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Effect of Combination Formula Cavendish Banana and Moringa Leaf Flour on HDL Levels in Obese Rats

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Abstract. Obesity leads to an increased risk of dyslipidemia, which is characterized by a decrease in High Density Lipoprotein (HDL) cholesterol levels. HDL is influenced, among other things, by the intake of foods containing fiber such as resistant starch and antioxidants such as flavonoids and vitamin C. Cavendish bananas contain resistant starch and Moringa leaves contain flavonoids and vitamin C. The aim of this study was to determine the effect of giving a formula of Cavendish banana flour and Moringa leaf flour on HDL levels in obese rats. This research is a true experiment with a Randomized Pre Test and Post Test with Control Group Design. Samples of 30 white Wistar rats were divided into 5 groups: positive control, negative control, K1 (75% banana:25% Moringa leaf), K2 (50% banana:50% Moringa leaf), K3 (25% banana:75% Moringa leaf) for 14 days. HDL levels were measured pre and post intervention using CHOD-PAP method. HDL levels are normal in the range of 35-85 mg/dL. The research results showed that HDL levels before and after the intervention had a significant increase ($p=0.000$). Giving different intervention doses also had a significant effect ($p=0.000$). The K3 group with a dose of 500 mg of cavendish banana flour and 375 mg of Moringa leaf flour was the most effective dose for increasing HDL levels. The dose conversion for humans is 28 g of banana flour and 21 g of Moringa leaf flour. The conclusion is cavendish flour and moringa leaf flour formula can increase HDL levels.

Keywords: Cavendish Banana Flour, Moringa Leaf Flour, HDL, Obesity

1 INTRODUCTION

Obesity is an abnormal condition characterized by the accumulation of excess fat tissue due to an imbalance between energy intake entering the body and energy released by the body [1]. Data from World Health Organization (WHO) in 2016 shows that 39% of people in the world are overweight and 13% are obese [2]. Based on the results of Riset Kesehatan Nasional (Riskesdas) (2018), the incidence of obesity in adults aged over 18 years has increased, namely in 2007 (10.5%), 2013 (14.8%), and 2018 (21.8%) [3]

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Obese nutritional status will lead to an increased risk of dyslipidemia. Dyslipidemia is a lipid metabolism disorder characterized by an increase or decrease in the lipid fraction in the blood. Components of dyslipidemia include low HDL cholesterol levels, and high LDL cholesterol levels [4]. Low HDL levels in the blood are associated with an increased risk of coronary heart disease (CHD), because low HDL levels trigger an atherogenic process or plaque formation on the walls of arteries [5]

Efforts that can be made for dyslipidemia patients are nutritional therapy by implementing eating arrangements and diet modifications, by limiting consumption of fat and cholesterol. Limiting fatty foods and cholesterol can be optimized by implementing therapy, one of which is consuming vegetables and fruits [6]

Cavendish banana (*Musa cavendishii*) is a fruit that contains fiber and is widely consumed by the public [7] Banana flour contains dietary fiber components such as resistant starch [8] The resistant starch content in cavendish banana flour is 40.14% [9] Resistant starch is able to increase the number of HDL particles [10].

Moringa leaves (*Moringa oleifera*) are a herbal plant that is often found and is known to have many benefits [11] Moringa leaves have a high antioxidant content, namely flavonoids and vitamin C. The flavonoid content found in dried Moringa leaves is 0.18% -1.64% [12]. Meanwhile, the vitamin C content in 100 g of dried Moringa leaves is 15.8 mg [13] The antioxidant content, namely flavonoids and vitamin C, can increase HDL cholesterol levels [14].

Cavendish bananas have the advantage of containing high levels of dietary fiber in the form of resistant starch. Moringa leaves contain antioxidant flavonoids and vitamin C. Therefore, it is hoped that the combination of cavendish banana flour and moringa leaf flour will be more effective in increasing HDL levels. Apart from that, Moringa leaf flour has a bitter taste and sharp aroma, so some people don't like it. It is hoped that the addition of banana flour can disguise the taste and aroma. This study aims to determine the effect of giving a formula of Moringa leaf flour and Cavendish banana flour on the HDL levels of obese Wistar rats by administering several different doses.

