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THE IN-VIVO POTENTIAL OF AVOCADO JUICE (*Persea americana* Mill) ON CHOLESTEROL LEVELS

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Abstract: An increase in blood cholesterol levels is one of the triggers for atherosclerosis. The use of natural components, such as avocado fruit juice, is an alternative to lowering cholesterol levels. Avocados contain hypolipidemic agents such as beta-sitosterol, omega-9, oleic acid, pantothenic acid (vitamin B5), niacin (vitamin B3), vitamins A, C, and E, cysteine amino acid, selenium, and fruit fiber. The study's goal was to examine the potential effects of providing avocado juice on cholesterol levels. The pretest-posttest design with a control group is used in this type of experimental research. The research sample consisted of 36 male white rats aged 2-3 months which were divided into 3 normal, positive, and negative control groups, and 3 treatment groups with avocado juice doses of 3.5mL, 4mL, and 4.5mL. The research phase involved acclimatization and generation of hypercholesterolemic rats by inducing quail egg yolk orally and assessing the effectiveness of avocado juice orally once a day for 7 days. Cholesterol levels were tested three times: after acclimation, induction by oral quail egg yolk, and administration of avocado juice. After egg yolk induction, the average cholesterol level in all treatment groups was 139.1 mgdL-1, 164.5 mgdL-1, and 190.1 mgdL-1, respectively. Following the administration of avocado juice, there was a decrease in all treatment groups with mean cholesterol levels of P1, P2, and P3, or 68.7 mgdL-1, 64.1 mgdL-1, and 42 mgdL-1. These findings are supported by test results from One Way Anova, which showed that after the administration of avocado juice, there were variations in cholesterol levels between groups. The study's finding is that consuming avocado juice can lower cholesterol levels.

Keywords: cholesterol levels, avocado juice, hypolipidemic agent, quail egg yolk

Introduction

Health problems cannot be separated from degenerative diseases, which have various contributing variables, including lifestyle, environmental conditions, physical activity, stress levels, and the consumption of high-fat diets that are not fiber-balanced. These disorders will cause degenerative diseases such as cardiovascular disease, coronary heart disease, and stroke (Yani, 2015). Based on Basic Health Research (Riskesdas) data, there was an increase in heart disease in 2018 by 1.5% compared to 2013 which was only 0.5% and this disease causes the death of 17.7 million people per year (Tarmizi, 2023). Survey Sample Registration System (SRS) in 2014 in Indonesia proved that coronary heart disease was the highest cause of death after stroke, which was 12.9% (Primadi, 2017). Coronary heart disease is typically caused by atherosclerosis, which develops as a result of plaque

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formation in the coronary arteries that deliver oxygen to the heart, causing hardening and thickening of blood vessel walls (Ghaniet al., 2016) (Hanifah et al., 2021).

The progress of atherosclerosis can be influenced by several major risk factors, including plasma total cholesterol concentration, distribution of cholesterol among lipoproteins, blood pressure, oxidized LDL, smoking habits, age and homocysteine levels. In addition to the basic components, various other factors, such as alcohol consumption, lifestyle, gender, stress level, and heredity, can cause atherosclerosis (Sargowo, 2015). Total cholesterol levels that experience an increase in the blood so that they exceed normal values are called hypercholesterolemia (Sinulingga, 2020). The increase in cholesterol levels can be caused by disturbances in cholesterol metabolism (Mayasari & Rahayuni, 2014). One sign of abnormal cholesterol metabolism is an increase in low density lipoprotein (LDL) levels. If the LDL level in plasma is high, it will be taken up by macrophages and foam cells form in the intima of the arteries through scavenger receptors as the start of atherosclerosis (Fairudz & Nisa, 2015). One of the treatments for hypercholesterolemic disorders that can be done is to change patterns of nutritional intake and food. (Sinulingga, 2020). This can be done by consuming natural food ingredients that can reduce cholesterol levels, one of which is avocado juice. Consumption of natural ingredients is also carried out to reduce the side effects of using the drug simvastatin as a cholesterol lowering which is popular in Indonesia (Hariadini et al., 2020).

