Hypolipidemic Effect of Avocado Peel (Persea americana Mill.) Extract in Rats with Dyslipidemia

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Abstract. Dyslipidemia is a disorder of lipid metabolism and one of the modifiable risk factors for cardiovascular disease. The prevalence of dyslipidemia based on raised total cholesterol in the world was 39%, of which 37% for men and 40% for women. Cholesterol-lowering drugs have been reported to have many side effects. One alternative medicine in reducing blood cholesterol levels by utilizing the potential of the avocado peel. This research was experimental with a pre-posttest control group design. This research was used 30 Sprague Dawley male rats divided into 6 groups, namely the normal control group (CG1), negative control (CG2), positive control (CG3) and 3 treatment groups (TG) who received avocado peel extract (APE) at 75, 150, and 300 mg/200gr bodyweight rats. Data were tested using a one-way ANOVA. The results showed that APE significantly reduced the levels of LDL-C (P<0.05) was 17.82±2.62, 29.62±2.00, and 36.33±4.47 mg/dl (TG1, TG2, TG3). Decreased triglycerides levels (P<0.05) of 1.62±4.17, 14.05±5.16, and 29.67±5.79 mg/dl (TG1, TG2, TG3). The increased levels of HDL-C (P<0.05) were 29.67±5.79, 22.63±4.82, and 34.35±2.72 mg/dl (TG1, TG2, TG3). Avocado peel (Persea americana Mill.) extract showed a hypolipidemic effect by reducing LDL-C, triglycerides, and increasing HDL-C levels in rats with dyslipidemia.

Keywords: Dyslipidemia, Persea americana Mill., Avocado peel extract

1. Introduction
Dyslipidemia is a disorder of lipid metabolism manifested by elevated levels of total cholesterol, low-density lipoprotein Cholesterol (LDL-C), triglycerides, and low of high-density lipoprotein Cholesterol (HDL-C) [1]. Dyslipidemia can contribute to the development of atherosclerosis, risk factors for cardiovascular disease [2]. The prevalence of dyslipidemia based on raised total cholesterol > 5.0 mmol/l in the world was 39%, of which 37% for men and 40% for women [3]. Pharmacological therapies for dyslipidemia can be given cholesterol-lowering drugs like a statin.[2] The problems today, cholesterol-lowering drugs have been reported to have many side effects such as...
disorders of the muscles, liver, and kidneys [4]. Currently, there is a tendency for people to consume alternative herbal medicine, due to changes in lifestyle back to nature [5]. Reasons for the increased popularity of alternative herbal medicines may include their relatively cheap, easy to obtain, and low side effects [1].

One of the alternative medicines to reduce cholesterol levels in the blood is by utilizing the potency of avocado peel. The recent study, potential avocado for hypolipidemic is still dominated from the flesh, seed, and leaves. Irma Antasionasti et.al showed a study that the avocado peel by methanol extract and fraction has the highest antioxidant activities, total phenolics contents, and total flavonoid contents [6]. The similar study by Wuri Marsigit showed that profil phenolics compound in avocado consist of catechin, hydroxybenzoic acid, flavonol and procyianid, and hydroxycinnamic acid [7]. Another study explain the extract of avocado peel that contains antibacterial compound such as flavonoid, saponin, and alkaloid [8]. The content of avocado peel may have the same work in reducing cholesterol levels in the blood. Therefore, the authors want to research the hypolipidemic effect of avocado peel (Persea americana Mill.) extract in rats with dyslipidemia.

2. Methods

2.1 Experiment Design
This research was experimental with a pre-posttest control group design. This research was used Sprague Dawley male rats divided into 6 groups, namely the normal control group who received aquades and standard feed (CG1), negative control who received high lipids feed and (CG2), positive control who received simvastatin 0.18 mg (CG3) and 3 treatment groups (TG) who received avocado peel extract (APE) at 75, 150, and 300 mg/200gr BW rats. This research has been approved by ethical committee from the Medical Faculty, Swadaya Gunung Jati University, Cirebon. Number 4/EC/FKUGJ/I/2020.

2.2 Criteria and Sample Size of Research Subject
The inclusion criteria of samples were male Sprague Dawley rat, age 2 months old, complete body organs, bodyweight of 200-250 grams. Meanwhile, the exclusion criteria were rats with disabilities and experience anatomic abnormalities. The formula for the number of samples used Federrer Formula: (t-n) (n-1) ≥ 15, [t: number of treatment groups, n: number of samples in each group]. The total sample was 30 rats.

