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The Effects of Okra (*Abelmoschus esculentus* L.) Immersion Water on Changes in High-Density Lipoprotein and Low-Density Lipoprotein Levels in High Fat Diet Wistar Rat Model

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ABSTRACT

Background. The mortality rate due to cardiovascular disease increases; one of the effects of hypercholesterolemia conditions, dyslipidemia, hypertriglyceridemia, and metabolic syndrome, is not handled properly and thoroughly. This phenomenon becomes the factor that the researchers took this research topic. This study aims to determine okra (*Abelmoschus esculentus* L.) immersion water on an increase in high-density lipoprotein (HDL) levels and decrease in LDL (low-density lipoprotein) levels in high-fat diet model Wistar rats.

Methods: This experimental study used 30 Wistar male rats (*Rattus norvegicus*) divided into six groups, namely normal rats (P1), positive control (P2), standard (P3), dose 1 treatment groups (P4), dose 2 treatment groups (P5), and dose 2 treatment groups (P6). The data were collected by measuring HDL and LDL levels before and after receiving okra (*Abelmoschus esculentus* L.) immersion water. The data were analyzed by paired sample t-test, Wilcoxon test, ANOVA test, and Kruskal-Wallis test. **Results:** This research showed a significant difference in the average level of HDL and LDL in all treatment groups (P4, P5, and P6) after induction of *Abelmoschus esculentus* L. in a high-fat diet rat model ($p < 0.05$).

Conclusion: It can be concluded that the administration of okra (*Abelmoschus esculentus* L.) immersion water could be used to increase HDL levels and decrease LDL levels in high-fat diet model Wistar rats ($p < 0.05$).

1. Introduction

Hyperlipidemia is a lipid metabolism disorder characterized by an increase in low-density lipoprotein (LDL) levels and a decrease in high-density lipoprotein (HDL) levels.¹ World Health Organization in 2008 stated that the global prevalence of hyperlipidemia in adults increased by 37% for men and 40% for women.² The people died of cardiovascular disease was 17.5 million and accounted for 31% of the causes of death globally in 2017.³

Many factors can affect the high amount of total cholesterol levels. It can be related to the increasing age and type of women calamine after menopause. The incidence of menopause is frequently associated with

increased cholesterol. It is because women who are still not menopausal are still protected by the hormone estrogen, which can prevent plaque formation in the arteries and increase HDL (high-density lipoprotein), while postmenopausal women experience more LDL and TG than pre-menopause.⁴

Unhealthy dietary habits, coupled with a lack of physical activity, can increase plasma lipid levels, called hyperlipidemia.³ Hyperlipidemia that is not handled correctly can increase the risk of forming atherosclerosis, which is the hardening of the arteries to block blood flow to the heart, which will later become the basis for cardiovascular diseases, myocardial

infarction, coronary thrombosis, and cerebrovascular diseases such as stroke.⁵ Consumption of vegetables that contain flavonoids and are rich in fiber can reduce cholesterol levels by increasing the excretion of bile.⁶ One of the vegetables that have high fiber content and flavonoids is okra (*Abelmoschus esculentus* L.).⁷ This study was aimed to determine okra (*Abelmoschus esculentus* L.) immersion water on an increase in HDL levels and decrease in LDL levels in high-fat diet model Wistar rats.

2. Methods

This research used an experimental laboratory design by using a pre-test post-test control group. The subjects were 30 Wistar rats (*Rattus norvegicus*) taken by stratified random sampling. The independent variable in this research was okra immersion water of various concentrations. The dependent variable was the HDL and LDL levels. The controlled variable was a trial animal with the same strain, gender, weight, age, feed, and individual cage.

This study used 30 male Wistar rats (*Rattus norvegicus*) aged between 2 - 3 months with \pm 150-200 g of weight and physical health as the sample. The exclusion criteria were rats that showed a decrease in physical condition during the adaptation phase. The rats were adapted for 7 days at Pusat Antar Universitas (PAU) building, Central Laboratory for Food and Nutrition Study, Universitas Gadjah Mada, Yogyakarta with adequate feeding and standard pellet rats diet, drinking, and lighting at room temperature.