2 METHOD

This research is a laboratory experimental research with a true experiment with a Randomized Pre Test and Post Test with Control Group Design. The independent variable was the administration of a formula of cavendish banana flour and moringa leaf flour for 14 days and the dependent variable was HDL levels. This research was carried out at the Experimental Animal Laboratory, Center for Food and Nutrition Studies, Universitas Gadjah Mada. The formula for Cavendish banana flour and Moringa leaf flour was made at the Food Laboratory at Universitas Muhammadiyah Semarang. Tests for the formula content of Cavendish banana flour and Moringa leaf flour were carried out at the Chem-Mix Pratama Laboratory. This research begins in July - August 2023.

The equipment used in making Cavendish banana flour and Moringa leaf flour is a blender, knife, tray, basin, drying cabinet, 100 mesh sieve, and gloves. The ingredients

used in making flour are Moringa leaves obtained from the Mranggen, Demak and Sunpride brand Cavendish bananas with a maturity level of 2 obtained from fruit suppliers, as well as 3% citric acid. Tools for raising rats are rat cages, digital animal scales, woven wire cage covers, husks, drinking bottles. Materials for raising rats are AD II feed, water, and male Wistar white rats. The tools used in analyzing HDL levels are a UV-Vis spectrophotometer (Hitachi Japan), micro pipette, yellow and blue tips, micro tube, micro capillary pipette, incubator, test tube, centrifuge and timer. The materials used in analyzing HDL levels are blood serum, cholesterol reagent and precipitate reagent. The tools used to test food fiber content are Erlenmeyer, water bath, filter paper, oven, desiccator and scales. The materials used in the food fiber content test were Cavendish banana flour, pH 7 phosphate buffer, alpha amylase enzyme, beta amylase enzyme, 1% pepsin enzyme, 1N NaOH, 1N HCL, ethanol, acetone and distilled water. The tools used to test the flavonoid content are Erlenmeyer, measuring flask, filter paper, test tube, vortex and spectrophotometer. The materials used in the food fiber content test were Moringa leaf flour, 96% ethanol, and 5% AlCl₃.

The stages in this research begin with the preparation of files and equipment, namely processing permits through ethical clearance as well as preparing equipment and materials that will be used during the research. The next stage is the maintenance of test animals, with the animals used being 30 male Wistar rats with the qualifications that the rats are 8 weeks old and weighing 150-200 grams, kept in cages with a total of 5 rats/cage or one group + 1 per group for dropped out.

Feeding was control feed using AD II feed and drinking water given ad libitum as well as high fat high glucose feed (HFHG) in the form of a mixture of pork oil given as much as 2 ml/200 g rat body weight, duck egg yolk 1 ml/200 g rat body weight, and glucose 1 ml/200 g rat body weight, given via gastric probe for 14 days [15]. Formula was given to the treatment groups, namely the positive group was only fed 10% of the rat's body weight ad libitum, the negative group was only fed 10% of the rat's body weight ad libitum, the intervention group 1 was given 10% control food + 1500 mg Cavendish banana flour + 125 mg Moringa leaf flour, intervention group 2 was given 10% control feed + 1000 mg Cavendish banana flour 250 mg Moringa leaf flour, and intervention group 3 was given 10% control feed + 500 mg Cavendish banana flour + 375 mg leaf flour Moringa.

Determination of the nutritional status of rats was carried out to determine the obesity status of rats. Measurements were carried out twice. The first was when the animals were tested after being given high-fat and high glucose diet for 14 days. Second, after being given intervention for 14 days. Determination of nutritional status using the Lee index calculation with the formula: $(\text{body weight (g)}^{1/3} / \text{nasoanal length (cm)}) \times 10^3$. Body weight was measured using a digital animal scale and nasoanal length was measured using a measuring tape. Nasonanal length is the length from the nose to the anus. Rats are said to be obese if they have a Lee index >300 [16].

