

Hypercholesterolemia is a metabolism disorder characterized by increased or decreased lipid fraction in plasma. Increased cholesterol levels refers to those with beyond normal limits >240 mg/dl.¹ The main lipid fraction disorders were increased total cholesterol, Low Density Lipoprotein (LDL) cholesterol, increased triglyceride levels, and decreased High Density Lipoprotein (HDL) levels. In a hypercholesterolemia process, everything plays an important role and is closely related to one another, so it is impossible to discuss it without discussing the others. All the three are known as Triad Lipids.² Hypercholesterolemia is a major factor in the formation of atherosclerosis that initiates cardiovascular disease, which is a response to injury to blood vessels.³ Products of fat oxidation (such as malonaldehyde and lipid peroxide) or protein oxidation (such as DOPA, dityrosin) and cholesterol, have been known to induce vascular injury. Therefore, it is very possible that the aforementioned oxidation products are toxic to cells, which can then trigger vascular injury. Atherosclerosis is a main factor causing cardiovascular disease, especially coronary heart disease (CHD) which is the number one silent killer. CHD is a common disease and a major killer in industrialized or developed countries.^{4,5} Attempts to lower cholesterol levels can be done with diet, exercise, or hypolipidemic medicines. If non-pharmacological therapy is not successful, we can give various normolipidemic treatments, depending on the type of hypercholesterolemia being treated. Some things we need to consider in giving treatments are the ability of the medicines to affect HDL, triglyceride, fibrinogen, LDL levels, not to mention to the side effects of these medicines. Currently, there are several medicine classifications, namely resins (sequestrants), nicotinic acid and acipimox, statins (HMG-CoA Reductase

Inhibitors), fibric acid derivatives, probucol.⁶ Rice bran, which is commonly used as animal feed, is the outer part or husk of rice which is a by-product of rice milling process. It is usually available in the form of a cream or light brown fine powder. The rice we commonly consume is often washed "too clean", even though it contains many important vitamins and nutrients with extraordinary properties. Rice bran is rich in vitamin B15 or pangamic acid. Based on its chemical structure, vitamin B15 is also called glucono-dimethylamino-acetic-acid.^{7,8} This research was conducted by feeding rice bran in various doses to determine whether rice bran can reduce cholesterol, LDL/HDL ratio, and increase HDL, in male white rats that were previously made to experience hypercholesterolemia by high-fat diet. Based on the above background, this research sought to determine the effect of feeding rice bran on cholesterol, HDL, and LDL/HDL ratios in male white rats (*Rattus norvegicus*) with hypercholesterolemia.

Research Method

Experimental Animals

We used adult male white rats (*Rattus norvegicus*) wistar strain, aged 2 months old, with initial body weight of 161-255 grams. The research samples were taken randomly from a population of male white rats (*Rattus norvegicus*) wistar strain as experimental animals. The samples were randomly assigned into 5 groups; group I as negative control (normal rats), group II positive control (hypercholesterolemia rats without rice bran), and 3 other groups (III, IV, V) with rice bran. The sample size used in this research, after being calculated using the sample size formula, was 20 rats. Thus, each group consisted of 4 rats. Rice bran was fed to group III, IV, V, respectively at 0.54g/bb; 1.08 g/bb; 1.62 g/bb.

Animal Feed

Atherogenic Feed

We applied a high-cholesterol high-fat diet to cause hypercholesterolemia on experimental animals, consisting of 50% *PARS feed*, 25% wheat flour, 2% cholesterol, 0.2% cholic acid, 5% pork oil, and 17.8% water. 40g of this feed was given daily 21 days.^{9,10}

Rice Bran Feed

We used rice bran obtained from grinding at the rice milling process in the form of a light brown fine powder. The rice bran was dissolved with water at a dose of 0.54g/bb; 1.08g/bb; 1.62 g/bb.