A total of 30 male Wistar rats were divided into 6 groups (n = 5) consisting of group 1: P1= normal rats, group 2: P2 = positive control, group 3: P3 = standard (hyperlipidemia rats + simvastatin 0.72mg/200KgBW/day), group 4: P4 = (hyperlipidemia rats + 0.18 gram of okra soaked in 3.6mL water/200 gr/BW/day), group 5: P5 (hyperlipidemia rats + 0.36 gram of okra soaked in 3.6mL water/200 gr/BW/day) and groups 6: P6 (hyperlipidemia rats + 0.36 gram of okra soaked in 3.6mL water/200 gr/BW/day). The treatment group received an okra immersion water on single dose per day by using sonde method for 28 days consecutively.

The material and tools used in this study included Okra (*Abelmoschus esculentus* L.), rat cages, gloves, scales, microtube, test tube racks, microhematocrit pipettes, timers, and spectrophotometers. Metabolic measurements used 5 mL sinus vein orbital blood collected in EDTA blood tubes after 8-10 hours fasting. HDL-Chol and LDL-Chol were measured by enzymatic colorimetric techniques based on the manual auto-analyzer procedure using a Dyasis Reagen kit.

The basic data of research with continuing variables were tested for normality using Shapiro-Wilk. If the data were not normal, then the transformation and normality tests were performed. Numerical variables were presented in the form of the mean (mean) and standard deviation (SD). Comparison of average HDL level and LDL level used paired t-tests and Wilcoxon-test. One Way ANOVA test was used to analyze the effectiveness of okra immersion water differences in HDL levels in the standard group compared to the treatment groups (P4, P5, P6). Kruskal-Wallis test was used to analyze the effectiveness of okra immersion water differences in LDL levels in the standard group compared to the treatment groups (P4, P5, P6). The results of statistical analysis were significant if the *p*-value was <0.05.

3. Results

This study is to determine the effect of okra on decreasing LDL levels and increasing HDL levels. In comparison to the positive control group, HDL levels increased after treatment compared to before the okra intervention (table 1). Table 2 shows that LDL levels after treatment decreased compared to before giving okra intervention compared to the positive control group. Levels of HDL after treatment in the standard group also increased and LDL levels are decreased compared to before giving okra.

The results of the Post-hoc test Tukey HSD data processing showed that there were significant differences between HDL levels on the standard group (P3) and the treatment group (P4, P5) with a *p*-value < 0.05. There was no significant difference between the standard group (P3) and the treatment group P6 with a *p*-value > 0.05 (Table 3).

The results of the Post-hoc test Tukey HSD data processing showed that there were significant differences between LDL levels on the standard group (P3) and the treatment group (P4) with a p-value < 0.05.

There was no significant difference between the standard group (P3) and the treatment group P5 and P6 with a p-value > 0.05 (Table 4).

Table 1. The average levels of pre-test and post-test HDL

Groups	Average levels of blood HDL (mg/dL) ± SD		p-values
	Pre-test	Post-test	
P1 (normal rats)	68.98 ± 3.61	66.10 ± 1.83	0.243 [#]
P2 (positive control)	26.12 ± 1.23	24.96 ± 3.67	0.130 [#]
P3 (standard)	24.49 ± 1.17	60.14 ± 1.36	0.043 [*]
P4 (dose 1 treatment)	25.57 ± 1.03	36.45 ± 3.67	0.002 [#]
P5 (dose 2 treatment)	23.94 ± 1.94	42.12 ± 1.70	0.000 [#]
P6 (dose 3 treatment)	24.35 ± 1.03	55.74 ± 2.38	0.000 [#]

The data were expressed as mean ± SD (Standard Deviation). Data would be significantly different if the p-value was <0.05. Confidence Interval is 95%.

* Wilcoxon Test

Paired T-Test

Table 2. The average levels of pre-test and post-test LDL

Groups	Average levels of blood LDL (mg/dL) ± SD		p values
	Pre-test	Post-test	
P1 (normal rats)	24.50 ± 2.21	27.00 ± 2.20	0.243 [#]
P2 (positive control)	75.70 ± 1.04	76.96 ± 2.07	0.130 [#]
P3 (standard)	76.95 ± 1.41	38.12 ± 1.38	0.043 [*]
P4 (dose 1 treatment)	76.12 ± 2.18	59.63 ± 1.73	0.002 [#]
P5 (dose 2 treatment)	75.15 ± 2.27	42.16 ± 1.49	0.000 [#]
P6 (dose 3 treatment)	77.23 ± 1.73	34.80 ± 2.67	0.000 [#]

The data were expressed as mean ± SD (Standard Deviation).

