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The effect of pindang patin intake on serum cholesterol and LDL levels of male mice (*Mus Musculus L.)*

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The effect of pindang patin intake on serum cholesterol and LDL levels of male mice (*Mus Musculus L.)*

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Abstract. High blood cholesterol level can cause atherosclerosis which is potentially cause coronary heart disease. Pindang patin is one of the favourite foods in Palembang which contain omega 3 fatty acids that can decrease blood cholesterol level. The aim of this study was to see the effect of pindang patin intake on total cholesterol and LDL. The type of this study was laboratory experimental using a pre-post-test randomized design with a control group in male mice (*Mus musculus L.*). The mice were given the high-fat diet by using egg yolk as much as 20% of body weight. The samples were divided into 5 groups: Negative Control, Positive Control, Dose I (0.26 ml/20 grams of body weight), Dose II (0.52 ml/20 grams of body weight) and Dose III (1.04 ml/20 grams of body weight). Blood samples were examined by using a spectrophotometer. Dose II and Dose III give the effect in decreasing the blood level of cholesterol in male mice, and Dose III give the effect in decreasing the blood level of LDL of male mice. Pindang patin can decrease the blood total cholesterol and LDL levels of male mice, and the best dose is in 1.04 ml/20 grams of body weight.

1. Introduction

The World Health Organization (WHO) estimates that in 2008 there were 17.3 million people died of cardiovascular disease (30% of all deaths in the world). Based on this case of death, 7.3 million died of CHD and stroke caused 6.2 million people died [1]. In 2015 based on WHO estimates an increase of 17.7 million people died of cardiovascular disease (31% of all deaths in the world). Based on this death case, 7.4 million of them died from CHD and 6.7 million due to stroke [2].

The occurrence of CHD cannot be separated from the processes that make the coronary arteries narrow. Atherosclerosis is actually normal in all people as we get older, it's just how the narrowing speed varies. Cholesterol is a type of lipid that has relatively important clinical significance with respect to atherogenesis [3]. This excess cholesterol can stimulate atherosclerosis which in turn has the potential to cause CHD [4].

Hypercholesterolemia is a condition in which an increase in the concentration of cholesterol in the blood that exceeds the normal value [5]. Normally, cholesterol is produced by the body in the right amount. But eating patterns tend to be animal-based foods with high-fat, causing cholesterol to be excessive in the blood [4].

According to the 2015 Palembang City Culture and Tourism Office, pindang is one of the favourite foods of the people of Palembang, South Sumatra in the form of processed special flavoured dishes.

The most famous Pindang in Palembang is pindang patin [6]. Patin fish is considered safer for health because cholesterol levels are lower than that of cattle. In patin fish meat, there are fatty acids free of omega-3 which are useful in suppressing cholesterol in the blood. The total fat content contained in patin fish meat is between 2.55% -3.42%, and unsaturated fatty acids above 50%. The oleic acid which is a monounsaturated fatty acid in patin fish meat reaches 8.43%. Based on the results of expert research, the nutrient content in patin fish in the form of unsaturated fats or unsaturated fatty acids (USFA 50%) is very good for preventing the risk of cardiovascular disease.[7]

There have been no special studies examining the effect of pindang patin intake on blood cholesterol and LDL levels, therefore this study needs to be done to see the effect of pindang patin on total cholesterol and LDL blood levels in male mice (*Mus musculus L*).

2. Methods

This type of research was laboratory experimental using a randomized pre-post-test design with a control group. The experimental animals used were male mice (*Mus musculus L.*). The study was conducted from June 207 to January 2018. The research will be carried out in two places, namely: Animal House, and Biochemistry Laboratory, Faculty of Medicine, Universitas Sriwijaya. The population of this study was male mice (*Mus musculus L.*) Swiss Webster strain aged 2-3 months. In this study 30 samples of mice were grouped into 5 groups, each group consisted of 6 mice.

To increase blood cholesterol levels in this study, animals were given high-fat feed, which was in the form of egg yolks. Egg yolks contained 48.5% water, 16.15% protein, 24.65% fat, 0.6% carbohydrate and 1.1% ash.8 Making high-fat feed was done by boiling chicken eggs, the yellow part was taken, then weighed and then given orally to experimental animals as much as 20% of the body weight (20% x 20 grams = 4 grams) for 21 days [8,9].