The rice bran doses in this research were based on the dose consumed by humans, namely 3 times a day 1 tablespoon, 3 times a day 2 tablespoons, 3 times a day 3 tablespoons, with 1 tablespoon of 10 ml is equivalent to 30, 60, 90 ml/day.¹¹ The rice bran doses for rats were calculated using the comparison/conversion table of human body weight of 70 kg to rat body weight of 200 grams, which is 0.018 ratio. Thus, the rice bran dose calculation was as the following: 30 ml x 0.018 = 0.54 g/bb, 60 ml x 0.018 = 1.08 g/bb, 90 ml x 0.018 = 1.62 g/bb.¹²

Normal Feed

An amount of 53% *PARS Comfeed* and 23.5% wheat flour and 23.5% water were given at 40g per day.

Blood Collection

After three weeks, the cholesterol levels in each group was measured to find out if the rats had reached hypercholesterolemia (cholesterol levels > 200 mg/dl), subsequently their cholesterol and triglyceride levels were measured. Data were collected by measuring total cholesterol and white blood serum triglyceride using CHODPAP (Cholesterol Oxidase Para Aminophenazone), which is recommended by the European Atherosclerosis Society.

1. Blood Cholesterol Test (CHOD-PAP): blood → centrifuging → serum + reagent →

incubation at 37C for 10' → measuring absorbance ($\lambda=546\text{nm}$)

- a. Total cholesterol = $\frac{As \times \text{standard mg/dl}}{Ast}$

- b. HDL Levels: Precipitant serum + reagent → 25C for 10' → centrifuging at 4000 rpm → taking supernatant → CHOD-PAP

2. HDL levels = $A \times F$ ($F(546\text{nm})=318\text{mg/dl}$)

- a. TG levels: serum + reagent → incubation at 37C for 5' → measuring absorbance

3. TG = $As \times 1403 \text{ mg/dl}$

LDL levels = total cholesterol – HDL – TG/5 mg/dl

Statistical Analysis

All data are expressed as mean ± SEM. Experimental groups were compared by one-way analysis of variance followed by Bonferroni's test for multiple comparisons. When the results were not normally distributed, statistical analyses were performed using Kruskal-Wallis one-way analysis of variance. The effect of SR9009 on the survival of mice was analyzed by Kaplan-Meier methods and compared by log-rank test. Differences were considered statistically significant at $p < 0.05$. The analysis was performed using IBM SPSS version 21.0 software (IBM Co., Armonk, NY, USA).

Results

The Effect of Rice Bran Extract on Total Cholesterol Levels

First, we investigated the total cholesterol levels of each group (figure 1). Total cholesterol levels was significantly decrease in group V (146.46 ± 7.20). After that, the obtained data were analyzed for normality and homogeneity to find out whether the data were feasible for ANOVA test. The normality test results (appendix 1) calculated that in the Kolgomorov-Smirnov test column, the sig value at $0.053 > p$ (0.01), meaning that the data were normally distributed. In addition, the homogeneity test results (appendix 1) calculated that the sig

value at $0.235 > p$ (0.01), meaning that the data variance was homogeneous. Therefore, it can be concluded that total cholesterol levels data can be analyzed using the ANOVA test.

The ANOVA test results (appendix 1) calculated that the sig value at $0.000 < p$ (0.01), meaning that there was a very significant effect of treatment on total cholesterol levels in white rat blood serum, specifically on total cholesterol parameter.

A further analysis using the *Honesty Significant Differences* (HSD) 5% test (appendix 1) explained that the rice bran treatment was able to reduce total cholesterol levels, but it had not yet reached normal level.

The correlation test results (attachment 1) calculated that the sig value (2-tailed) = 0.000, less than p (0.01), meaning that there was a very significant correlation. The value of *person correlation* = -0.982, indicating that there was a very strong inverse correlation between rice bran doses and total cholesterol levels, as the increasing rice bran doses could reduce total blood cholesterol levels in white rats.

