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Okra Powder Effect to Low Density Lipoprotein and Triglycerides in Diabetic Wistar Rat

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ABSTRACT

This study aimed to find out the effect of okra powder treatment on the changes of the concentration of the low density of lipoprotein (LDL) and triglycerides in diabetic Wistar rats. The research was an experimental research with the re-post test control group design. The research subjects were 15 furrow Wistar rats. The experiment animals were made diabetes by injecting alloxan 140 mg/kg. The subjects were divided into three groups: group 1 (moderate dosage of 100 mg/kg), group 2 (high dosage of 200 mg/kg), and group 3 (control). The intervention was administered in 14 days. The data analysis used the paired T-test to test the subjects before and after the treatment, and the One-Way ANOVA test was used to test the difference between the groups. The research results indicated that the treatment with okra powder had a significant effect on the concentration of LDL in group 1 (from 38.60 ± 6.693 to 24.80 ± 2.86 , $p=0.005$) and in group 2 (from 39.00 ± 7.483 to 24.80 ± 2.490 , $p=0.004$); on the other hand, there happened a significant increase in control group (from 42.20 ± 3.347 to 71.40 ± 1.817 , $p=0.000$). The research result of triglycerides concentration indicated a significant effect before and after the treatment with okra powder in group 1 (from 125.40 ± 13.6 to 53.20 ± 24.056 , $p=0.000$) and in group 2 (126.80 ± 14.20 to 49.20 ± 11.735 , $p=0.000$), and in control group experienced an increase though not significant (from 127.80 ± 18.53 to 163.20 ± 29.141 , $p=0.028$). The post hoc analysis indicated an effect of the moderate dosage decrease, and the high dosage of LDL did not show a significant difference of LDL ($p=0.800$) nor did the high dosage of triglycerides ($p=0.864$). Thus the okra powder could reduce LDL and triglycerides in diabetic Wistar rats.

CCS Concepts

- Social and professional topics → User characteristics

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Keywords

Okra; rats; LDL; Triglycerides.

INTRODUCTION

Diabetes mellitus (DM) is a chronic syndrome in which carbohydrate, protein and fatty metabolism disorders are caused due to lack of insulin hormone or insulin retention [1]

Diabetes Mellitus is one of the most common problems challenging public health in the 21st century. Metabolic disorders characterized by high blood sugar (hyperglycemia), or when the pancreas is unable to produce enough insulin (DM type 1) or the body can not effectively use insulin product (type 2 DM) [2].

Diabetes mellitus is usually characterized by hyperglycemia. Chronic hyperglycemia in diabetes mellitus is associated with long-term damage, dysfunction or failure of several organs, especially the kidneys, nerves, heart and blood vessels [3].

Hyperglycemia will aggravate and aggravate the formation of oxidation to produce Reactive Oxygen Species (ROS) through several mechanisms. ROS will increase the expression of Tumor Necrosis Factor- α (TNF- α) and exacerbate oxidative stress. TNF- α may lead to insulin resistance by decreasing autophosphorylation (auto-phosphorylation) from insulin receptors, substrate insulin receptor changes to insulin receptor tyrosine kinase activity inhibitors, insulin-sensitive glucose transporter (GLUT-4) decrease, increase fatty acid circulation, alter function β cells, increase triglyceride levels and decrease HDL levels [4].

Diabetes Mellitus can not be cured, but can be managed by compliance with four pillars of DM management that include health education, Medical Nutrition therapy, physical exercise and pharmacological therapy. The combination of the four pillars of DM handling is expected to overcome the incidence or death due to DM [5].

The use of natural materials as traditional medicine in Indonesia has been done by our ancestors since centuries ago and the return of public attention on the treatment of using natural ingredients known as "back to nature" is caused because traditional medicine has relatively few side effects compared to synthetic drugs [6].

Diabetes Mellitus (DM) prevalence in Indonesia is high. As one of the ten DM countries, the prevalence of DM in Indonesia has increased over the years. In 1983, the prevalence of DM in Indonesia was 1.63%, an increase of 5.7% in 2007 and is expected

to be 6.0% by 2030. Therefore, the prevalence of DM in Indonesia is potential to be higher than the available data [7].

Okra (*Abelmoschus esculentus*), often called lady's finger, is one of the vegetables grown in tropical and subtropical regions. Okra comes from India and is eaten as a vegetable in salads and soups and is usually used as an implantation in various countries. Okra is believed to lower blood sugar levels [8].

