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Research Article

Analog Rice Reduces Weight and Total Cholesterol Levels in Overweight and Hypercholesterolemic Rats

Beras Analog Menurunkan Berat Badan dan Kadar Kolesterol Total pada Tikus *Overweight* dan Hiperkolesterolemia

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ABSTRACT

Overweight, whose prevalence is increasing, is caused by unbalanced consumption patterns such as high consumption of fat and can trigger hypercholesterolemia. Analog rice (AR), which has better nutritional content than ordinary rice, is thought to have beneficial effects on health. This study aimed to determine the potential of analog rice in reducing body weight and total cholesterol levels. A total of 24 male Wistar rats were divided into four groups, namely: I) control, II) ordinary rice treatment, III) analog rice 1 treatment (PBA1) and IV) analog rice 2 treatment (PBA2). The control group was given standard feed for 40 days ad libitum. Groups II, III, and IV were given a combination induction of high-fat diet (HFD) for 40 days ad libitum and intraperitoneal injection of streptozotocin (STZ) 35 mg/kg single dose on day 33. All groups were then given the treatment of diet for three weeks according to their respective groups, namely the standard, ordinary rice, analog rice 1, and analog rice 2 feed. Body weight and total cholesterol levels were measured in the pre-induction, post-induction/pre-treatment (pre-test), and post-treatment (post-test). The results of the study showed that the induction of the HFD-STZ combination carried out resulted in a significant increase in body weight and total cholesterol levels. Measurements made after dietary therapy showed that body weight and total cholesterol levels in the I and II groups did not change significantly, whereas those in the III and IV groups decreased significantly (p<0.05). This study concluded that the administration of analog rice could reduce body weight and total cholesterol levels in overweight and hypercholesterolemia rats.

Keywords: *Analog rice, high-fat diet, hypercholesterolemia, overweight, streptozotocin*

ABSTRAK

Overweight yang prevalensinya semakin meningkat diakibatkan oleh pola konsumsi tidak seimbang, seperti konsumsi tinggi lemak, dapat memicu hiperkolesterol. Beras analog (BA) yang memiliki kandungan gizi lebih baik dari beras biasa, diperkirakan memiliki efek yang menguntungkan bagi kesehatan. Penelitian ini bertujuan untuk mengetahui potensi BA dalam menurunkan berat badan (BB) dan kadar kolesterol total. Sebanyak 24 ekor tikus Wistar jantan dibagi menjadi 4 kelompok, yaitu: I) kontrol, II) perlakuan beras biasa (PBB), III) perlakuan beras analog 1 (PBA1), dan IV) perlakuan beras analog 2 (PBA2). Kelompok kontrol diberi pakan standar selama 40 hari *ad libitum*. Kelompok II, III, dan IV diberi induksi kombinasi berupa *high-fat diet* (HFD) selama 40 hari *ad libitum* dan injeksi *streptozotocin* (STZ) intraperitoneal 35 mg/kgBB dosis tunggal pada hari ke-33. Semua tikus selanjutnya diberi perlakuan pemberian pakan/diet selama 3 minggu sesuai dengan kelompoknya masing-masing, berturut-turut yaitu pakan standar, beras biasa, BA1 dan BA2. Berat badan dan kadar kolesterol total diukur pra-induksi, pasca-induksi/ pra-perlakuan (*pre-test*), dan pasca-perlakuan (*post-test*). Hasil penelitian menunjukkan induksi kombinasi HFD-STZ yang dilakukan mengakibatkan peningkatan BB dan kadar kolesterol total yang bermakna. Pengukuran yang dilakukan pasca terapi diet menunjukkan BB dan kadar kolesterol total kelompok I dan II tidak mengalami perubahan bermakna, sedangkan kelompok III dan IV mengalami penurunan yang bermakna (p<0,05). Kesimpulan pada penelitian adalah pemberian BA mampu menurunkan BB dan kadar kolesterol total pada pada tikus yang mengalami *overweight* dan hiperkolesterolemia.

Kata Kunci: Beras analog, *high-fat diet*, hiperkolesterolemia, *overweight*, streptozotocin

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INTRODUCTION

Overweight is a condition of body weight that exceeds the normal limit. The prevalence is increasing both in developed and developing countries such as Indonesia. In 2016, more than 1.9 billion adults aged 18 and over suffered from overweight. Unhealthy eating patterns, such as the tendency to consume foods high in fat, high in calories, low in fiber, are one of the triggers for increased overweight and obesity among teenagers and young adults. Overweight can develop into obesity and trigger hypercholesterolemia (1-3). The prevalence of hypercholesterolemia among Indonesian inhabitants over 15 years is 35.9% (4).