The regression test results (figure 2) showed that there was an effect between the rice bran doses and total blood serum cholesterol levels, and it could be explained in a mathematical/regression equation as follows:

$Y = 340.686 - 114.040 (X)$ with a coefficient of determination $R^2 = 0.964$

Where

Y = total cholesterol levels (mg/dl)

X = rice bran dose (g/head)

The equation was found fit as this can be seen from the ANOVA regression table where the value of sig = $0.000 < p$ (0.01), meaning that the regression model fitted very significantly. This was also supported by the *t-table coefficient test* showing that the sig value (*constant*) = 0.000 and the sig value (*dose*) = 0.000, meaning less than the p -value (0.01).

The Effect of Rice Bran Extract on HDL Levels

We next investigated HDL levels of each group (figure 3). HDL levels was significantly

increased in group V (77.43 ± 2.28). The obtained data were then analyzed for normality and homogeneity to find out whether the data were feasible for ANOVA test. The normality test (appendix 2) calculated that the Kolgomorov-Smirnov test column showed sig value at $0.200 > p$ (0.01), meaning that the data were normally distributed. The homogeneity test results (appendix 2) calculated the sig value at $0.630 > p$ (0.01), meaning that the data variance was homogeneous. Therefore, it can be concluded from the previous test results that the HDL data can be analyzed using the ANOVA test.

The ANOVA test results (appendix 2) calculated that the sig value was $0.000 < p$ (0.01), meaning that there was a very significant effect of the treatment on HDL cholesterol levels in white rats blood serum.

The HSD 5% test results (appendix 2) explained that the rice bran treatment was able to increase HDL levels.

The correlation test results (appendix 2) calculated that the sig value (2-tailed) = 0.000 was less than p (0.01), meaning that there was a very significant correlation. The *Pearson correlation* value = -0.975 indicated that there was a very strong direct correlation between rice bran doses and HDL cholesterol levels, as an increase in rice bran dose could increase HDL cholesterol levels in white rats.

The regression test results (figure 4) explained that there was an effect between rice bran doses and HDL cholesterol levels by using the following equation:

$Y = 32.279 - 29.560 (X)$ with a coefficient of determination $R^2 = 0.951$

Where

Y = HDL cholesterol levels (mg/dl)

X = rice bran dose (g/head)

The equation was found fit. This can be seen from the ANOVA regression table where the sig value = $0.000 < p$ (0.01), meaning that the regression model fit very significantly. This was also supported by the *t-table coefficient test* (appendix 2), showing that the sig value

(*constant*) = 0.000 and the sig value (*dose*) = 0.000, which was less than the p-value (0.01).

The Effect of Rice Bran Extract on LDL/HDL Ratio

We also investigated the LDL/HDL ratio of each group (figure 5). The LDL/HDL ratio decreased the most in group V (0.59 ± 0.15). The obtained data were analyzed for normality and homogeneity to find out whether the data were feasible for ANOVA test. The normality test results (appendix 3), specifically the Kolgomorov-Smirnov test column, calculated the sig value at $0.026 > p$ (0.01), meaning that the data were normally distributed. The homogeneity test results (appendix 3) calculated the sig value of 0.01 p (0.01), meaning that the data variance was homogeneous. Therefore, it can be concluded that the LDL/HDL ratio could be analyzed using the ANOVA test.

The ANOVA test results (appendix 3) showed that the sig value was $0.000 < p$ (0.01), meaning that there was a very significant effect of treatment on the LDL/HDL ratio in white rats.

The HSD 5% follow-up test (appendix 3) obtained that there was no significant difference found in its results.

The correlation test (appendix 3) calculated the sig value (2-tailed) = 0.000 which was less than p (0.01), meaning that

there was a very significant correlation. The *Pearson correlation* value = -0.914 indicated that there was a very strong inverse correlation between rice bran dose and LDL/HDL ratio, as an increase in rice bran dose could reduce blood LDL/HDL ratio in white rats.