Human dose conversion (70kg) to mice (20gr) based on Laurence and Bacharach formula was 0.0026 [10]. The average portion of patin fish for one person was 100 grams of patin fish, then the dose of patin fish in mice = 100 grams x 0.0026 = 0.26 grams/20 grams body weight [11]. The dose was lowered and raised according to the measurement series to 0.26 gr/20 grams body weight, 0.13 gr/20 grams body weight and 0.52 gr/20 grams body weight.

The diet given to mice was in the form of processed pindang patin, patin fish meat separated from the bone. Then, put it in a blender together with the pindang sauce, and smooth it. As for 100 grams of patin fish meat plus 100 ml of shredded broth produced 200 ml of pindang patin processed, so that 1 gram of pindang patin was contained in 2 ml of pindang patin processed. Therefore, the dosage of processed pindang patin given to mice was 0.26 ml/20 grams body weight, 0.52 ml/20 grams body weight, 1.04 ml/20 grams body weight. How to provide processed pindang patin was using stomach sonde for 21 days [12]. The drug used to reduce cholesterol in the positive control group was simvastatin 10 mg. Simvastatin in adults was given at a dose of 10 mg, then the dose of simvastatin given to mice (added 10 ml 1% CMC or 1 mg/ml) = 10 ml x 10 mg x 0.0026 = 0.26 ml [13].

Animals were adapted first for 7 days with standard feed and drink *ad libitum*. Then, animals were given high-fat feed for 7 days. Blood samples were taken from the orbital sinus section using a hematocrit pipette and obtained 0.1 ml of blood, and were examined and analyzed for total cholesterol and LDL levels. After getting total cholesterol and LDL blood levels to become high, then animals were treated with pindang patin processed. The experimental animals were grouped into 5 groups: Group I was not reduced in total cholesterol and LDL cholesterol while in group II was given simvastatin suspension as much as 0.26 ml/20 grams body weight. Group III (dose I group) was given 0.26 ml/20grams body weight of pindang patin processed. Group IV (dose II group) was given 0.52 ml/20 grams body weight pindang patin processed. Group V (dose III group) was given 1.04 ml/20grams body weight of pindang patin processed. The five groups were treated for 21 days. Furthermore, blood samples were taken and put into a cuvette and checked for total cholesterol and LDL levels using cholesterol and LDL-cholesterol assay kits.

The data obtained were processed with the SPSS 22 program. In this study, after high-fat feeding data was taken which was the pretest data, then after the treatment was given the data would be taken as post-test data. Pretest and post-test data will be analyzed by Paired t-Test. Post-test data will then be analyzed using parametric statistics, namely analysis of variance (ANOVA). Then, data were analyzed by the Bonferroni Post Hoc.

3. Results

The results of the examination of serum cholesterol levels could be seen in table 1. From table 1 it could be seen that the average of the results of examination of total blood cholesterol levels of mice after high-fat feeding was obtained at 108.53 mg/dl. Normal levels of total blood cholesterol in mice were 26-82.4 mg/dl, so that the total blood cholesterol level of mice was high [14].

Table 1. Serum total cholesterol levels of mice.

Group	Average Cholesterol Levels (mg/dl) ± SD			
	Pre-test	Post-test	Difference	p[a]
I (Negative Control)	93.45 ± 17.17	89.55 ± 14.14	3.9 ± 2.76	0.062
II (Positive Control)	131.79 ± 16.3	83.5 ± 11.4	48.3 ± 34.14	0.000
III (Dose I)	72.15 ± 13.93	66.1 ± 10.96	10.18 ± 4.28	0.091
IV (Dose II)	99.49 ± 7.44	82.86 ± 10.04	16.63 ± 11.76	0.020
V (Dose III)	141.63 ± 18	101.63 ± 17.3	40 ± 28.3	0.002
Total	108.53 ± 28.48	84.73 ± 16.71	23.38 ± 19.63	

[a]Paired t-test, significant if $p<0.05$

From the results of Paired t-test analysis in table 1 it could be said that there were significant differences between total pre-test and post-test cholesterol levels ($p<0.05$) in Positive Control, Dose II and Dose III, and there was no significant difference between pre-test total cholesterol levels and post-tests ($p> 0.05$) in Negative Control and Dose I.