Analog rice is a rice-grain-like processed product that can be made from partially or wholly non-rice ingredients, and the nutrition can be modified (5,6). The content of food fiber (7.8) and Resistant starch (RS) (8.9) of AR (analog rice) is generally higher than ordinary rice. Food fiber, which can be grouped into soluble fiber and insoluble fiber, is a residue from plant cell walls composed of carbohydrates, consumable, and resistant to the process of digestion and absorption in the human small intestine. Food rich in fiber can reduce body weight and total cholesterol levels (2), while RS is starch, and its degradation products cannot be absorbed in the small intestine (10). High RS levels in the diet can increase fatty acid oxidation, and trigger satiety, so consumption of a diet rich in RS can be used to treat obesity (11).

Several studies regarding the potential of AR in several medical conditions have been carried out. Corn analog rice (Smart Rice) is able to provide hypoglycemic effects in diabetes mellitus patients with an average of 24.2 percent after a three-week consumption (7). Analog Rice with main compositions of sago starch, fresh cassava, coconut pulp, and rice bran given to Sprague Dawley rats for four weeks was able to reduce total cholesterol and Low-Density Lipoprotein (LDL) levels and increase High-Density Lipoprotein (HDL) levels compared to the group control (12). Administration of AR made from a mixture of sago starch and red bean flour for four weeks caused a reduction in total cholesterol in prediabetes patients (13). This study used AR containing the main raw materials of MOCAF flour, corn flour, rice flour, and sodium alginate, with two variations in the composition of raw materials (BA1 and BA2). The calorie content in both types of AR was made equal to ordinary rice by regulating the composition of raw materials.

METHOD

Experimental Animals

This study was a pure experiment using a pre-test post-test control group design. The study used 24 white male rats (Rattus norvegicus), Wistar strain, ± three months old, and 150-200 grams body weight. Rats were obtained from the Biochemical Laboratory of the Faculty of Medicine, Airlangga University. Ethical Clearance research was obtained from the Ethics Commission of the Faculty of Medicine, University of Jember through a decree number 1176/H25.1.11/KE/2018. After the rats were adapted for seven days, the rats were randomly divided into four groups (each group consisted of 6), namely: I) control group, II) ordinary rice treatment (PBB), III) analog rice treatment 1 (PBA1), and IV) analog rice treatment 2 (PBA2). Every rat was placed in a cage with the same shape, size, and material. During the adaptation period, mice got standard feed and distilled water ad libitum.

Overweight and Hypercholesterolemia Induction

The induction of overweight and hypercholesterolemia was carried out for the treatment groups (II, III, and IV) with an induction combination of HFD and STZ injection (14-16). The high-fat diet provided contained 22.8% pork fat (table 1) and was administered for 40 days, while STZ was given intraperitoneally once on the 33rd day calculated from the first day of HFD administration. The STZ dose used was a low dose, which was 35 mg/kg BW (8,14). Induction of a combination of low-dose HFD and STZ has been proven to cause mice to experience hyperglycemia, weight gain (14-16), hypercholesterolemia, and hypertriglyceridemia (14,15). STZ was dissolved in citric buffer with a concentration of 0.05 M and pH 4.5 (15,16). Confirmation of overweight and hypercholesterolemia was done after induction, after the rats were first fasted for 12 hours. Cholesterol levels were measured through blood obtained from the tails of the rats and were measured using a digital cholesterol level meter (15). Rats were classified as hypercholesterolemia if they have total cholesterol levels >111 mg/dl (11). Rats that gained weight over the mean of +2SD of the control group were considered overweight. Overweight and hypercholesterolemia rats obtained were used as subjects in this study by providing dietary therapy for three weeks.

Table 1. Composition of pellets per 100 grams

Component	Standard pellet	HFD pellet	Rice pellet	BA1 pellet	BA2 pellet
Standard pellet [1]	100	77,2	60	60	60
Lard	0	22,8	0	0	0
Ordinary rice	0	0	40	0	0
BA1	0	0	0	40	0
BA2	0	0	0	0	40
Total	100	100	100	100	100

Note: 1=standard pellet containing 60% carbohydrate, 23% fish flour, 6% soybeans and other additives, HFD=High fat diet, BA1=analog rice 1, BA2=analog rice 2

Analog Rice and Pellet Preparation

Analog rice in this study was artificial rice made utilizing a hot extruder using Subagio Technique (7) with the main raw materials of MOCAF, corn flour, rice flour, and an addition of sodium alginate with two different composition variations. The composition was listed based on a formula from one of the Smart Rice variants modified by Hairrudin et al. (Table 2). Analog rice 1 had less MOCAF content but more rice flour, while analog rice 2 had more MOCAF content but less rice flour content (8).