HDL levels were checked twice. Firstly, after the test animals were given high fat and high glucose diet on the 17th day, and secondly after the test animals were given an intervention formula of Cavendish banana flour and Moringa leaf flour on the 31st day. Test HDL levels using the Cholesterol Oxidase– Peroxidase Aminoantipyrene (CHOD-PAP) method. Test the dietary fiber content in Cavendish banana flour using

the multienzyme method and test the total flavonoid content in Moringa leaf flour using the spectrophotometric method.

The next stage is data collection, processing and analysis. Data analysis carried out in this study used Shapiro Wilk to see the normality of the data with results of $p \geq 0.05$ said to be normally distributed. Next, to see the difference in HDL levels before and after the intervention, use the Paired t-test, where the results are said to be significant if $p \leq 0.05$. Next, to see the difference in the effect of intervention doses of Cavendish banana flour and Moringa leaf flour, use the One Way Anova test for normally distributed data and the results are said to be significant if $p \leq 0.05$. If there is a difference then continue using the Post Hoc Duncan test.

3 RESULTS AND DISCUSSION

This study aims to determine the effect of giving a combination formula of cavendish banana flour and moringa leaf flour on HDL levels in obese rats. The research was carried out on 30 male Wistar rats adapted to a control diet for 3 days. Next, the rats were divided into 5 groups, namely groups K+, K-, K1, K2 and K3. The positive control group (K+) was given control feed for 14 days. Meanwhile the negative control group (K-) and intervention groups (K1, K2 and K3) were given high fat and high glucose (HFHG) diet for 14 days.

After administering HFHG, the initial Lee index was measured to determine the nutritional status of obesity in rats. Rats are declared obese if they have a Lee index >300.16 . Furthermore, groups K+ and K- were given control feed, while groups K1, K2, and K3 were given control feed and intervention with cavendish banana flour and moringa leaf flour for 14 days. Group K1 was given 1500 mg cavendish banana flour and 125 mg moringa leaf flour or a dose of 75 %: 25%. Group K2 was given 1000 mg cavendish banana flour and 250 mg moringa leaf flour or a dose of 50 %: 50%. The K3 group was given 500 mg Cavendish banana flour and 375 mg Moringa leaf flour or a dose of 25 %: 75%. Next, the final Lee index was measured to determine the nutritional status of the rats after the intervention.

The results of the analysis of dietary fiber and flavonoid content carried out at the Chemix Pratama Yogyakarta Laboratory showed that Cavendish banana flour contained 5.71% dietary fiber and 0.92% flavonoids. This means that group K1 was given a formula containing 85.6 mg of dietary fiber and 1.2 mg of flavonoids. The K2 group was given a formula containing 57.1 mg of dietary fiber and 2.3 mg of flavonoids. The K3 group was given a formula containing 28.5 mg of dietary fiber and 3.4 mg of flavonoids.

3.1 Description of changes in nutritional status of rats during treatment

The result of the initial Lee index measurement before the intervention (after giving high fat high glucose diet) and the final Lee index after the intervention can be seen in Table 1.

Table 1. Rat Lee Index During Treatment

Treatment group	Baseline Lee index Mean±SD	Final Lee index Mean±SD
K+	287.68 ± 1.83	288.80 ± 2.35
K-	334.88 ± 2.25	337.11 ± 2.18
K1	333.24 ± 3.09	300.61 ± 2.40
K2	330.80 ± 2.25	292.58 ± 2.07
K3	331.36 ± 4.05	289.13 ± 2.88

Based on Table 1, the K+ group before and after the intervention had a Lee index <300. This is because the K+ group was only given control feed. Meanwhile, groups K-, K1, K2, and K3 had a Lee index >300, which indicated that the rats were obese. This is because the K-, K1, K2 and K3 groups were given high fat high glucose diet. Induction of a high-fat diet consisting of pork fat and duck egg yolk, where pork fat contains 2% myristic fatty acid, 25% palmitic fatty acid, 15% stearic fatty acid, 45% oleic fatty acid, and 9% linoleic fatty acid, while Duck egg yolk contains 17 g protein, 35 g fat and 884 mg/100 g cholesterol which can increase body weight [17]. The high fat and high glucose diet used in this study contained 58.75% total fat or 2.35 grams of fat in each feeding. This fat content is higher than the control feed which contains 7% fat or 1.49 grams of fat in each feeding. A diet high in fat will result in increased fat deposition in organs in the body which will trigger obesity [18]. The research results of Fernández et al. (2018) also explained that giving a high-fat diet combined with glucose showed better results in making rats obese [19].