Furthermore, the post-tests data was in the form of data on the difference in total cholesterol level or large total cholesterol reduction by One-Way ANOVA test. The One-Way ANOVA test results obtained $p=0.000$ ($p<0.05$) so that it could be said that there was a significant difference in each treatment group. Then, the data proceed by using the Bonferroni Post Hoc test to find out the significant differences between the two unpaired groups. Post Hoc test results could be seen in table 2.

Table 2. Results of total cholesterol levels Bonferroni Post Hoc test.

Group	I (Negative Control)	II (Positive Control)	III (Dose I)	IV (Dose II)	V (Dose III)
I (Negative Control)		0.000[a]	1.000	0.453	0.000[a]
II (Positive Control)	0.000[a]		0.000[a]	0.000[a]	1.000
III (Dose I)	1.000	0.000[a]		1.000	0.001[a]
IV (Dose II)	0.453	0.000[a]	1.000		0.008[a]
V (Dose III)	0.000[a]	1.000	0.001[a]	0.008[a]	

[a]Significant if $p<0.05$

The result of table 2 showed the difference in the total difference in cholesterol levels in the Control Group with Positive Control, Positive Control with Dose I, Positive Control with Dose II, Negative Control with Dose III, Dose I with Dose III Dose II with Dose III with $p<0.05$, whereas Negative Control with Dose I, Positive Control with Dose III, Negative Control with Dose II and Dose I with Dose II showed no significant difference in total cholesterol levels ($p>0.05$).

The results of the examination of serum LDL levels could be seen in table 3. From table 3 it could be seen that the average of the results of blood LDL levels after high feeding was obtained at 16.43 mg/dl. Normal levels of rat blood LDL are 7-27.2 mg/dl, so it could be said that LDL blood levels of mice were still within normal limits, but treatment is continued because the total blood cholesterol levels of mice are in the high category [15].

Table 3. Serum LDL levels of mice.

Group	Average LDL Levels (mg/dl) ± SD			
	Pre-test	Post-test	Difference	p[a]
I (Negative Control)	17.38±0.73	16.78±0.84	0.6±0.43	0.055
II (Positive Control)	15.45±2.79	11.24±1.93	4.21±2.98	0.001
III (Dose I)	14.03±1.59	12.96±1.6	1.07±0.76	0.071
IV (Dose II)	17.08±1.45	15.44±2.36	1.64±1.15	0.065
V (Dose III)	18.2±2.45	15.04±2.07	3.16±2.23	0.014
Total	16.43±2.43	14.3±2.6	2.13±1.75	

[a]Paired t-test, significant if $p<0.05$

From the analysis of Paired t-test in table 3, it was found that there were significant differences between LDL pre-test and post-test levels ($p<0.05$) in Positive Control and Dose III, and there was no significant difference between LDL pre-test and post-test levels ($p<0.05$) in Negative Control, Dose I and Dose II.

Furthermore, the post-test data was in the form of data on the difference in LDL levels or the amount of LDL reduction carried out by One-Way ANOVA test. The One-Way ANOVA test results obtained $p=0.000$ ($p<0.05$) so that it could be said that there was a significant difference in each treatment group. Then, the data proceed by using the Bonferroni Post Hoc test to find out the significant differences between the two unpaired groups. Post Hoc test results could be seen in table 4.

Table 4. Results of LDL levels Bonferroni Post Hoc test.

Group	I (Negative Control)	II (Positive Control)	III (Dose I)	IV (Dose II)	V (Dose III)
I (Negative Control)		0.001[a]	1.000	1.000	0.027[a]
II (Positive Control)	0.001[a]		0.004[a]	0.025[a]	1.000
III (Dose I)	1.000	0.004[a]		1.000	0.112
IV (Dose II)	1.000	0.025[a]	1.000		0.555
V (Dose III)	0.027[a]	1.000	0.112	0.555	

[a]Paired t-test, significant if $p<0.05$.

The result of Table 4 showed the differences between LDL and Negative Control with Positive Control, Positive Control with Dose I, Positive Control with Dose II and Negative Control with Dose III with $p<0.05$, while Negative Control with Dose I, Negative Control with Dose II, Positive Control with Dose III, Dose I with Dose II, Dose I with Dose III, Dose II with Dose III showed no significant difference in LDL content ($p> 0.05$).

