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Abstract. Atherosclerosis is a significouldt health problem in developed countries and is a major cause of morbidity and mortality, especially in developing countries. Metabolic disorders which include increased levels of LDL and lipoprotein and decreased levels of HDL could cause atherosclerosis which is an inflammatory process due to the formation of plaques on the large artery walls. Black rice is a source of dietary fiber and anthocyanin could act as an antioxidant, which if consumed regularly could improve lipid profiles and blood antioxidants. This is expected to reduce the rate of disease caused by atherosclerosis. The purpose of this study to determine the potential of black rice on HDL and LDL levels in Sprague dawley mice with a high cholesterol diet. This study was in vivo experimental in Sprague dawley rats with a randomized post-test only control group design using 4 groups of rats weighing 175-200g. The group consisted of negative controls, positive controls, simvastatin and black rice drugs. The results showed that rats with hypercholesterol diet which were intervened with black rice feed for 12 weeks decreased LDL and increased HDL. Statistical tests showed that there was a significouldt effect between the treatment of feed on HDL and LDL levels. Further tests showed differences in each group. Conclusion of this study is black rice consumption could regulate lipid profiles by lowering LDL, increasing HDL levels and inhibiting the increase in fat oxidation results in the body with the MDA indicator.

Keywords: *Black rice, HDL, LDL, MDA*

1. Introduction

Increase in blood cholesterol or hypercholesterolemia is a major cause of morbidity and mortality in both developed and developing countries.[1] High LDL cholesterol levels are a risk for CHD. High-cholesterol foods such as meat, liver, brain, and innards cause excess cholesterol in the body.[2] The state of hypercholesterolemia in animals occurs when the total cholesterol level in the blood exceeds normal. Mice have normal total cholesterol levels with a value of 10-54mg / dl.[3] Hypercholesterolemia also causes HDL levels in the blood to decrease. Normal HDL cholesterol level in rat blood plasma is ≥35 mg / dL.[4] The normal threshold of LDL in rats is 7-27.2 mg / dl.[5]

Delivered that the use of the drug hypercholesterolemia in experimental animals managed to control and reduce cholesterol levels in the blood, but in the use of drugs long-term hypercholesterolemia will cause side effects. [6] The choice of high fiber and anthocyanin intake as a source of antioxidants could be considered as one of the options for treating hypercholesterolemia. Food which is still a staple food source in Indonesia is rice. One rice variety that has higher fiber and antioxidant content is black rice.

Compared to white rice, black rice contains higher fiber. This is caused by the epidermis which is still attached because of black rice not through ignition. Fiber content of food includes

soluble, insoluble fiber and total dietary fiber of 0.93; 10.79 and 11.72%. Food fiber in black rice consists of soluble fiber and insoluble fiber. The mechanism of soluble fiber in reducing cholesterol levels is by binding to fat in the small intestine, so that cholesterol levels in the blood could be lowered. [8] Furthermore, in the intestine or digestive tract, fiber could bind bile salts which are the final cholesterol products and are released with feces. Thus food fiber will reduce cholesterol levels in blood plasma. Another mechanism is that when there is an increase in cholesterol excretion through feces, the amount of cholesterol that leads to the liver will decrease. With the reduction in the amount of cholesterol in the liver will cause an increase in cholesterol uptake in the blood which will be synthesized into bile acids, causing cholesterol levels in blood plasma to decrease.[9] The high fat in the body could also trigger an oxidation reaction, which is described by the high number of plasma Malondealdehyde (MDA).

MDA is the result of the oxidation process of plural unsaturated fats by free radical compounds in the body. Free radicals are defined as atoms or molecules that have one or more unpaired electrons in their atomic structure so they are reactive. Free radicals could interact with cell membranes and macromolecules such as lipids and proteins.[10] Malondealdehyde (MDA) is a fat oxidation product that could induce blood vessel injury.[11] The high levels of free radicals in the body could be demonstrated by the low activity of antioxidant enzymes and high levels of malondialdehyde (MDA) in plasma.[12] This study studied the role of fiber in black rice for the treatment of animal models of hypercholesterolemia in terms of HDL, LDL and Malondealdehyde (MDA) levels.