In Turkey, okra seeds are boiled with water, then the stewed water is usually drunk by patients with high blood sugar levels. So far, okra has been recognized as one of the plants for traditional Turkish medicine [9].

In studies conducted by Khatun et al in the year 2010, it is known that Viscous Soluble Dietary Fibers (VSDF) from okra can decrease the absorption of glucose in the intestine. Fiber content of the okra are 67.50% α -cellulose and 15.40% hemicellulose, of which two contents The VSDF has an anti-diabetic effect. The okra fruit containing the fiber helps to stabilize blood sugar by limiting the rate of sugar absorption in the intestinal tract, thereby decreasing postprandial blood glucose levels (2 h after meals) by reducing glucose diffusion and delaying carbohydrate absorption and digestion [10].

Research conducted by Sabitha et al. [11] to see the potential of okra as antidiabetes and antihyperlipidemia by using the fruits and seeds separated okra then made into a flour given to diabetic rats. The results obtained were diabetical significant rats ($P < 0.001$) increasing cholesterol, triglyceride, LDL, VLDL and decreased HDL levels compared with normal mice as controls.

Currently, research on the administration of okra powder on triglyceride concentrations and LDL concentrations in Aloxan-induced diabetes-induced wistar rats has not been done. Thus, this study aimed to find out the effect of okra powder treatment on the changes of the concentration of the low density of lipoprotein (LDL) and triglycerides in diabetic Wistar rats. The results are expected to provide additional types of herbal remedies that can be used to suffer Diabetes Mellitus.

2. METHODOLOGY

2.1. Research Type

This study is done using design pre-post test with control group design.

2.2. Location and Time of Study

This research was done from March 2016 until April 2016. The manufacture of okra flour is done in Nutrition Culinary Laboratory of Faculty of Public Health Unhas. Adaptation and animal treatment is done in Biopharmaceutical Laboratory of Hasanuddin University. The examination of LDL and Triglyceridaheawan concentration is done in Makassar Health Laboratory. Examination of macro and micro nutrients at Hasanuddin University's Animal Feed Chemicals Laboratory and Antioxidant Antioxidant Inspection at the Bogor Agricultural Institute's Bogor Study Center for Biopharmaceutical Laboratory.

2.3. Research Sample

Subjects in this study were male Wistar rats with body weight of 150-200gram (adult body weight). Samples used were taken at random from the affordable population. Sizing of sample was done based on the minimum size sample requirement of 5 set by WHO [12]. The treatment was done in 2 groups and 1 control group with total of 3 groups, so the total sample size was 15 rats. However, to avoid the exclusion criteria, an addition of 5 rats

sample was done so the total subjects used were 20 rats. The division of rats in each group was done by simple random by marking the rat's tail and withdrawal based on lucky number selection.

2.4. Materials and Sample Preparation

a. alloxan

Preparation of Diabetes Mellitus mice by inducing with single dose alloxan 140 mg / kg bb intraperitoneal (injection performed in the abdominal cavity).

b. Okra flour

A fresh standard size okra were used. Okra is processed by washing cutting and dried twice using microwave at high temperature for 10 minutes. Next the okra was blended and was smoothen using 80 mesh sieve. Okra flour given to mice once a day with medium and high dose.

c. Rats

All the rats that will be samples of the research are first adapted at Unhas Biopharmaceutical Laboratory for 1 month at room temperature (25-27°C) with adequate air and light ventilation. Rats are kept in plastic enclosures and covered with chaff. To ensure the rats are under Diabetes Mellitus condition, the following steps were carried out

ci. Weigh BB (weight) and measure the blood glucose concentration.

cii. Inject all mice with aloxan dose 140 mg / kg BB intraperitoneally (i.p), after 1 week measured fasting blood glucose level using glucometer.

d. Okra Flour Suspension

okra flour is given in the form of suspension in order to facilitate its administration to mice. Suspension is given orally using sonde. Suspension of okra powder was given to mice to see its effect on LDL concentrations and triglycerides in diabetic mice. The dosage of okra flour given to the treatment group was 100 mg / KgBW, the dose was categorized in moderate doses. For a high dose, the dose was 200 mg / Kg BW.