The regression test results (figure 6) showed that there was an effect between rice bran dose and blood LDL/HDL ratio, and it could be explained in the mathematical/regression equation as follows:

$Y = 7.995 - 5.068 (X)$ with a coefficient of determination $R^2 = 0.836$

Where

Y = LDL/HDL ratio

X = rice bran dose (g/head)

The equation was revealed fit. This can be seen from the ANOVA regression table where the sig value = $0.000 < p$ (0.01), meaning that the regression model fit very significantly. This was also supported by the t-table *coefficient test* which showed that the sig value (*constant*) = 0.000 and the sig value (*dose*) = 0.000, which means it was less than the p-value (0.01).

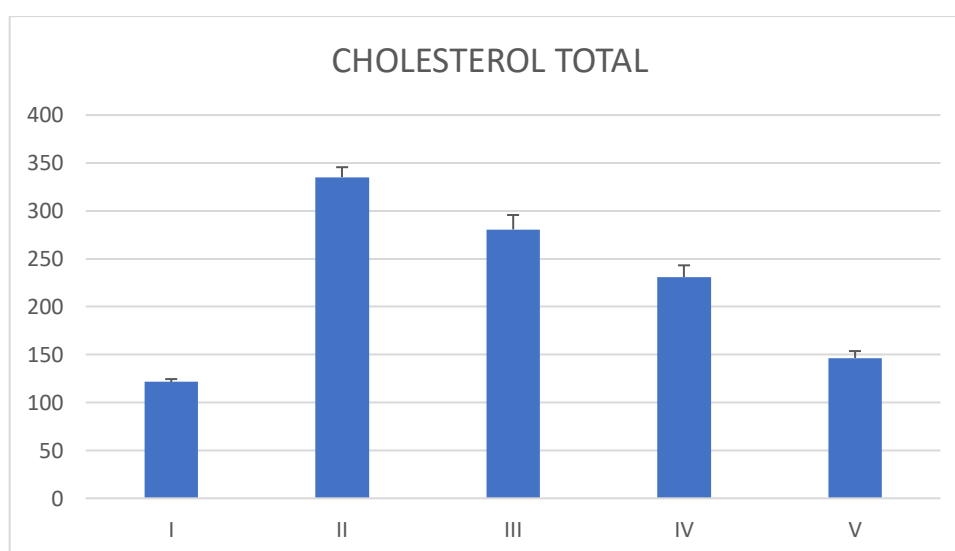


Figure 1 The effect of rice bran extract on total cholesterol levels in hypercholesterolemia rats.