2. Method

This research is an experimental study with a research design Pre Test and Post Test Control Group Design with a Completely Randomized Design (CRD). The experimental animals used in this study were 24 Sprague Dawley male rats, equipped with research ethics information number: 111 / KEPK-FKM / UNIMUS / 2018 issued by the Health Research Ethics Commission (KEPK) Faculty of Public Health, University of Muhammadiyah Semarang. Weight inclusion criteria. between mice 175-200 g, male, looks active. Exclusion criteria namely; the weight of rats decreased (less than 175gr), died during the study period, experienced diarrhea during the study took place, at autopsy found a congenital abnormality that could affect the results of the examination Preparation of experimental animals The preparation phase of the experimental animal includes acclimatization for 5 days and then intervenes according to treatment. Plasma HDL, LDL, cholesterol and MDA levels were measured at the beginning before intervention (week 0), then at weeks 4, 8, 9, 10, 11 and 12. Hypercholesterol cholesterol diet by giving 1.25% cholesterol and 0.5 % cholic acid / day, the percentage calculated from adlibitum feed. Simvastatin medication is given at a dose of 0.18 mg/ 200 g BB / oral / day in a sonde. Group animal experiments and interventions Rats were divided into 4 groups, each group consisting of 6 rats each.

1. Negative control: (AIN 93 M standard diet)
2. Positive control (standard AIN 93 M + atherogenic diet)
3. Drugs (standard AIN 93 M + atherogenic + simvastatin diet at doses of 0.18 mg / 200 g BB / oral/day.
4. Black rice (standard diet + atherogenic + black rice flour).

Diet is given iso calories and protein. Black rice diet groups contain higher fiber than other groups. This is in accordance with the calculation of the amount and calories of feed and aims to find out how the black rice diet with higher fiber content could have an impact on the levels of HDL, LDL, cholesterol and MDA plasma experimental animals.The composition of each feed group was calculated based on the similarity of protein and calorie levels presented in Table 1.

Table 1. Composition of Standard Diet AIN 93 M.

No	Material	Standard AIN 93M Feed (g)	Black Rice Feed (g)
1	Maezena	62,5	0
2	Black rice flour	0	93,3
3	Casein	14	6,7
4	Sucrose	10	0
5	Soybean oil	4	0,23
6	Fiber	5	0
7	Mineral mix	3,5	3,5
8	Vitamin mix	1	1
9	L-Cistin	0,18	0,18
10	Cholin Bitartrate	0,25	0,25
	Total (g)	100,40	105,16

Methods of examination of HDL, LDL and MDA (CHOD-PAP by enzymatic ***spectrophotometry*** principle.[14] Analysis of HDL and LDL is done by Precipitant and Standard for Use with Human Cholesterol Liquicolor Test Kit. 200 µl of serum was added with 500 µl of precipitation, mixed until homogeneous, then allowed to stand for 10 minutes at a temperature of 20-25^{o}C. The tube is then centrifuged for 10 minutes with 4000 rpm. The supernatant is prepared from the precipitate within one hour after centrifugation. 100 µl of the supernatant plus 1000 µl of reagent were mixed, incubated for 10 minutes at a temperature of 20-25^{o}C. Absorbance is read within one hour at a wavelength of Hg 546 nm. For blanks the reagent was made from 100 µl of distilled water plus 1000 µl of reagent kit. HDL and LDL levels are calculated as follows:

$$\text{HDL cholesterol (mg/dl)} = \text{Standard concentration} \times \frac{\text{Absorbance of the sample}}{\text{Absorbansi standar}}$$

$$\text{HDL cholesterol (mg/dl)} = \text{total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

Plasma Malondialdehyde (MDA) levels (*Thiobarbituric Acid*)

Data analysis

Numerical data from the examination of HDL, LDL and MDA levels in blood in each group were tested using the SPSS version 17.0 program. If the results of the analysis of variance have a significouldt (significouldt) effect, then proceed with the Duncould test $\alpha = 5\%$. to find out which treatment is different.

3. Results and Discussion

HDL and LDL levels in hypercholesterol cholesterol animals with a black rice diet. Levels of High Density Lipoprotein (HDL) or often referred to as good fats in the blood. Before getting feed intervention in each group, Pre Test Examination was conducted which aims to determine whether Sprague Dawley rats used in this study were in normal conditions and there were no disturbances related to cholesterol synthesis. The results of the preliminary examination before intervention found that all groups of mice were in normal condition and did not experience any disturbances associated with cholesterol levels in the blood. The results of the examination are said to be normal if the HDL and LDL levels are still within normal limits, namely 35-85 mg / dl for HDL levels and 2-27 mg / dl for LDL levels. Blood HDL levels of experimental animals are presented in Figure 1.