2.5. Analysis of Blood Samples

Samples of blood of experimental animals are analyzed to determine the concentration of LDL and triglyceride experimental animals

a. LDL analysis

blood specimens were left ½ - 1 hour, then dicentrifuge for 5-10 minutes at 3000 rpm to get the serum. Prepare the test tube / bio cup then insert the serum sufficiently (at least 500 μ L), then the sample is ready to be inserted into the tool ABX pentra C-200 for analysis.

b. Triglycerides Analysis

blood specimens were left ½ - 1 hour, then dicentrifuge for 5-10 minutes at 3000 rpm to get the serum. Prepare the test tube / bio cup then insert the serum sufficiently (at least 500 μ L), then the sample is ready to be inserted into the tool ABX pentra C-200 for analysis. The reagent needle / reagent sample took 290 μ L triglyceride reagents after it was incubated for 2 minutes then the needle again took 3 μ L samples and then mixed by steerer then incubated for 5 minutes then read on 2 wavelengths 510 nm and 700 nm.

2.6. Data Analysis

1. Test the normality of the variables by using the Shapiro-Wilk test for a small sample ($n \leq 50$)

2. Normal data distribution

a. Data analysis measured pre and post LDL and triglyceride concentrations using paired t test.

b. The analysis for the test differs between the groups with an ANOVA or ANOVA one-way ANOVA, if there is a significant difference followed by the Tukey-LSD post hoc test

3. RESULT AND DISCUSSION

3.1. Okra Flour Nutrient Content

The results of testing the nutrient content of okra flour conducted was conducted in the Laboratory of Animal Feed Chemicals Hasanuddin University and is listed in Table 1. From the test results of nutrient content, it is observed that okra flour has high crude fiber content, Vitamin A and also Vitamin C high. In addition, antioxidants also contained in the tested okra. Testing of antioxidant content in okra flour was done at Bogor Agricultural Institute's Bogor Agricultural Research Center Laboratory. Based on Table 1, it is observed that the okra flour contained antioxidant flavonoids, phenols, and tannins.

Table 1. Okra Flour Nutrient Composition (Abelmoschus esculentus) per 100 g

Nutrient Content	Total
Water	8.46 g
Protein	19.03 g
Fat	3.16 g
Coarse Fiber	10.59 g
Carbohydrate	61.24 g
Phosphor	58 mg
Vitamin A	360 mg
Vitamin C	214 mg
Potassium	36 mg
sodium	14 mg
22	42.84 ppm
Flavonoid	2.6 mg
Phenol	11.2 mg
Tannin	16 mg

3.2 Effect of Okra Flour on LDL Concentrations and Triglycerides

4 this study 15 male rats wistar rats was used as a subject that is divided into 3 groups of 5 each. The study group consisted of 28 up 1 moderate dose of okra powder (100 mg / kg BW), group 2 high dose of okra flour (200 mg / kg BW) and group 3, control group. Intervention is done for 14 days. Adaptation and testing was done for 1 month. measurements of fasting blood sugar of rats was taken. Next, the rats was into diabetes by inducing alloxan that causes the rat pancreatic β cells to be damaged. Once declared diabetes, then rats examined LDL and triglyceride levels (pre test) by taking blood from rats through the tail.

a. The Effect of Okra Flour on LDL Concentrations

LDL concentration was measured 2 times before (pre) and post intervention for the divided 3 groups. The results of pre and post measurements of LDL concentration blood in rats is shown in table 2. In group 1 the average LDL concentration decreased by 38.34%, for group 2 also decreased LDL concentration by 36.41%

and for control group 3, there was increased LDL concentration by 69.1%.

Table 2. Mean LDL Concentrations of Rats

Groups	Mean LDL concentration		% Change
	Pre mean \pm SD	Post mean \pm SD	
1 (Moderate Dose)	38.60 \pm 6.693	24.80 \pm 2.864	38.34
2 (High Dose)	39.00 \pm 7.483	24.80 \pm 2.490	36.41
3 (Control)	42.20 \pm 3.347	71.40 \pm 1.817	69.1

Then, the LDL concentration data of the experiment 4 animals were tested for difference between the groups with One Way Anova test. The test was performed to see the difference of LDL concentration between treatment groups. Meanwhile, to assess the difference before and after treatment (mean \pm SD) in each study group was done by paired T-test. The difference can be seen in table 3. Results in Table 3 showed that based on One-Way ANOVA test results, there are significant differences between groups on the condition after treatment with p value equal to 0.000 < 0.05. This suggests that changes after treatment, between groups experienced a marked difference. This difference can be seen in conditions before and after treatment in each group tested by paired T-test.