Table 2. Calorie content and analog rice composition per 100g

Ingredients	Ordinary rice	BA1	BA2
MOCAF	0	27	54
Rice flour	100	36	9
Corn flour	0	27	27
Soy Protein	0	6,5	6,5
Palm Oil	0	2,3	3,5

Table 2. Calorie content and analog rice composition per 100g (Continued)

Ingredients	Ordinary rice	BA1	BA2
Sodium alginate	0	1,2	0
Total	100	100	100

Note: MOCAF = Modified cassava flour, ordinary rice, BA1 = analog rice 1, BA2 = analog rice 2. Sources: (8)

The calorie content, both BA1 and BA2, was made equal to ordinary rice. Analog rice had a fiber content of 1.77% in analog rice formula 1, 0.86% in analog rice formula 2, and 0.41% in ordinary rice. The fiber and RS content of ordinary rice, BA1, and BA2 can be seen in Table 3. Regular rice and AR were then used as pellets by mixing them with standard pellets with a ratio of 60% ordinary rice or AR and 40% standard pellets.

Table 3. Nutrient content in rice, BA1, and Ba2

Group	Fiber	RS
Ordinary rice	0,41	0,01
BA1	1,77	16,83
BA2	0,86	11,46

Note: RS = Resistant starch, BA1 = analog rice 1, BA2 = analog rice 2

Measurement of Body Weight and Total Cholesterol Levels

Weight measurements were carried out three times that were during the pre-induction (initial), post-induction/pre-treatment diet (pre-test), and post 3-week treatment diet (post-test). Weight measurements were carried out using a digital scale (Camry). Measurement of total cholesterol levels was done with an electrode-based biosensor method using a digital cholesterol gauge or cholesterol control check (Easy Touch) and blood cholesterol test strip. Measurement of total cholesterol levels was carried out twice, post-induction/pre-treatment diet (pre-test) and post 3-week treatment diet (post-test). Before the blood was obtained, the rats were fasted overnight (12 hours). Blood collection was done through the tail cut ± 0.5 cm. Blood drops were inserted into the cholesterol test strip that was attached to a digital cholesterol gauge to calculate the total cholesterol levels.

Statistical Analysis

The data obtained were summarized and presented in a tabular form, and the statistical analysis was carried out using IBM SPSS version 21 with a confidence level of 95% ($\alpha = 0.05$). Data normality distribution was assessed using the Shapiro-Wilk, while data homogeneity was assessed using the Levene test. The One-Way ANOVA test with Bonferroni posthoc was carried out on initial body weight and pre-test as well as pre-test total cholesterol levels to determine the success of the combination induction. The Paired T-test was carried out on body weight and total cholesterol levels of pre-test and post-test to see the difference between before and after the 3-week diet treatment to know the success of treatment with analog rice.

RESULTS

This study aimed to determine the potential of analog rice with the main components of MOCAF flour, corn flour, rice flour, and sodium alginate in reducing body weight and total cholesterol levels in overweight rats with hypercholesterolemia. The rats were obtained by induction of HFD-STZ combination. After the induction, the body weight and total cholesterol levels of the rats increased. The next stage was giving intervention to the rats in the form of diet therapy for three weeks. A summary of the research results can be seen in Table 4 and 5.

Body Weight

The results of animal body weight measurements during the study can be seen in Table 4. Body weight was measured three times that were in pre-induction (initial), pre-test, and post-test. The mean body weight of the treatment rats (group II, III, and IV combined) at the beginning of the study was 160.28±11.51gram. After the HFD-STZ induction, the mean body weight of the rats increased significantly to 208.00±26.03gram, while the weight of the control group did not increase significantly. The One-Way ANOVA test results on all body weight groups after induction (pre-test) obtained p = 0.002, which means that there were significant differences in mean body weight between groups after HFD-STZ induction. Body weight differences among the groups were tested using the Bonferroni posthoc test. The Bonferroni posthoc test results for BW after induction among the induced groups (II, III, and IV) showed no significant differences, whereas among all the induced groups and group I (control) there were significant differences (I vs. II p = 0.008; I vs. III p = 0.007; I vs. IV p = 0.013). The results of the analysis showed that body weight between the control group and all HFD-STZ-induced groups experienced significant differences, whereas between the treatment groups, there were no significant differences. These facts prove that the induction of the HFD-STZ combination carried out increased the body weight.