4. Discussions

In table 1, it was obtained after feeding high-fat, obtained high total cholesterol. High total blood cholesterol after being fed high in fat due to the cholesterol esters in food is hydrolyzed to cholesterol which is then absorbed by the intestine along with un-esterified cholesterol and other lipids in food. Together with the cholesterol synthesized, cholesterol is then put into the chylomicron. Cholesterol absorbed 80-90% has esterification with long chain fatty acids in the intestinal mucosa. Ninety-five percent of chylomicron cholesterol is channelled to the liver in the residual form of chylomicrons, and most of the cholesterol secreted by the liver in the form of VLDL is maintained during IDL formation and eventually, LDL is absorbed by LDL receptors in the liver and extrahepatic tissue [16].

From table 1, in the Positive Control group, the results of Paired t-test were obtained with $p=0.000$, which means that there were significant differences between the total pre-test and post-tests cholesterol levels. In the Positive Control group, Simvastatin is used as a cholesterol-lowering drug. Simvastatin works by inhibiting HMG-CoA reductase. HMG-CoA reductase inhibitors function to inhibit cholesterol synthesis in the liver and result in lower plasma LDL levels. Reductase inhibitors induce an increase in high-affinity LDL receptors. These effects increase both LDL fractional catabolism velocity and liver LDL precursor extraction (residual VLDL), thereby reducing plasma LDL deposits. In addition, there was also a slight decrease in plasma triglycerides and a slight increase in HDL cholesterol levels. This drug causes a decrease in cholesterol by increasing the number of LDL receptors, so that there will be a decrease in cholesterol levels (LDL) [17].

Paired t-test test results in Dose II group (0.52 ml/20 grams body weight) and Dose III (1.04 ml/20 grams body weight) showed that there were significant differences between total pre-test and post-test cholesterol levels with p=0.020 and p=0.002 which means that Dose II and Dose III have the effect of reducing total cholesterol levels. However, the Paired t-test test group Dose I (1.04 ml/20 grams body weight) showed no significant difference between total pre-test and post-tests cholesterol levels with p=0.091 which means that Dose I did not give a decrease in total cholesterol effect. On the One-Way ANOVA test, it was obtained p=0.000 ($p<0.05$) so that it can be said that there was a significant difference in the reduction in total cholesterol in male mice (*Mus musculus L.*) based on the five treatment groups. Because there are significant differences, then the Post Hoc test is continued.

Based on the significance value of the Post Hoc test in table 2 shows that the decrease in total cholesterol levels between group, dose III with dose I and dose III with dose II had a significant difference ($p<0.05$). This shows that the dose reduction effect of cholesterol levels with Dose I is very different as well as in Dose III with Dose II. Unlike the case with the Dose I and Dose II groups which showed a non-significant difference ($p> 0.05$), which means that the effect of reducing cholesterol levels in Dose I and Dose II was not different, therefore pindang patin gave a decrease in blood cholesterol levels in male mice (*Mus musculus L.*) in Dose II and Dose III, and statistically the most significant is Dose III.

This is supported by Sukarsa's research which states that omega-3 fatty acids from fish can reduce the biochemical components of blood rats such as cholesterol. The result was that rats fed dietary casein saturated fatty acid (control) obtained total cholesterol levels of 63.11 mg/dl, in rats fed a diet with a relative percentage of omega 3 fish 10% obtained total cholesterol levels of 56.10 mg/dl, and in rats fed a diet with a relative percentage of omega 3 fish 30% obtained total cholesterol levels of 46.22 mg/dl [18]. This is in accordance with the theory that patin fish meat has good nutritional content. In patin fish meat there are fatty acids free of omega-3 which are useful in suppressing cholesterol in the blood. The benefits of patin fish for health are characterized by a lower fat content than other types of fish, especially essential fatty acids docosahexaenoic acid (DHA) 7.74% and Eicosapentaenoic acid (EPA) 0.31%. Both types of omega-3 fatty acids are usually produced from types of fish that live in cold water, such as salmon, tuna and sardines [7].

In addition to omega-3, the amino acid content in patin fish, like arginine is one of the factors that might influence the decline in total cholesterol levels in this study. According to research by Borsheim et al who administer 11 grams of essential amino acid supplements twice a day for 16 weeks. The results of this study showed that amino acid supplements reduced plasma triglycerides (TG) (<0.001), total cholesterol (p=0.048) and VLDL ($p<0.001$) [19].