The result of paired T-test showed that the group experiencing significant differences in the conditions before 7 d after treatment occurred in all groups, in group 1 ($p = 0.005 < 0.05$), group 2 ($p = 0.004 < 0.05$) and group 3 ($p = 0.000 < 0.05$). However, groups 1 and 2, those given the intervention of octopus powders, gave a very good effect of decreasing LDL concentrations. While the control group experienced a total increase in LDL in which the group was not subjected to okra flour intervention.

The mean Δ value was used to see the 5 difference in LDL concentrations before and after treatment in the treatment group and control group. In the 1st group treated with medium-dose aerospace suspension, a decrease in LDL concentrations with a large difference was 14.8. For group 2 treated with high-dose okra flour suspension, a decrease in LDL concentrations with a large difference was 14.2 and group 3 as a control group experienced an increase in LDL concentrations with large difference is 29.2.

Table 3. Differential Test Results of LDL Concentrations

Groups	LDL concentration		Δ mean	p
	Pre mean \pm SD	Post mean \pm SD		
1 (Moderate Dose)	38.60 \pm 6.6 93	24.80 \pm 2.8 64	13.8 \pm 3.8 29	0.005 *
2 (High Dose)	39.00 \pm 7.4 83	24.80 \pm 2.4 90	14.2 \pm 5.1 67	0.004 *
3 (Control)	42.20 \pm 3.3 47	71.40 \pm 1.8 17	29.2 \pm 3.1 97	0.000 *
p	0.607**	0.000**	0.002*	

n=5, *paired T-test, *One-way Anova

To know the different groups significantly then the follow-up test of anova that is post hoc was done and the result is shown in Table 4. Based on Table 4, it is observed that groups 1 and group 2 differ significantly with the control group. We know that group 1 and group 2 are the groups given the okra flour intervention in which the okra flour causes the LDL concentration to decrease

while the control group which is not in the powdered okra concentration of LDL increases. While in the posthoc test results, group 1 and group 2 did not significantly differ from LDL-lowering effects in both groups.

Table 4. Post Hoc Test LDL Concentrations

Group	1(Moderate Dose)	2(High Dose)	3(Control)
1(Moderate Dose)		0.8000	0.007*
2(High Dose)	0.800		0.002*
3(Control)	0.007*	0.002*	

b. The Effect of Okra Flour on Concentration Triglycerides

After the rat tried through maintenance and adaptation for 1 month, pre triglyceride concentrations were measured. Then after treatment on each group for 14 days, the post triglyceride concentration were measured. The results of pre and post triglyceride concentration in the rats is shown in Table 5.

In group 1 the average triglyceride concentration decreased by 57.4%, for group 2 also decreased the triglyceride concentration of 61.2% and in the control increased triglyceride concentration of 27.7%.

Table 5. Mean Experimental Trial of Animal Trials

Groups	Mean triglyceride concentration		% Change
	Pre mean±SD	Post mean±SD	
1(Moderate Dose)	125.40±13.6	53.20±24.056	57.4
2(High Dose)	126.80±14.20	49.20±11.735	61.2
3 (Control)	127.80±18.53	163.20±29.141	27.7

The data of triglyceride concentration of animal try to test the difference between the groups with One Way Anova test. This test was done to see the difference of triglyceride concentration between treatment groups. While to assess the difference before and after treatment (mean ± SD) in each study group was done by paired T-test. The difference can be seen in the Table 6. Results in Table 6 shows that based on One-Way ANOVA test results, there are significant differences between groups on the condition after treatment with p value equal to 0,000 <0.05. This suggests that changes after treatment, between groups experienced a marked difference. This difference can be seen in conditions before and after treatment in each group tested by paired T-test.

The paired T-test results showed that all groups experienced significant differences in the conditions before and after treatment, in group 1 (p = 0,000 <0.05), group 2 (p = 0,000 <0.05) and group 3 (p = 0,028 <0.05).

Groups 1 and 2 which are the groups given intervention have a decreasing effect of good concentrations after the administration of okra powder. While the control group experienced an increase in triglyceride concentration in which the group was not subjected to okra flour intervention. Δ Mean values were used to see the magnitude of the difference in triglyceride concentrations before and after treatment in the treatment group and control group. In the 1st group treated with medium-dose okra flour suspension, a decrease in triglyceride concentration with a large difference was 72.2. For group 2 treated with high-dose okra flour suspension, a decrease in triglyceride concentration with a large difference was

77.6 and group 3 as the control group experienced an elevated triglyceride concentration with a large difference of 35.4