Table 4. Mean of the weight measurement result

Group	Initial BW (Mean±SD)	*Pre-Test* BW (Mean±SD)	*Post-Test* BW (Mean±SD)
I (Control)	161,33±11,15	152,83±20,13	154,17±19,82
II (PBB)	159,00±10,16	208,83±30,71*	192,00±34,92
III (PBA1)	161,17±11,81	209,83±15,99**	190,17±09,83*
IV PBA2)	160,67±14,31	205,33±33,03*	186,67±41,70**

Note: BW=body weight, SD=standard deviation, PBB=ordinary rice treatment, PBA1=analog rice treatment 1, PBA2=analog rice treatment 2, *=significant difference between initial BW and pre-Test, **=significant difference between BW pre-Test and pre-Test

After the diet treatment for three weeks, group II, III, and IV experienced a significant decrease in body weight, while group I (control) experienced an increase but not significant. The changes in body weight from each group before and after the diet were analyzed using the Paired T-test. The test results showed a significant decrease in weight in group III (p = 0.003) and group IV (p = 0.007), but not significant in group II (p = 0.350), while group I actually experienced an increase in weight but not significant (p = 0.401). This showed that the provision of analog rice could significantly reduce body weight while the provision of ordinary rice did not reduce body weight in overweight rats. The difference was due to differences in fiber content and RS. The greater the fiber and RS content in the feed consumed, the greater the effectiveness of weight loss.

Total Cholesterol Levels

Total cholesterol levels in normal Wistar rats is 103.8±7.2 mg/dl (17). This fact shows that the highest normal total cholesterol levels is 111 mg/dl. In this study, the normal value of total cholesterol levels used the average cholesterol level of the control group, which was 116 mg/dl. Rats in the treatment groups (II, III, and IV) had hypercholesterolemia since they had total cholesterol levels higher than 116 mg/dl. The results of measurements of total cholesterol in rats during the study can be seen in Table 5. Total cholesterol levels were measured twice before and after being given the diet. Differences in cholesterol levels per group before and after diet (pre-test and post-test) were analyzed using the Paired T-test. The test results showed that total cholesterol levels in group III and group IV had a significant decrease with the same p-value (0.045). The level of total cholesterol in group II increased but was not significant (p = 0.622), while the total cholesterol levels in group I did not experience a significant change (p = 0.849). This showed that the provision of analog rice could significantly reduce total cholesterol levels. The fact is because AR has high fiber and RS content, the greater the fiber and RS content in the diet consumed, the greater the effectiveness of decreasing total cholesterol levels.

Table 5. Mean of the total cholesterol measurement result

Group	*Pre-Test cholesterol* (Mean±SD)	*Post-Test cholesterol* (Mean±SD)
I (Control)	116,00±08,63	116,83±06,24
II (PBB)	145,17±28,74*	151,67±12,71
III (PBA1)	176,33±30,81*	142,33±28,48**
IV PBA2)	152,00±24,20*	120,83±07,14**

Note: SD=standard deviation, PBB=ordinary rice treatment, PBA1=analog rice treatment 1, PBA2=analog rice treatment 2, *=significant difference between initial and pre-Test BW, **=significant difference between pre-Test and pre-test BW

DISCUSSION

In this study, the induction of HFD-STZ combination performed was proven to significantly increase the body weight and total cholesterol levels in white rats (Rattus norvegicus). Overweight conditions in rats are caused by excess fat accumulation in the body due to high-fat consumption in HFD (15,17). This study used HFD that contained 22.8% lard. The administration of HFD results in increased absorption of triacylglycerol (TG) so that the levels in lipoproteins increase. This condition results in TG hydrolysis by increased lipoprotein lipases in the capillaries. The hydrolysis releases free fatty acids so that the levels in the blood increase (17,18). Some of the free fatty acids are re-formed into TG and stored in adipocytes (17). Prolonged administration of HFD results in more TG deposits resulting in overweight (18). Overweight can lead to obesity and is one of the triggers of insulin resistance that can trigger hypercholesterolemia. The combination of intraperitoneal HFD and low dose STZ injection has been shown to increase insulin resistance (8), thus causing glucose metabolic disorders marked by hyperglycemia (14-16), overweight (14,16), and hypercholesterolemia (15-16). Repeated administration of HFD results in increased accumulation of fat in adipose tissue and increased levels of free fatty acids and fatty acid oxidation. Fatty acid oxidation increases the production of acetyl Co-A (18). It stimulates endogenous cholesterol synthesis or triggers excessive increases in total cholesterol in the blood resulting in hypercholesterolemia (15-16). One way to handle overweight and hypercholesterolemia is to consume a diet that contains high fiber (2,12,19,20) and RS.