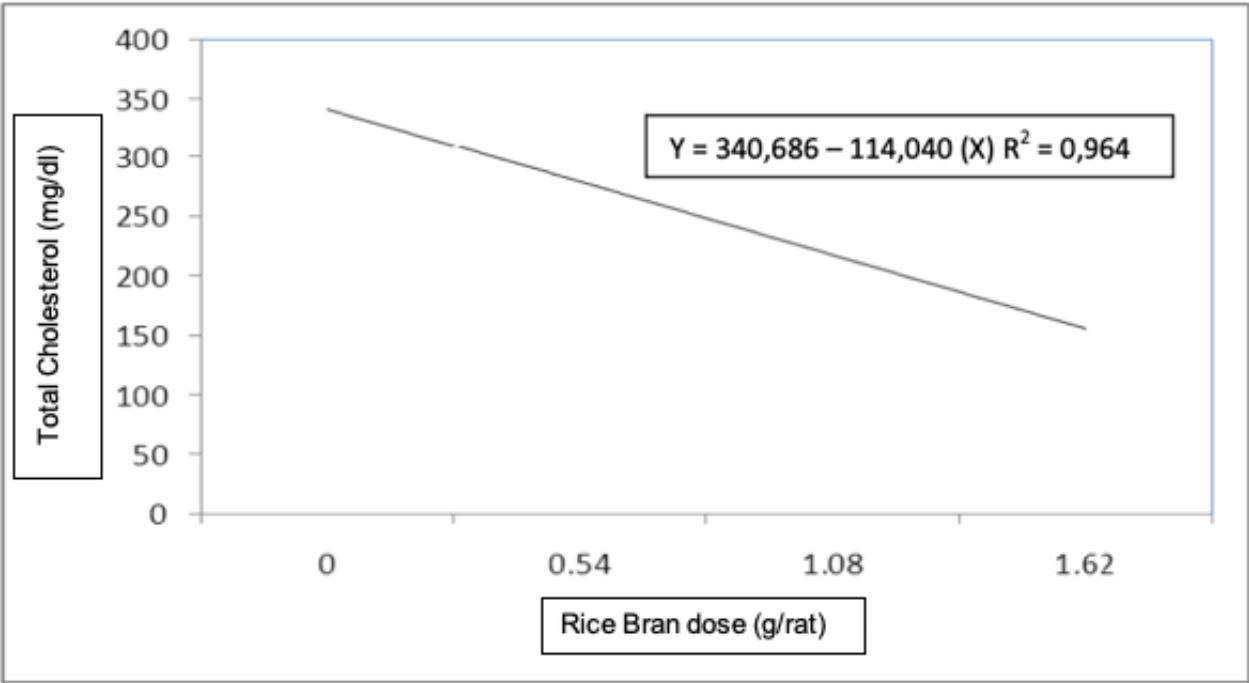


Figure 2 The regression graph that shows the correlation between rice bran dose (g/rat) and total cholesterol levels in white rats. The correlation curve between rice bran doses with total cholesterol levels.

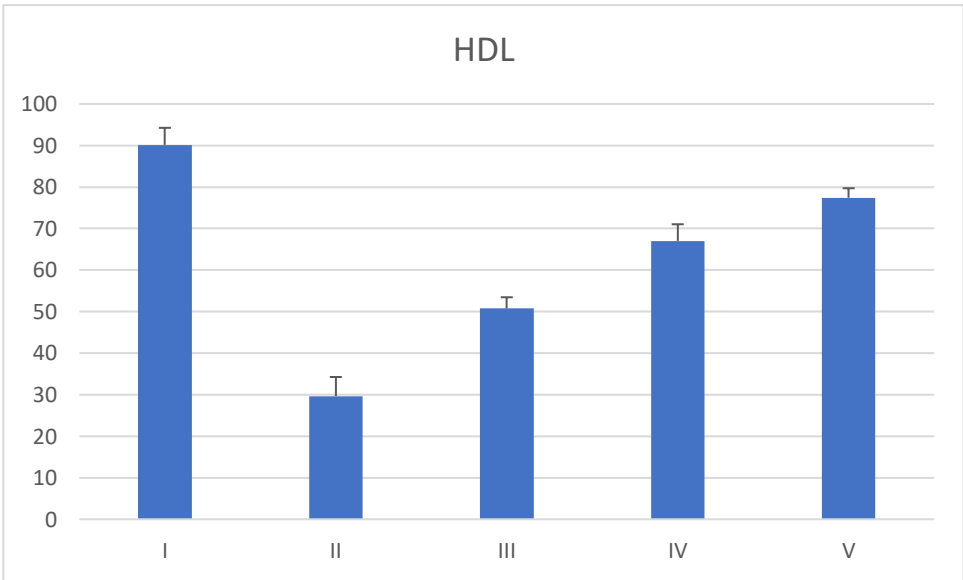


Figure 3 The effect of rice bran extract on HDL levels in hypercholesterolemia rats