Data would be analyzed with Paired T-Test with a significant difference if the p-value was <0.05.

Table 3. The results of the inter-group comparison test of HDL levels

Groups	Inter-group	p values*
P3	P4	0.000
	P5	0.000
	P6	0.052
P4	P3	0.000
	P5	0.010
	P6	0.000
P5	P3	0.000
	P4	0.010
	P6	0.000
P6	P3	0.052
	P4	0.000
	P5	0.000

*Post Hoc Test Tukey HSD with p<0.05 showed a significant difference. Confidence Interval is 95%.

Table 4. The results of the inter-group comparison test of LDL levels

Groups	Inter-group	p values*
P3	P4	0.000
	P5	0.059
	P6	0.353
P4	P3	0.000
	P5	0.000
	P6	0.000
P5	P3	0.059
	P4	0.000
	P6	0.002
P6	P3	0.353
	P4	0.000
	P5	0.002

*Post-hoc test Tukey HSD with $p < 0.05$ showed a significant difference. The confidence interval is 95%.

4. Discussion

Low-density lipoprotein (LDL) is a lipoprotein with a diameter of 18-30 nm, having a density of 2.029-2.063 g / mL. LDL cholesterol contains 34-45% cholesterol, 4% triglycerides, 22-26% phospholipids and 22-26% protein.⁸ Low-density lipoprotein (LDL) is frequently referred to as bad cholesterol as high LDL levels are associated with cardiovascular disease, one of which is the occurrence of clogged arteries when LDL levels are too high.⁹

High-density lipoprotein (HDL) mainly contains protein. The HDL is produced in the liver and small intestine. High-Density Lipoprotein takes cholesterol and phospholipids in the blood and delivers them to other lipoproteins to be transported back or excreted from the body.¹⁰ To assess the high and low HDL, a standard number from the National Centers for Environmental Prediction (NCEP) ATP III was used, namely low HDL levels, < 40 mg / dL and high HDL levels, ≥ 60 mg / dL.

This research showed the difference between pre-test and post-test HDL-Chol levels in rats' high-fat diet model induction of okra immersion water ($p < 0.05$) on P4, P5, and P6 groups analyzed by paired t-test. Table 2 showed significant differences in LDL-Chol level in rats' high-fat diet model induction of okra immersion water ($p < 0.05$) on P4, P5, and P6 groups with paired t-test for each standard group and the treatment group with okra immersion water ($p < 0.05$).

The okra flour (*Abelmoschus esculentus* L.) contains high fiber and good bioactive content, one of which is flavonoids. The flavonoid content in fresh okra is quercetin in the amount of 60-75%. Quercetin

compounds can play a role in preventing the oxidation process of LDL by capturing free radicals. The dose of okra flour 36 g/200 g BW per day could increase HDL levels with a result of $P < 0.05$.¹¹

In the previous research, giving okra (*Abelmoschus esculentus* L.) could reduce cholesterol in the blood ($P < 0.05$).¹² The flavonoid content in the ethanol extract of okra fruit also has a good working mechanism by controlling simvastatin positively in reducing total cholesterol levels. The mechanism of action is by inhibiting the activity of the 3-hydroxy-3-methylglutaric-CoA enzyme, which can cause inhibition of cholesterol synthesis.¹³ Apart from flavonoids, the ethanol extract of okra fruit has alkaloids, polyphenols, tannins, and steroids. This tannin compound will coat the intestinal wall and bind to proteins in the body so that fat absorption can also be inhibited.¹⁴

Okra (*Abelmoschus esculentus* L.) is rich in flavonoid compounds that have antioxidant activity.¹⁵⁻¹⁶ Previous studies have shown that the flavonoid content in okra could normalize blood glucose.¹⁷ The main flavonoid compounds, which are okra (isoquercitrin and quercetin), can reduce blood glucose levels, improve glucose tolerance and reduce triglyceride levels by increasing lipoprotein lipase (LPL) activity.¹⁸⁻¹⁹ Research conducted by Ngoc in 2008 revealed that extracts of okra plants (*Abelmoschus esculentus* L.) could significantly reduce total cholesterol and triglyceride levels in rats.²⁰

5. Conclusion

Based on the result of the research, it can be concluded that the administration of okra

(*Abelmoschus esculentus* L.) toward rats' high-fat diet model could increase HDL levels and reduce LDL levels.