The ethanol extract of avocado flesh contains alkaloids, flavonoids, tannins, saponins and steroids/triterpenoids (Di Stefano et al., 2017) (Simarmata et al., 2018) (Shivering et al., 2018). Active ingredients in avocados such as omega-9 oleic acid, phantenin, niacin (vitamin B3), beta sitosterol, vitamins C, E, A, pantothenic acid, MUFA group, oleic acid, amino acids and fiber can help lower total and LDL cholesterol levels, also increase HDL levels (Setianingsihet al., 2017). According to Wardani's research (2014), patients with mild hypercholesterolemia who consumed avocados for 3 days had a 17% decrease in total blood cholesterol levels and a 22% decrease in LDL and triglycerides, as well as an increase of 11% HDL. Based on Anggraheny's research (2007), showed that administration of avocado juice at doses of 2mL/day, 3mL/day and 4mL/day for 14 days in rats was shown to significantly reduce cholesterol levels with the highest reduction in cholesterol levels in mice given 4mL/day treatment. day. Based on the preliminary test, it was shown that giving 4 mL of fresh avocado flesh within 7 days could lower cholesterol levels compared to giving 3 mL and 5 mL. Therefore, the volume variations carried out in this study were 3.5 mL administration; 4 mL and 4.5 mL of avocado juice (Persea americanaMill). The aim of the study was to determine the potential of avocado juice on cholesterol levels with a concentration of 3.5 mL; 4 mL and 4.5 mL.

Materials and Method

This study is experimental research with a complete-random group design. The study's subjects were male white rats (Rattus norvegicus) weighing 200-250 grams and aged 2-3 months. The study was divided into six groups (Table 1), 3 control groups and 3 treatment groups, each with six rats. The tools used in the study were a photometer (Mindray BA88a), centrifuge, micropipette, hematocrit capillary tube, blender, and sonde needle. In contrast, the materials needed are avocado, BR-II standard feed, quail egg yolk, simvastatin, and cholesterol reagent (Diasys FS). The research method begins with measuring cholesterol levels before acclimation. Acclimatized for 7 days, then measured cholesterol levels. The next stage was giving quail egg yolks to each treatment except the normal

control group, 2 mL every day for 7 days. Furthermore, cholesterol levels were measured to determine that all groups had hypercholesterolemia. The avocado juice test was conducted for 14 days, according to the treatment table. The negative group received only 5% Na-CMC; the positive control group received simvastatin; and the treatment groups received avocado juice in 3.5 mL, 4 mL, and 4.5 mL, respectively. The final cholesterol level measurement was performed 14 days later. Examination of cholesterol levels using serum samples and a photometer (Mindray BA88a). Blood sampling through the supraorbital vein. Administering quail egg yolk, CMC, simvastatin, and avocado juice orally using a sonde. Data on cholesterol levels are shown in the table as means for each group. Research data were analyzed using SPSS, normality test using Shapiro-Wilk, and homogeneity test with Levene test. Data analysis continued with One Way Anova and Post Hoc LSD test.

Table 1. Group of Research

Group	Description
Normal Control (NC)	Normal rats without any treatment
Negative Control Group (-C)	Rats were administered 2mL of quail egg yolk each day (7 days) and Na-CMC 5%.
Positive Control Group (+C)	Rats were administered 2mL of quail egg yolk each day (7 days) and simvastatin 0,18 mg/ 200gW each day (14 days).
Treatment 1 (T1)	Rats were administered 2mL of quail egg yolk each day (7 days) and 3.5 mL avocado juice each day (14 days).
Treatment 2 (T2)	Rats were administered 2mL of quail egg yolk each day (7 days) and 4 mL avocado juice each day (14 days).
Treatment 3 (T3)	Rats were administered 2mL of quail egg yolk each day (7 days) and 4.5 mL avocado juice each day (14 days).