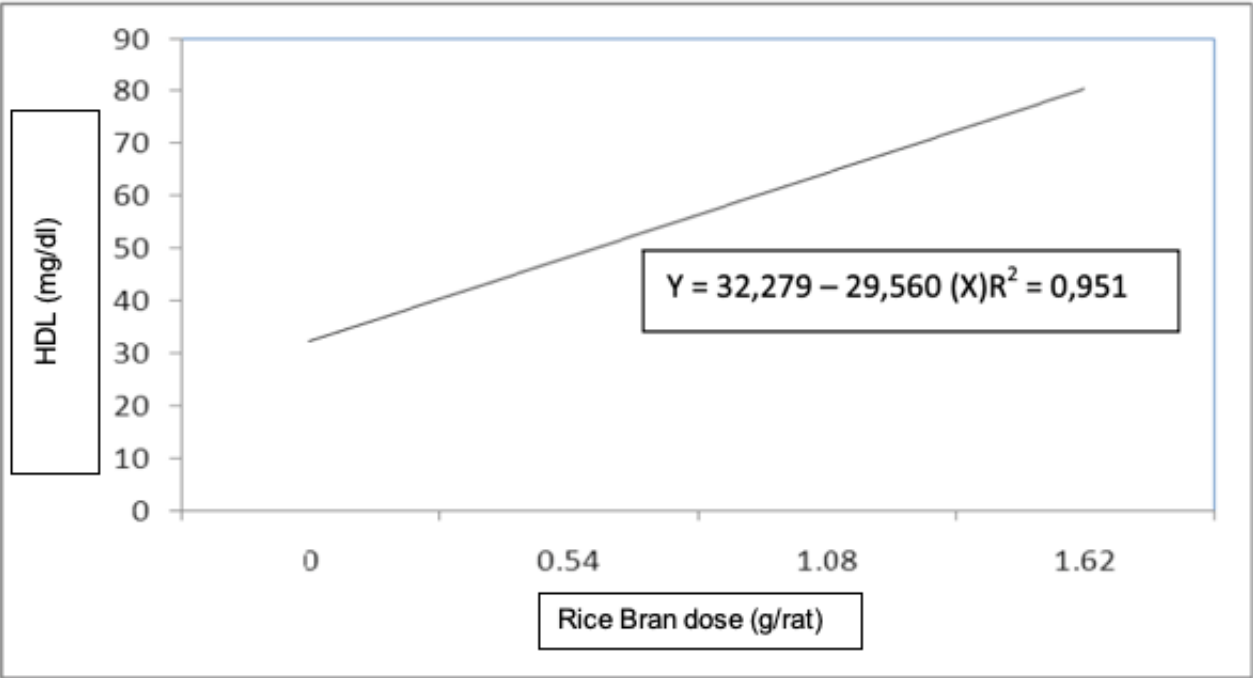


Figure 4 The regression graph that shows the correlation between rice bran dose (g/rat) and HDL levels in white rats. The correlation curve between rice bran doses with HDL levels.

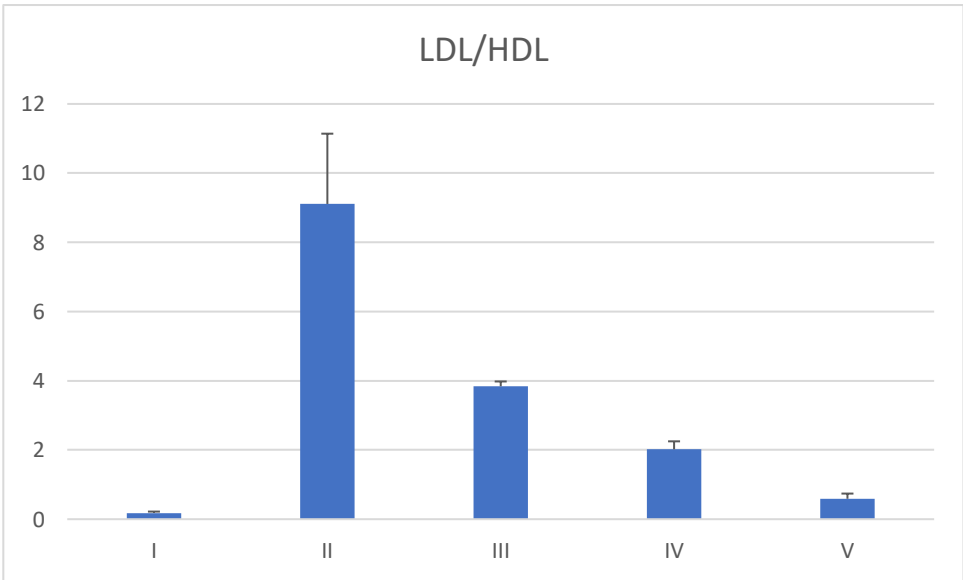


Figure 5 The effect of rice bran extract on LDL/HDL Ratio levels in hypercholesterolemia rats