The mean Lee index measurement at the end of treatment explains a decrease in the Lee index in the three intervention groups. The K3 group that was given the intervention dose of Cavendish banana flour and Moringa leaf flour 500 mg: 375 mg had the lowest mean Lee index, close to K+, while the K1 group that was given the intervention dose of Cavendish banana flour and Moringa leaf flour 1500 mg: 125 mg had the highest mean Lee index. high among the three intervention groups. Meanwhile, the K+ and K- groups who were not given the intervention of cavendish banana flour and moringa leaf flour experienced an increase in the Lee index.

3.2 Description and Differences in Rat HDL Levels Before and After Intervention

The results of measuring HDL levels before and after the intervention can be seen in Table 2. Based on Table 2, the results of measuring HDL levels before intervention in the K-, K1, K2, and K3 groups were lower compared to the K+ group. The K+ group which was only given control feed had HDL levels of 80.95 mg/dL which was included in the normal category. Normal HDL levels in rats are 35-85 mg/dL [20] Meanwhile, in the group that had been adapted to control feed for 3 days and then continued with high fat and high glucose diet for 14 days, there was a decrease in HDL levels below normal values. After administering the intervention of Cavendish banana flour and Moringa leaf flour for 14 days, it could be seen that there was an increase in HDL levels to the normal threshold in groups K1, K2 and K3. The K+ and K- groups who were not

given the cavendish banana flour and Moringa leaf flour intervention experienced a decrease in HDL levels.

Table 2. Rat HDL Levels Before and After Intervention

Treatment Group	HDL levels (mg/dl)			p^*
	Pre Intervention	Post Intervention	Delta	
K+	80.95 ± 2.15 ^a	80.00 ± 1.99 ^c	-0.95 ± 0.44	0.009
K-	24.38 ± 2.55 ^b	23.37 ± 2.25 ^d	-1.01 ± 0.38	0.004
K1	26.29 ± 2.52 ^b	57.88 ± 2.68 ^e	31.58 ± 2.04	0,000
K2	24.22 ± 0.91 ^b	69.02 ± 1.75 ^f	44.80 ± 2.05	0,000
K3	24.06 ± 2.06 ^b	78.90 ± 3.26 ^c	54.84 ± 4.00	0,000
p^{**}	0,000	0,000	0,000	

Note: *Paired t-test ($p \leq 0.05$ significant), **One Way Anova test ($p \leq 0.05$ significant), ^{a,b,c,d,e,f} Values with different lowercase superscripts showed a real difference ($p \leq 0.05$) after analysis using Post Hoc Duncan

Analysis of differences in HDL levels before and after intervention began with a normality test using the Shapiro-Wilk test because the sample was <50 . The normality test results showed that HDL level data for all groups were normally distributed ($p \geq 0.05$) so that it could be continued with the Paired sample T-test and One Way Anova test to see the comparison of the averages for each group.

Paired t-test analysis in groups K1, K2, and K3 showed that there was a significant mean difference between the conditions before and after the intervention of Cavendish banana flour and Moringa leaf flour for 14 days with a value of $p = 0.000$ ($p \leq 0.05$). The largest increase in delta HDL levels occurred in the K3 group, namely 54.84 ± 4.00 mg/dL. Meanwhile, the lowest increase in HDL levels was in the K1 group, namely 31.58 ± 2.04 mg/dL.