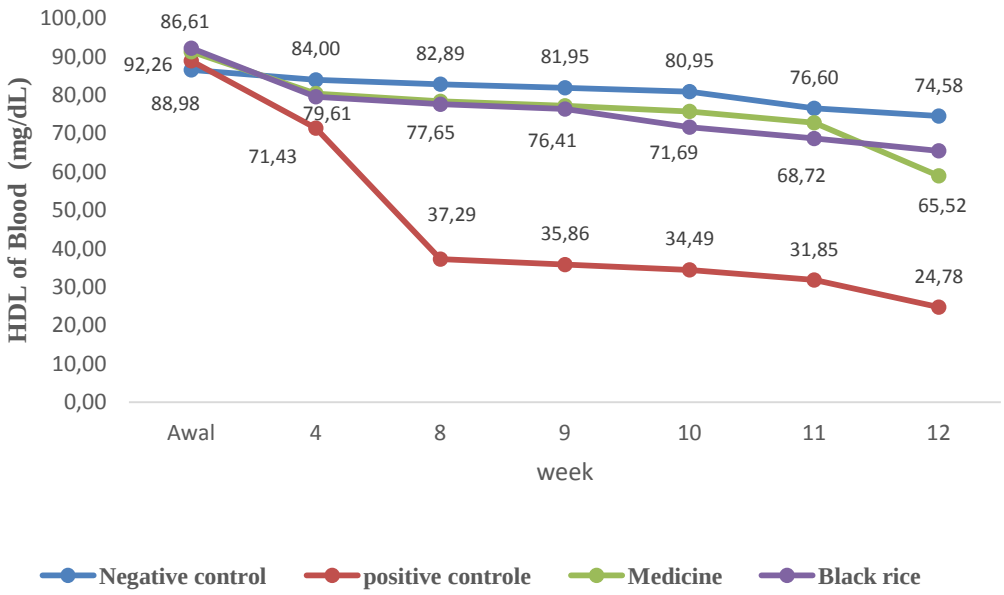

Figure 1. HDL in hypercholesterol rats with a black rice diet

Figure 1. shows a tendency to decrease HDL levels in all groups of experimental animals. The highest decrease in the positive control group, namely hypercholesterol cholesterol mice with a standard diet, decreased HDL to 73,14%, while the experimental group with a hypercholesterol cholesterol and black rice diet only experienced a 24,35% reduction almost the same as the Simvastatin drug interaction. This shows that black rice could play a role in controlling the decrease in HDL levels.

Statistically there was an effect of treatment on HDL levels from the 8th week. At the 8th and 9th week the black rice group was the same as the group of mice given the sivastatin drug. Weeks 10 to 12 there are differences in each group.

Hypercholesterolemia tends to result in LDL oxidation through increasing substrate, LDL conformational changes that are more susceptible to oxidation and increased O_2 vascular production OxLDL could stimulate a number of redox-sensitive processes that have a negative impact on endothelial function.[15]

In this study, dietary fiber in brown rice could stabilize LDL blood in mice. There are several mechanisms for decreasing LDL cholesterol levels by dietary fiber, among others; fibers could modify lipid absorption and metabolism; Short chain fatty acids as a result of fermentation of fiber in black rice could affect cholesterol metabolism and lipoprotein; changing the absorption and metabolism of bile acids and fiber could change insulin or other hormone concentrations or tissue sensitivity to hormones that regulate cholesterol metabolism.[16, 17]

Exposure in Figure 2. shows the levels of Low Density Lipoprotein (LDL) or commonly referred to as bad fat in the experimental group with drug intervention and black rice is much lower under positive control. Black rice diets in group 4 compared to positive controls were able to reduce LDL levels by 52% at the end of the study or at 12 weeks. Almost the same as negative controls. Statistically there is an effect of giving a diet on LDL levels and further testing shows there are differences in each group.