Result and Discussion

Table 2. The Average of cholesterol level (mgdL-1) on each research group

Group	Average of cholesterol level (mgdL-1)				Decline (%)
	Before Acclimation	After Acclimation	After Administration of quail egg yolk	After Administration of Avocado Juice	
NC	41.5a	58.7	-	-	-
-C	34.8	52.5	177.6	176.4b	0,68%
+C	28.8	49.7	156.5	63.7c	59,29%
T1	35.5	47.3	139.1	68.7c	50,61%
T2	21	48.9	164.5	64.1c	61,03%
T3	20	43.9	190.1	42a	77,90%

According to the results table 2, all treatment groups experienced a decrease in cholesterol levels. Giving 3.4 mL (T1) and 4 mL (T2) of avocado juice can lower cholesterol levels similar to the positive control group, whereas giving 4.5 mL of avocado juice has cholesterol levels similar to the normal control group. The results of the one-way ANOVA test obtained a significance value of 0.007,

so there was a significant difference. While Pos Hoc LSD knew that the T1 group was not significantly different from the T2 group (0.179), the T1 and T2 groups were significantly different (0.031 and 0.040) from T3.

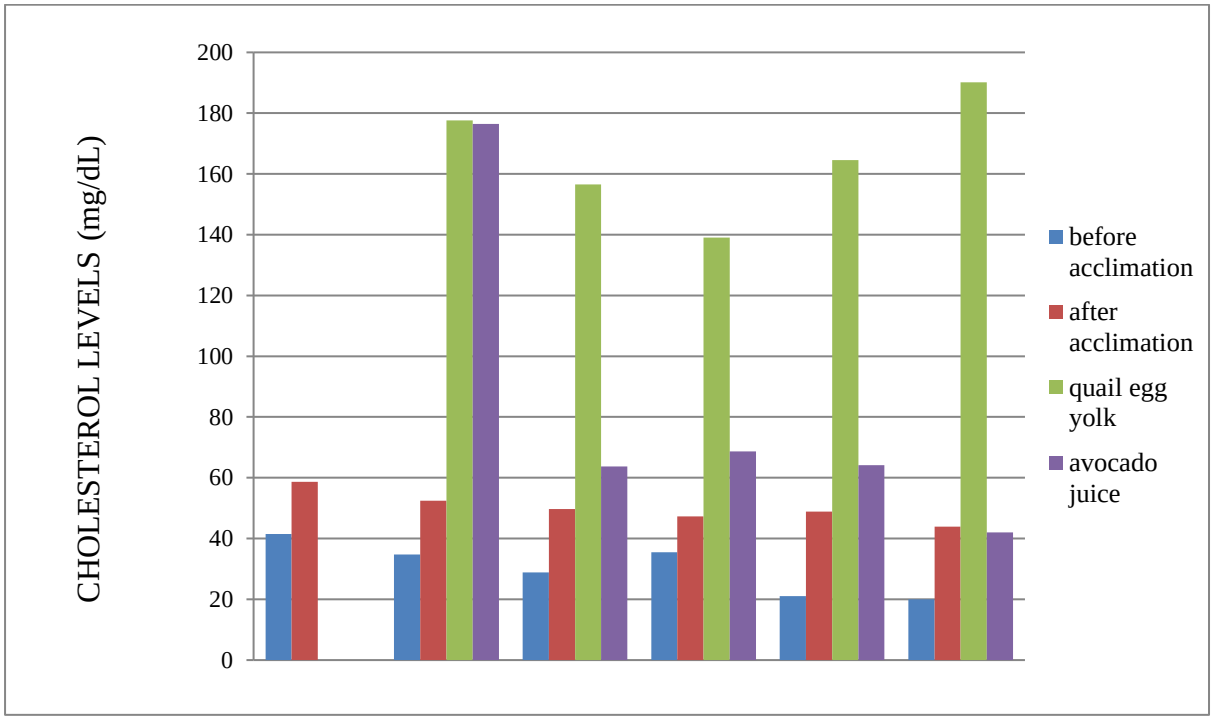

Figure 1. Graph of the average of cholesterol level (mgdL-1) on each research group