2.3 Research Procedure
The research procedure was started by preparing 30 male Sprague Dawley rats divided into 6 groups, then preparing the tools and materials used. The rats that have been prepared will be adapted for 7 days ad libitum. Furthermore, the avocado peel is extracted which will be given to the research subjects using the maceration method.

The rats will be made dyslipidemia by given high lipids fed for 7 days. On day 14 the rats were fasted for 12 hours to take blood samples from the orbital plexus for pretest. The levels of LDL-C and HDL-C were determined by CHOD-PAP method (Cholesterol Oxidase Peroxidase Aminoantipyrine Phenol) and the measurement of triglycerides levels was determined by the enzymatic colorimetric assay by 3-Phosphatase Oxidase-Paminophenazone (GPO-PAP). Furthermore, the avocado peel extract and high lipids feed were given to 3 treatment groups (TG1, TG2, TG3) for 14 days. On day 28, the rats fasted again for 12 hours and their blood serum was taken to get LDL-C, triglycerides, and HDL-C as posttest values. Then the data obtained will be analyzed.

2.4 Data Analysis
First, all data obtained were analyzed statistically with the normality test by Shapiro Wilk test. Data for control groups (CG) were statistically analyzed by independent T-test. Hypolipidemic activity data were statically analyzed by one-way analysis of variance (ANOVA).
3. Results and Discussion

Table 1. Effect of avocado peel extract on lipid profile of rats

| Groups                      | APE dose  | LDL-C (mg/dl) | Triglycerides (mg/dl) | HDL-C (mg/dl) |
|-----------------------------|-----------|---------------|-----------------------|---------------|
| CG 1 (Normal control)       | -2.75 ± 2.25 | 5.03 ± 3.34*  |                       | 0.57 ± 2.43   |
| CG 2 (high lipid fed)       | 8.50 ± 4.08* | 14.40 ±1.00*  |                       | -1.73 ± 1.78  |
| CG 3 (Simvastatin 0.18 mg)  | 43.02 ± 1.82* | -31.07 ± 4.00* | 40.49 ± 1.99*        |               |
| TG 1                        | 75        | -17.82 ± 2.62* | -1.62 ± 4.17         | 6.29 ± 1.68*  |
| TG 2                        | 150       | -29.62 ± 2.00* | -14.05 ± 5.16*       | 22.63 ± 4.82* |
| TG 3                        | 300       | -36.33 ± 4.47* | -29.67 ± 5.79*       | 34.35 ± 2.72* |

Data expressed as mean ± standard deviation; n=6, analyzed by independent T test for control group and ANOVA test for treatment group. *P<0.05; significant difference to the pre and posttest. CG, Control Group. TG, Treatment Group. APE, Avocado Peel Extract. LDL-C, Low-density Lipoprotein Cholesterol. HDL-C, High-density Lipoprotein Cholesterol.

Normal control (CG1) indicated that the average levels of LDL-C, triglycerides, and HDL-C on rats were given standard feed and aqudest. The negative control (CG2) statistically significantly increases the LDL-C and except in triglycerides the decreased HDL-C levels but not significant. In this research, high lipids feed was made from quail egg yolk 10 g/day and cooking oil. Egg yolks contain lots of cholesterol, can increase the amount of cholesterol in the serum [9]. Rats that have been fed high lipids will be prepared according to the group. Simvastatin 0.18 mg statistically significantly reduces levels of LDL-C, triglycerides, and increases HDL-C. Statin can reduce the LDL and triglycerides by inhibiting of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase).[10] Statins vary markedly in their ability to raise the level of HDL-C.[11] Another study showed that treatment with atorvastatin, fluvastatin, pravastatin, and simvastatin results in an elevation in HDL-cholesterol that varies between 3% and 12% in type IIA and type IIB hyperlipidemia [12]. In this research, simvastatin 0.18 mg get from conversion doses from 10 mg/kg human to rats by Laurence and Bacharach 1964 [13]. The hypolipidemic effect of APE was evaluated at doses at 75, 150, and 300 mg/200 g BW rats.