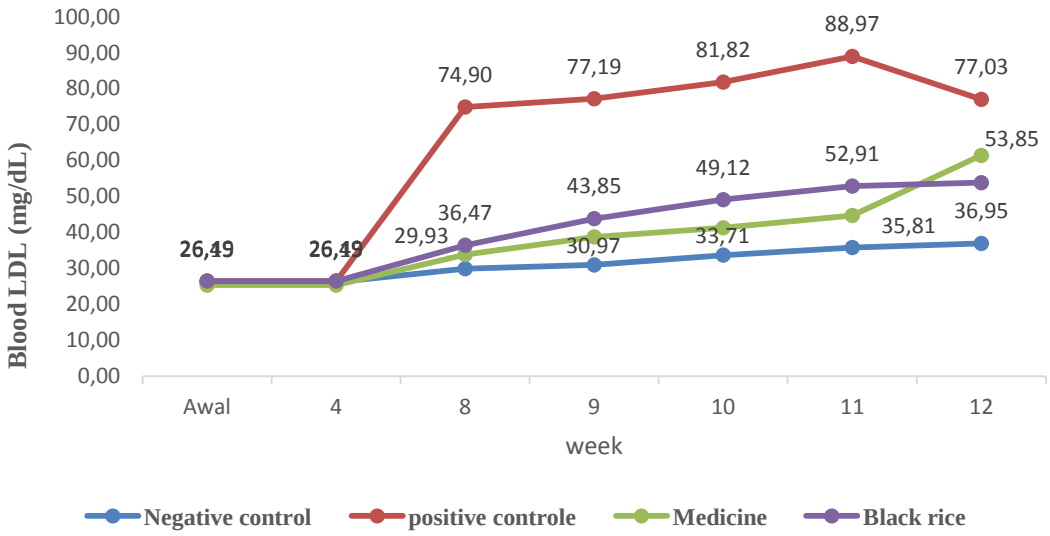

Figure 2. LDL in hypercholesterol rats with a black rice diet

Increased LDL levels and decreased HDL levels are caused by excessive amounts of cholesterol, which causes cholesterol buildup in the body.[18] The results of this study indicate that they are still in normal condition according to the limits of LDL levels 2-27.2 mg / dl or <100 mg / dl.

Increased LDL levels in the blood are caused by high cholesterol levels. This causes VLDL to form LDL. High levels of LDL make HDL depressed and could not get rid of excess cholesterol contained in the blood, so that HDL decreases. Hypercholesterolemia results in impaired lipoprotein metabolism, which includes increased LDL levels and decreased HDL levels.[19]

Malondealdeida (MDA)

Malonaldehyde (MDA) is the result of the oxidation process of plural unsaturated fats by free radical compounds in the body. Free radicals are defined as atoms or molecules that have one or more unpaired electrons in their atomic structure so they are reactive. Free radicals could interact with cell membranes and macromolecules such as lipids and proteins.[10] Malonaldehyde (MDA) is a fat oxidation product that could induce blood vessel injury.[11] The high levels of free radicals in the body could be demonstrated by the low activity of antioxidant enzymes and high levels of malondialdehyde (MDA) in plasma.[12] MDA plasma hypercholesterol mice with a black rice diet are presented in Figure 3.

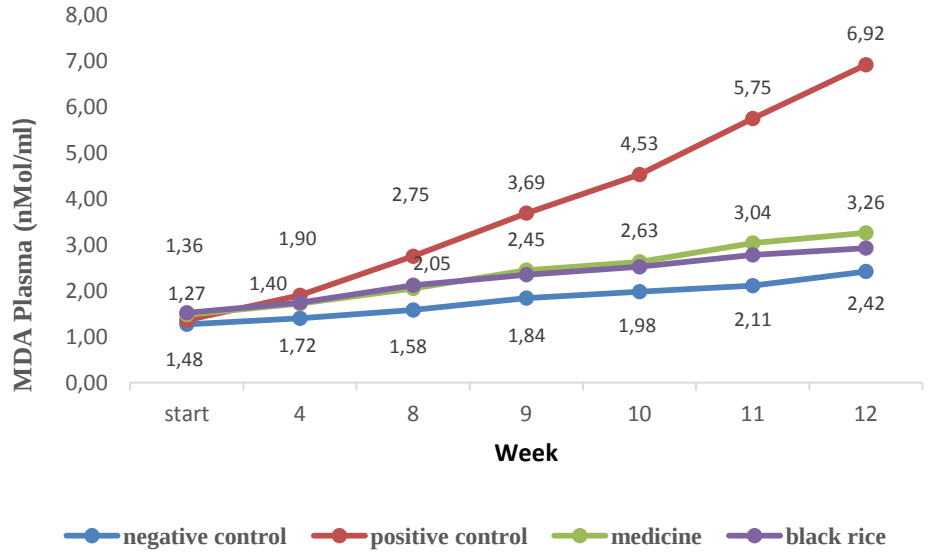

Figure 3. Levels of Malondealdehyde (MDA) in hypercholesterol rats with a black rice diet

Figure 3. shows the trend of increasing MDA of rats in all treatments. The highest increase was in the positive control group where rats were given a high cholesterol diet with standard feed. The other group was negative control, the interaction with the drug simastatin and black rice also increased but was lower than before the intervention.

In the group of positive control rats there was an increase in MDA levels reaching 408.8%, whereas in rats with black rice intervention the increase was only 89%, lower than mice that were interfered with simvastatin drugs which reached 17.1%. Mice with high cholesterol feed could cause the oxidation process of plural unsaturated fats by free radical compounds in the body. With the intervention of black rice in feed containing anthocyanins it could act as an antioxidant so that it could reduce the occurrence of the oxidation process. Brown rice added with fiber from carrageenan and anthocyanin extract could reduce MDA formation in diabetic rats.[7]

4. Conclusion

Black rice consumption could regulate lipid profiles by lowering LDL, increasing HDL levels and inhibiting the increase in fat oxidation results in the body with the MDA indicator.

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