The results of this study prove that diet intervention in the form of analog rice for three weeks can reduce body weight and total cholesterol levels in male Wistar strain rats that are overweight and hypercholesterolemia due to the induction of the HFD-STZ combination. After the 3-week diet, group III (PBA1) experienced body weight decrease by 19.67 grams, and group IV (PBA2) by 18.67 grams. Group II (PBB) did not experience a significant decrease in weight, while group I (control) experienced an increase in weight but not significant, which was as much as 1.33 grams. The mean total cholesterol levels of group III (PBA1) experienced a significant decrease of 34 mg/dl and group IV (PBA2) of 31.17 mg/dl. The mean total cholesterol levels in group II (PBB) increased but not significant as much as 6.5 mg/dl, while group I (control) experienced a decrease in total cholesterol but not significant as much as 0.83 mg/dl. The decrease in body weight and total cholesterol is inversely proportional to the fiber content contained in the diet. This shows that the higher the fiber content in the feed given, the greater the effect on weight loss and total cholesterol.

The food fiber content in analog rice plays an important role. c flour and MOCAF flour are alternative carbohydrate sources that have a higher fiber content compared to ordinary rice. Sodium alginate added to BA1 plays an important role in increasing its fiber content. MOCAF has a high crude fiber content of 3.4% to 12% (21,22). Corn flour is one source of food fiber in the form of soluble and water-insoluble fiber (23), while sodium alginate contains water-soluble fiber (24).

Water-soluble fiber triggers gel formation in the digestive tract that functions to slow gastric emptying and maintain satiety. Fiber-rich foods can support the peptide release in the form of cholecystokinin that is involved in the regulation of gastric emptying and stimulate the nucleus of fullness in the hypothalamus. Satiety induced by dietary fiber shows the potential for sustainable weight loss without hyperphagia and weight re-bound in obesity cases (2,25,26).

Fiber can bind with fatty acid salts in the small intestine and is released and become a food source of microflora in the colon through the fermentation process. The microflora produces fermented products from fibers in the form of Short Chain Fatty Acids (SCFA), such as propionic acid (8). Propionic acid has activity as an inhibitor of the enzyme 3-Hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, thus inhibiting cholesterol synthesis. Insoluble fiber also has the effect of increasing the expression of Glucose Transporter Type 4 (GLUT-4), resulting in improved insulin sensitivity or reduced insulin resistance. Decreased insulin resistance results in the inhibition of cholesterol synthesis so that the hypercholesterolemia condition can be prevented (2).

The RS content in AR also has an important role in reducing body weight and total cholesterol levels. The RS content in AR is quite high, while rice contains almost no RS (Table 3). Hot-extrusion technology used in the AR manufacturing process resulted in the starch in AR undergoing gelatinization and retrogradation (7). Retrogradation occurs because the technology goes through the stages of heating and cooling which facilitate amylose retrogradation. Retrogradation produces RS so that the level increase (10,27). Sodium alginate contains water-soluble fiber (24) which triggers gel formation in the gastrointestinal tract, thereby increasing RS production. This results in the RS level in BA1 becomes higher than BA2 because sodium alginate is added in BA1 composition.

High RS level causes inhibited digestion and absorption of food in the intestine (11), resulting in the synthesis of fatty acids, lipogenesis, free fatty acids, and decreased energy storage pathways which in turn causes decreasing energy storage in the form of TG in adipocytes, so body weight also decreases. The reduction in free fatty acids subsequently results in decreased fatty acid oxidation and reduced acetyl-CoA production. Acetyl-CoA is the raw material for cholesterol synthesis in the body. A decrease in acetyl-CoA production results in a decrease in total cholesterol levels (11,18).

This study concludes that the provision of analog rice 1 and analog rice 2 can significantly reduce body weight and total cholesterol levels in overweight and hypercholesterolemic rats. Analog rice 1 has a higher fiber and RS content than analog rice 2, so it gives a better effect on weight loss and total cholesterol levels. This shows the potential of analog rice made from MOCAF, corn flour, rice flour, and the addition of sodium alginate as an alternative solution for dietary therapy that can be utilized in the health sector, especially in overweight and hypercholesterolemia.

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