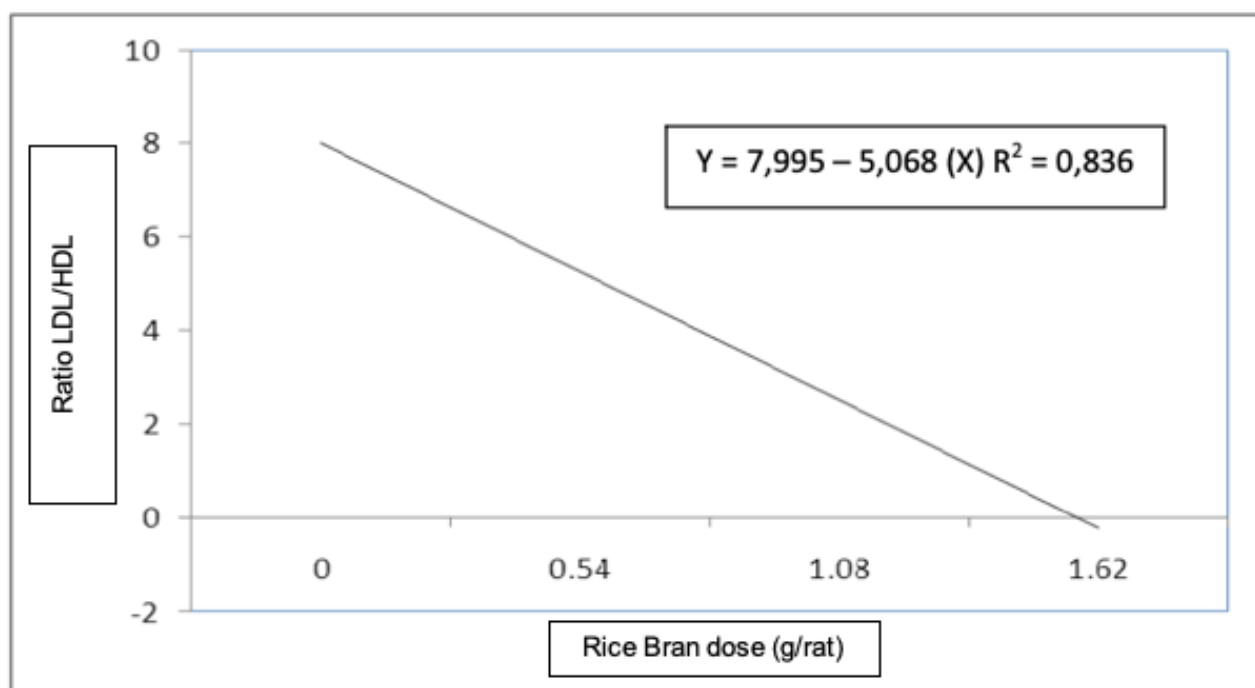


Figure 6 The regression graph that shows the correlation between rice bran dose (g/rat) and LDL/HDL ratio levels in white rats. The correlation curve between rice bran doses with LDL/HDL ratio levels.

Discussion

This research has proven that rice bran can reduce total cholesterol levels, increase HDL levels, and reduce LDL/HDL ratio.

In this particular research, the plasma lipoprotein levels were examined and the plasma LDL/HDL ratio was calculated in rats treated with atherogenic diet for 21 days. The following results were obtained: with normal feeding (group I), total cholesterol, HDL, and LDL/HDL ratios were normal, while the atherogenic diet (groups II, III, IV, and V) showed hypercholesterolemia.