Table 6. Different Test Results of Triglycerides Concentrations

Groups	LDL concentration		Δ mean	p
	Pre mean±SD	Post mean±SD		
1(Moderate Dose)	125.40±13.6	53.20±24.056	72.2±14.23	0.005*
2(High Dose)	126.80±14.20	49.20±11.735	77.6±7.301	0.004*
3 (Control)	127.80±18.53	163.20±29.14	35.4±23.56	0.000*
p	0.971**	0.000**	0.003*	

n=5, *paired T-test, **One-way ANOVA

To know the different groups significantly then the follow-up test of anova is post hoc that can be seen in the table 7. Based on table 7, it is observed that groups 1 and group 2 differ significantly with the control group. We know that group 1 and group 2 are the groups given the okra flour intervention in which the okra powder causes the triglyceride concentration to decrease while a control group that was not given wheat flour triglyceride concentration increased. While on the result of group posthoc test 1 and group 2 did not significantly differ from the effects of triglyceride reduction in both groups.

Table 7. Post Hoc Result

Group	1(Moderate Dose)	2(High Dose)	3(Control)
1(Moderate Dose)		0.864	0.011*
2(High Dose)	0.864		0.004*
3(Control)	0.011*	0.004*	

* significant p ≥ 0.05

3.3 Overall Discussion

The results showed that average LDL concentrations of mice after exposure to diabetes were higher than the normal LDL concentrations of LDL > 27 mg / dL. However, after 14 days were treated with okra flour in Group 1 the mean LDL concentration decreased by 38.34% and statistically significant (p = 0.005 <0.05), for group 2 also decreased LDL concentration by 36.41% and statistically significant (p = 0,004 <0.05) in the control group had an increase of LDL concentration of 69,1 which was statistically significant (p = 0,000 <0.05).

For triglyceride concentration values of rats after expressed high diabetes which exceeded the concentration of triglyceride normaltriglyceride > 120 mg / dL. Namun, after 14 days were treated with okra flour in group 1 that is the dose of okra dose medium showed decreased triglyceride concentration of 57.4% and statistically significant (p = 0,000 <0.05). The same was also shown in group 2, which was high doses of okra concentration where the triglyceride concentration decreased to 61.2% and statistically significant (p = 0.001 = 0 <0.05). In group 3, control group showed that triglyceride concentration increased by 27.2% and there was influence before and after measurement (p = 0,028 > 0,05) because in this group was not given treatment any. From the test results of nutrient content of okra flour is fiber that can help lower triglyceride and LDL level so as to prevent the occurrence

of cardiovascular disease (Ngoc et al., 2008) it is indicated that fiber content in okra **11** is 10,59 gram. There are some decreasing mechanism LDL cholesterol lev **11** by dietary fiber, among others, fibers are able to **6** alter the absorption and metabolism of bile acids; fiber can modify lipid absorption and metabolism; Short chain fatty acids as a result of fiber **20** mentation affect the metabolism of cholesterol and lipoproteins; and fiber may alter insulin or other hormone concentrations or tissue sensitivity to hormones [13]. Food fibers will stay in the digestive tract in a relatively short time so that the absorption of nutrients is reduced. In addition, foods that contain relatively high fiber will provide a sense of satiety because the complex carbohydrate compositio **13** s to stop appetite, resulting in lower consumption of food. Foods with relatively high crude fiber content typically contain low calories, low sugar and fat levels that **can** help you lose weight, reduce obesity and heart disease. The antioxidant content of okra can lower LDL concentration and increase triglyceride concentration, including flavonoids, phenols, tannins, vitamins A and C. flavonoid content to okra flour is likely to cause a decrease in LDL concentrations in mice [14]. Where flavonoids can improve blood vessel endothelial function, can reduce the sensitivity of LDL so as to reduce total cholesterol levels of triglycerides, and increase HDL by inhibiting the enzyme HGM CoA reductase [15]

Tanin is able to prevent oxidative stress by inhibiting fat oxidation (Dorland, 2002). The tannin is a class of polyphenol compounds that act as antioxidants so that through the mechanism of tannin antioxidants can prevent the **6** crease of total LDL by increasing the mechanism of LDL into **bile acids** and increase **the** excretion of **bile acids** through the feces

4. CONCLUSION

Based on the findings of this study, it can be concluded that Okra flour can reduce LDL concentrations significantly in diabetic wistar rats. In addition, Okra flour may decrease **19** triglyceride concentrations significantly in diabetic wistar rats. There was no significant difference in the effect of LDL and triglyceride reduction on moderate and high-dose okra flour.

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