6. References

1. Velayutham P, Anand B, Dongmin L. Green tea catechins and cardiovascular health. *Curr Med Chem.* 2008; 15(18): 1840-50.
2. WHO. Raised cholesterol situation and trends. Global Health Observatory (GHO) data. 2008.
3. WHO. Cardiovascular disease (CVDs). WHO Media Centre. 2017.
4. Kurniawan F, Slamet S, Kamilla L. 2019. Relationship between age, gender, and obesity with total cholesterol levels of teachers of SMAN 1 Sei Raya Pontianak. *Jurnal Laboratorium Khatulistiwa.* 2019; 2(2): 72-5.
5. Goldstein JL. Hyperlipidemia in coronary heart disease. *Dep Med Genet Univ Wash Seattle.* 1973:52.
6. John D, Brunzell MD. Hypertriglyceridemia. *New Eng J Med.* 2007; 357: 1009-17.
7. Axe A. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench. in streptozotocin induced diabetic rat. *J Pharm BioAllied Sci.* 2011.
8. Mishra N, Kumar D, Rizvi SI. Protective effect of *Abelmoschus esculentus* against alloxan-induced diabetes in Wistar strain rats. *J Diet Suppl.* 2016; 13, 634-46.
9. Ratnayake WMN, Galli C. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. *Ann Nutr Metab.* 2009; 55: 8-43.
10. Stancu C, Sima A. Statins: mechanism of action and effects. *J Cell Mol Med.* 2001; 5: 378-87.
11. Febriyatna A, Widyawati A. Okra flour (*Abelmoschus esculantus*) decreased the ratio of LDL levels to HDL in hypercholesterolemic rats. *Indonesian J Nutr Diet (Jurnal Gizi dan Dietetik Indonesia).* 2017; 5(1): 17-22.
12. Waskito RH, Purwanto NH, Supriani A, Rosyidah NN. Changes in blood cholesterol after giving okra fruit soak (*Abelmoschus*

- esculantus* (L). *J Ners Com.* 2020; 11(1): 43-51.
13. Sutjiatmo AB, Sukandar EY, Sinaga R, Hernawati R, Vikasari SN. Anti-cholesterol effect of ethanol extract of cerme leaves (*Phyllanthus acidus* (L.) Skeels) on female Wistar rats. *Kartika J Farmasi.* 2013; 1-7.
14. Arief MI, Novriansyah R, Budianto IT, Harmaji MB. Potential of karamunting flower (*Melastoma malabathricum* L.) against total cholesterol and triglyceride levels in hyperlipidemic male white rats induced by propylthiouracil. *Unlam.* 2012.
15. Khomsug P, Thongjaroembuangam W. Antioxidative activities and phenolic content of extract okra (*Abelmoschus esculentus* L.). *Res J Biol Sci.* 2010; 5: 310-3.
16. Sudheesh S, Presanakumar G, Vijayalakshmi NR. Hypolipidemic effect of flavonoids from *Solanum melongena*. *Plant Foods Hum Nutr Abstracts.* 1997; 51(4): 321-30.
17. Ngoc TH, Ngoc QN, Van ATT, Phung NV. Hypolipidemic effect of extracts from *Abelmoschus esculentus* L. (Malvaceae) on tyloxapol-induced hyperlipidemia in mice. *Mahidol Univ J Pharm Sci* 2008; 35: 42-6.
18. Anjani PP, Damayanthi E, Rimbawan R, Handharyani E. Potential of okra (*Abelmoschus esculentus* L.) extract to reduce blood glucose and malondialdehyde (MDA) liver in streptozocin-induced diabetic rats. *J Gizi Pangan.* 2018; 13(1).
19. Herowati R. Antidiabetic activity of okra fruit (*Abelmoschus esculentus* (L) Moench) extract and fractions in two conditions of diabetic rats. *Indo J Pharm.* 2020; 31(1).
20. Elkhalfifa AEO, Alshammari E, Adnan M, Alcantara JC, Awadelkareem AM, et al. Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. *Molecules.* 2021; 26(3): 696.