The research continued by feeding rice bran with various doses for 28 days, with the following results were obtained: by feeding rice bran at a dose of 0.54 g/bb rat/day (group 3), there was a decrease in total cholesterol levels, decrease LDL/HDL ratio, and an increase in HDL levels. The results were more significant at bran dose of 1.62 g/bb rat/day (group 5).

This research sought to determine whether the hypocholesterolemia content of rice bran in the form of tocopherol antioxidant (vitamin E), tocotrienols, oryzanol, and pangamic acid (vitamin B15) can reduce total cholesterol, reduce LDL/HDL ratio, and increase HDL. These compounds are found in rice bran that can lower blood cholesterol levels, LDL, and increase blood HDL levels. The rice bran fat fraction ability is due to its unspecifiable material. In addition, the non-fat fraction of rice bran also poses the same hypolipidemic effect as the fat fraction. The mechanism of reducing blood lipids is processed through an increase in the binding capacity of LDL receptors. Another mechanism that also plays a role in lowering blood cholesterol is an increase in the activity of the enzyme *cholesterol-7 alpha-hydroxylase* (Cyp7a1), an enzyme that is responsible for the biosynthesis of bile acids. Increased activity of this enzyme will stimulate the conversion of cholesterol into bile acids, thereby preventing its reabsorption in the intestine and finally excreted. It is concluded

that not only an increase in bile acid synthesis, but a decrease in cholesterol absorption also has a cholesterol-lowering effect so that it could lead to a decrease in blood cholesterol. The non-fat fraction is obtained by extraction using ethanol followed by extraction using *Driselase*. *Driselase* is the name of a commercial enzyme product for the degradation of plant cell walls consisting of cellulase, silanase, and laminarise.

This research revealed a very strong correlation, as evidenced by the effect of rice bran dose on total cholesterol reduction levels ($r = -0.982$; $\text{sig} = 0.000 < 0.01$; $R^2 = 0.964$), the effect of rice bran dose on LDL/HDL ratio levels ($r = -0.914$; $\text{sig} = 0.000 < 0.01$; $R^2 = 0.836$), and the effect of rice bran dose on HDL increase ($r = -0.975$; $\text{sig} = 0.000 < 0.01$; $R^2 = 0.951$). However, these strong effects had not been able to reduce total cholesterol levels, LDL/HDL ratio, and increase HDL levels completely or 100% to match the negative control group (group I). This is presumably due to the relationship between the stability of plasma lipoproteins with each other, for example levels of plasma lipoproteins, total cholesterol, and HDL cholesterol. This relationship can be seen in the formula for calculating LDL cholesterol levels:

$$\text{LDL-C} = \text{TC} - (\text{HDL-C}) - \text{TG}/5 \text{ (mg/dl)}$$

Therefore, although this research revealed that there was a very significant increase in HDL cholesterol levels, but if it was not followed by an optimal decrease in total cholesterol and triglyceride levels, the LDL cholesterol level would become abnormal and subsequently affect the plasma LDL/HDL ratio. This is in accordance with NCEP ATP III which stated to decrease LDL cholesterol and increase HDL cholesterol, the LDL/HDL ratio should be below 3.^{13,14}

From the various facts obtained through this research and various pre-existing theories, the hypothesis about rice bran can reduce total cholesterol levels, reduce LDL/HDL ratio, and increase HDL levels has

been proven correct. In this research, it is not determined yet what the effective dose of rice bran is considered as hypocholesterolemic, neither is toxic. In addition, this research has not been able to determine the exact mechanism of rice bran in reducing total cholesterol levels, reduce LDL/HDL ratio, and increase HDL levels, thus requiring further research.

Conclusions

Based on the research results and discussion, it can be concluded that rice bran can reduce total cholesterol levels, reduce LDL/HDL ratio, and increase HDL levels in the blood of hypercholesterolemia rats.

However, further research is needed to determine the exact mechanism of rice bran in reducing total cholesterol levels, reducing LDL/HDL ratio, and increasing HDL levels.

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