The graph shows that after being given quail egg yolks, all groups' cholesterol levels increased to hypercholesterolemia, but after being given avocado juice treatment for 14 days in each group, cholesterol levels decreased into the normal range. According to The Clinical Laboratory Parameters the normal range cholesterol levels around 24-73 $mgdL^{-1}$ (Giknis, M L A., Clifford, 2008).

The study found that the average cholesterol level of white rats decreased in all treatment groups (T1, T2, and T3). This decrease occurred because avocados are hypolipidemic agents, which contain active ingredients including beta-sitosterol, unsaturated fatty acids, Omega-9 oleic acid, pantothenic acid (Vitamin B5), niacin (Vitamin B3), Vitamin A, Vitamin C, Vitamin E, the amino acid cysteine, selenium, and fruit fiber are contained in avocado flesh (Figueroa & Segura-carretero, 2017a, 2018b) (Tremocoldi *et al*., 2018) (Salazar-lópez *et al.*, 2020).

Beta-sitosterol compounds have been shown to block cholesterol absorption and promote cholesterol excretion in the intestine, resulting in lower cholesterol levels. Beta-sitosterol is able to inhibit the absorption of cholesterol which is produced from the liver. The reduction in cholesterol levels occurs by inhibiting the hydroxylase and reductase enzymes used to convert HMG CoA into HMG *mevalonate*, so that the production of cholesterol will be inhibited. Monounsaturated fatty acids *(MUFA)* such as oleic acid does not stimulate cholesterol synthesis in the body so that total cholesterol in the blood does not increase. The omega-9 oleic acid found in avocados can keep the

LDL receptor active, hence increasing the cholesterol-taking cycle (Elbadrawy & Shelbaya, 2013) (Wardani, 2014) (Bhuyan *et al.,* 2019).

Niacin decreases cholesterol levels by inhibiting fatty acid release as well as the synthesis of fatty acids and triglycerides (Wardani, 2014) (Setianingsih *et al.,* 2017). Pantothenic acid can prevent lipid metabolism (Wardani, 2014). Free radicals can be stabilized by vitamin A. Vitamin C contained in avocados helps in the hydroxylation reaction for the formation of bile acids so that it will increase cholesterol excretion and lower cholesterol levels in the blood (Setianingsih *et al.,* 2017). Vitamin C also protects cells from free radical damage and promotes total cholesterol metabolic waste removal in the blood (Grosso *et al.*, 2013). Selenium binds to plasma proteins, forming selenoproteins that can inhibit LDL oxidation (Wardani, 2014) (Setianingsih *et al.*, 2017). Studies show that β-glucan contained in fiber can reduce LDL (Low-Density Lipoprotein) cholesterol levels without affecting HDL (High-Density Lipoprotein) or triglyceride levels (Kaczmarczyk et al., 2012). The best dose in this study that was able to reduce cholesterol levels in white rats was 4.5 mL. Based on the results of the study it can be seen that the higher the dose of avocado given the more active substance contained in the avocado given will be more effective in lowering cholesterol levels. The statistical test results also showed that there were differences between the control group and the treatment group. Likewise, there were differences between the treatment groups, the T1 and T2 treatments were significantly different from the T3 group. These results indicated that there was a decrease in cholesterol levels after administration of avocado juice. High doses of avocado juice may lower cholesterol levels, but further research is needed.

Conclusion

It has been demonstrated that consuming avocado juice lowers cholesterol levels; the more avocado juice consumed, the greater the reduction in cholesterol levels.

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