3.1 LDL-C

![Average of LDL Cholesterol (mg/dl)](image)

Figure 1. The average levels of LDL-C between pre and post-test during treatment of APE. TG 1= Treatment Group 1; TG 2= Treatment Group 2; TG 3= Treatment Group 3.
Figure 1 explains the average levels of LDL-C decreased from 76.82 to 58.99 mg/dl between pre and posttest on TG1 (APE 75 mg/200grBW rats), TG 2 (APE 150 mg/200grBW rats) decreased from 74.05 to 44.42 mg/dl, and TG 3 (APE 300 mg/200gr BW rats) decreased from 75.66 to 39.33 mg/dl. All of this group statistically significant (P<0.05) (Table 1). The mechanism for reducing LDL-C levels comes from the content of catechins and flavonoids in an avocado peel [7]. The content of avocado having a high phenol and also containing flavonoids which play a role in antioxidants.[6] The results of the phytochemical test by Ernawati's study also obtained the same results where the content of avocado peel contains flavonoids, alkaloids, and saponins [8]. Catechins increases the work of the LDL-C receptor by binding to the LDL-C receptor. Furthermore, the increased expression of LDL-C receptors will trigger an increase in LDL-C metabolism so that LDL-C levels in plasma decrease [14]. Catechins along with carbohydrates and gut microbiota can increase fat metabolism by activating Adenosine Monofosphate Protein Kinase (AMPK) [15]. AMPK works in the liver by inactivating Acetyl-CoA Carboxylase to reduce fat synthesis, and decreasing and even inactivating the enzyme HMG-CoA reductase so that the cholesterol ordering process decreases [16].

Flavonoids have been reported to reduce cholesterol synthesis by the enzyme 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA reductase) [17]. Besides, flavonoids can inhibit the secretion of Alpha lipoprotein-B100 (Apo-B100) into the intestine, thereby reducing the amount of Apo-B which forms very-low-density lipoproteins (VLDL) and LDL-C [18]. Research Onoja et al. Shows that avocado peel can reduce oxidative stress (free radicals) in the heart and kidney of rats [19]. Panche et al. have similar studies that describe the function of polyphenols to reduce the LDL oxidation process [20].

3.2 Triglycerides

![Average of Triglyceride (mg/dl)](image)

Figure 2. The average levels of triglycerides between pre and posttest during treatment of APE. TG 1 = Treatment Group 1; TG 2=Treatment Group 2; TG 3=Treatment Group 3.

The Figure 2 explain the average levels of triglycerides between pre and posttest. TG 1 decreased from 126.78 to 125.17 mg/dl, TG 2 decreased from 126.08 to 112.03 mg/dl, and TG3 decreased from 129.86 to 100.18 mg/dl. The TG 1 (APE 75 mg/200grBW rats) decreased levels of triglycerides but not significant. The groups of TG 2 and TG 3 group statistically significant with (P<0.05) (Table 1).

The dietary fat before it was emulsified with the help of bile salts was mostly triglycerides. Triglycerides are broken down into monoglycerides and free fatty acids and form micelles. Micelles
help the absorption of fat in the intestinal lumen and after absorption, it will be reconstituted into triglycerides in the form of chylomicrons [21]. In this study, the decrease in triglycerides levels was due to the chemical content of avocado peel including polyphenols, flavonoids, and saponins.

Saponins inhibit cholesterol absorption in the intestine by forming complex insoluble bonds with cholesterol, binding to bile acids, and increasing the binding of cholesterol by fiber [11]. This study is in line with Ali et al. which shows that saponins can increase the excretion of triglycerides through feces in reducing cholesterol levels in the blood [22].

3.3 HDL-C

The average levels of HDL-C (Figure 3) in TG 1 was increased from 25.17 to 31.46 mg/dl. TG 1 increased from 23.81 to 46.44 mg/dl. TG 3 increased from 25.17 to 59.49 mg/dl. All of this group statistically significant ($P<0.05$) (Table 1).

![Average of HDL Cholesterol (mg/dl)](image)

**Figure 3.** The average levels of HDL-C between pre and posttest during treatment of APE. TG 1 = Treatment Group 1; TG 2=Treatment Group 2; TG 3=Treatment Group 3.

The increase in HDL levels is due to the chemical content of avocado peel, namely flavonols. Research states that flavonols increase HDL levels by increasing the activity of Paraoxonase 1 (PON 1 [23]. The similiar study by Manolescu et al. that the enzyme Paraoxonase 1 (PON 1) after leaving the hepatocyte cells will combine with HDL. This incorporation process aims to keep HDL from being oxidized, so that HDL levels will increase [24].

4. Conclusion

Avocado peel (*Persea americana* Mill.) extract (APE) showed a hypolipidemic effect by reducing LDL-C, triglycerides, and increasing HDL-C levels in rats with dyslipidemia. This effect could be attributed to the phenolic content, flavonoids content, antioxidant activity. Further research is required to identify the components of APE that are responsible for the observed hypolipidemic effects.

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