One Way Anova test analysis of HDL levels before intervention showed a significant value with $p = 0.000$ ($p \leq 0.05$), which means there was a real difference between groups. Post hoc Duncan's follow-up test results on HDL levels before intervention showed that there were real differences between the K+ and K-, K1, K2, and K3 groups. This means that HDL levels decreased after being given feed high in fat and glucose. This is in line with research by Khoiriyah et al. (2020) which showed a decrease in HDL levels in white rats after being given a High Fat Fructose Diet (HFFD) in the form of pork oil, egg yolk and fructose for 14 days [21] In theory, high intake of cholesterol and fatty acids from high-fat feed causes blood cholesterol to increase. Chylomicrons formed in the intestinal mucosa transport cholesterol absorbed from the intestine and then transport triglycerides to adipose tissue and intrahepatic tissue to become raw materials for the formation of cholesterol in the liver. As a result, the amount of cholesterol in the blood increases. In addition, saturated fatty acids have the ability to reduce levels of Apo A-1, which is a precursor to HDL formation. As a result, HDL cholesterol synthesis is stopped and causes a decrease in the amount of HDL in the blood [22]. Giving a high-fat diet combined with glucose showed better results in making rats dyslipidemic [19].

One Way Anova test analysis of HDL levels after intervention showed a significant value with $p = 0.000$ ($p \leq 0.05$), which means there was a real difference between groups. Post hoc Duncan's follow-up test results on HDL levels after intervention showed that there were real differences between groups K1 and K2, K1 and K3, and K2 and K3. This means that there are differences in the effect of dose in each treatment group. Meanwhile, the K+ and K3 groups showed no significant differences. The K3 group has a mean that is closest to the K+ group. This indicates that the intervention in the K3 group with a dose of 500 mg of Cavendish banana flour and 375 mg of Moringa leaf flour or a dose of 25 %: 75% had the most similar mean to the K+ control group which was not given high fat high glucose diet and had normal HDL levels before and after. after intervention. This means that the K3 group with a dose of cavendish banana flour and Moringa leaf flour of 25 %: 75% was the most effective dose for increasing HDL levels in obese rats.

The large dose of Cavendish banana flour and Moringa leaf flour formula had an effect on increasing HDL levels in the intervention group. The fiber intake needed by adults to control cholesterol is 25-35 g/day [23]. If converted to a rat dose, the required fiber content is 450 mg/day. When compared with the highest amount of dietary fiber in group K1 with a dietary fiber content of 85.6 mg, the amount of dietary fiber contained is quite far from the required dose. Meanwhile, in the research of Li et al. (2013), flavonoid intake of 165.6 mg/day in humans can prevent cardiovascular disease and improve lipid profiles [24] If converted into a dose for rat, the required flavonoid content is 3 mg/day. When compared with the highest amount of flavonoids in the K3 group of 3.4 mg, this amount is sufficient for flavonoid needs per day. Therefore, the formula containing higher levels of Moringa leaf flour had a more effective effect in increasing HDL levels than the group containing higher levels of Cavendish banana flour.

In line with research by Rupiasa (2022), supplementation with Moringa leaf flour at a dose of 100 mg/100 g BW and 200 mg/100 g BW for 14 days can increase HDL cholesterol levels in rats [14]. Increasing HDL cholesterol levels through administering Moringa leaf flour is associated with the antioxidant content in Moringa leaves. The antioxidant content, namely flavonoids and vitamin C, can increase the activity of Lecithin-cholesterol acyltransferase (LCAT). LCAT is an enzyme that can convert free cholesterol into more hydrophobic cholesterol esters, so that it can bind to lipoprotein core particles and form new HDL cholesterol [25]. The higher the LCAT activity, the more new HDL is formed. The LCAT enzyme also increases the production of Apo A-1 which plays a role in increasing serum HDL cholesterol levels. High Density Lipoprotein (HDL) containing Apo-A1 is protective against atherosclerosis. The formation of HDL is the body's defense mechanism in maintaining the balance of fat in the body. HDL returns excess cholesterol to the liver, which is then converted into bile salts and excreted through the intestines [14].

4 CONCLUSION

Based on the research that has been carried out, it can be concluded that there was a significant increase in HDL levels after giving the intervention of cavendish banana flour and moringa leaf flour for 14 days. Giving different intervention doses has a significant effect. The largest increase in HDL levels occurred in the K3 group, so the dose of cavendish banana flour and moringa leaf flour 25 %: 75% was the most effective dose for increasing HDL levels in obese rats. Providing a formula of cavendish flour and Moringa leaf flour can increase HDL levels.

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