Not only patin fish can reduce total cholesterol, but other herbs and spices which are the basic ingredients for making pindang patin have an effect on decreasing total cholesterol, such as garlic, red chili and pineapple. This is supported in the research of Wignjosoesastro, Arieselia and Dewi which states that the administration of garlic at a dose of 125 mg/kg, 250 mg/kg body weight, and 500 mg/kg body weight affects the prevention of hypercholesterolemia in rats that have been given garlic and exposed to a high cholesterol diet [20]. In the research of Rahmi, Aria and Rahmi, it was found that the ethanol extract of red chili (*Capsicum annuum L*) fruit was proven to reduce cholesterol levels of hypercholesterolemia experimental animals, with variations in the dose given did not affect blood cholesterol levels [21]. In the Putri, Hermayanti and Fathiyah studies were found Pineapple fruit extract results can reduce LDL levels, triglycerides, and total cholesterol male white rats dyslipidaemia, can increase HDL levels of male white rats dyslipidaemia, and the use of effective pineapple extract dose in repairing lipid profiles to near normal is a dose of 4 g/head/day [22].

From table 3, the Positive Control group obtained the results of Paired t-test with p=0.001 which means that there is a significant difference between the total pre-test cholesterol level and the post-tests on Positive Control. In the Paired t-test in the Dose III group, the results showed that there were significant differences between LDL pre-test and post-tests levels with p=0.014, which means that Dose III had the effect of decreasing LDL levels. In contrast to the results of Paired t-test Dose I and Dose II groups which showed no significant difference between LDL pre-test and post-tests levels with p=0.065 and p=0.071 which means that Dose I and Dose II did not give an effect on LDL reduction.

In the One-Way ANOVA test, it was obtained p=0.000 ($p<0.05$) so that it can be said that there were significant differences in the reduction of LDL male mice (*Mus musculus L.*) based on the five treatment groups, then continued the Post Hoc test.

Based on the Post Hoc test in table 4, the decrease in LDL levels between Doses I group with Dose II, Dose I group with Dose III and Dose II and Dose III groups showed a non-significant difference ($p>0.05$) which means the effect between the three doses was no different Therefore, it can be said that pindang patin gives effect on decreasing blood LDL of male mice (*Mus musculus L.*) in Dose III, and statistically the most significant is Dose III.

This is supported by the study of Micallef and Garg, who stated that omega 3 supplementation was given for 21 days in people with hyperlipidaemia with a standard diet, the result was a decrease in serum LDL cholesterol by 12.5% [12]. This is in accordance with the theory that the clinical effect from omega-3 fatty acids in lowering blood cholesterol levels is thought to be due to its effect on the mechanism of the production of lipoprotein transport in the liver which is secreted into the blood. Unsaturated fatty acids, especially omega-3, can inhibit VLDL synthesis and consequently LDL production decreases. High levels of VLDL and LDL secreted can cause cholesterol deposits in the blood, because VLDL and LDL are transport proteins that carry triglycerides, cholesterol and phospholipids from the liver to the entire tissue. Whereas HDL will actually transport cholesterol into the liver, then it is broken down into bile acids and removed through body excretion [23]. In addition to the content of omega 3, the oleic acid contained in patin fish is proven to reduce blood LDL levels. This is supported by Haryanti's research, which states that giving avocado meat containing the oleic acid can significantly reduce LDL levels ($p<0.05$) [24]. Decreasing LDL levels by the oleic acid may affect structural functions, namely the cell membrane as a transduction signal and regulating function, which is maintaining the moisture of the membrane so as to maintain the function of the LDL receptors that are in the cell membrane. This can speed up the cycle of taking cholesterol. Furthermore, LDL cholesterol from the circulation gets more into the liver cells and LDL cholesterol in the circulation decreases [25].

In this study, it was obtained the results that pindang patin has the effect of reducing blood total cholesterol and LDL levels so that people can use pindang patin as an alternative to prevent hypercholesterolemia.

In this study, the results of cholesterol induction (pre-test results) were not homogeneous/different in each sample so that this was a weakness of the study, besides measuring triglycerides and HDL blood levels of male mice (*Mus musculus L.*), so it was unknown how is the other mechanism for patin to reduce total cholesterol. In addition, because there was no HDL value, there was no ratio of total cholesterol/HDL or LDL/HDL, so further research was needed.

5. Conclusions

The conclusion that can be drawn in this study is that pindang patin gives effect to lower total cholesterol and LDL blood levels in male mice (*Mus musculus L.*), with the best reduction effect, namely at dose III (1.04 ml/20 grams body weight).

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