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Effect of Juice Berry Fruit (*Antidesma bunius* (L.) Spreng.) on Total Cholesterol Levels in Mice (*Mus musculus* Linnaeus, 1758) with Hypercholesterolemia

Pemberian Jus Buah Buni (*Antidesma bunius* (L.) Spreng) terhadap Kadar Kolesterol Total pada Mencit Jantan (*Mus musculus* Linnaeus, 1758) Hiperkolesterolemia

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Abstrak

Hiperkolesterolemia dipengaruhi oleh konsumsi lemak berlebih. Untuk menurunkan kadar kolesterol dalam darah bisa dilakukan terapi dengan obat dan tanpa obat. Salah satu tanaman yang berpotensi menurunkan kadar kolesterol yakni tanaman buni (*Antidesma bunius* (L.) Spreng.). Penelitian ini bertujuan mengetahui pengaruh jus buah buni dan konsentrasi jus yang paling efektif menurunkan kadar kolesterol total pada mencit jantan hiperkolesterolemia. Penelitian ini menggunakan desain acak lengkap terdiri dari 5 kelompok masing-masing diperulang 5 kali. Hewan coba diberikan Makanan Diet Tinggi Lemak (MDTL) yang memiliki komposisi propyltiourasil (PTU) pada dosis 1,8 mg/200g BB yang disuspensikan dalam Pure Grain Alcohol (PGA) 2% dan kuning telur puyuh 10 mL/KgBB. Perlakuan terdiri dari kontrol negatif (pakan standar); kontrol positif (MDTL dan simvastatin 0,52 mg); P1 (MDTL dan 1 ml jus buah buni 20%); P2 (MDTL dan 1 ml jus buah buni 25%); dan P3 (MDTL dan 1 ml jus buah buni 30%). Perlakuan P1, P2, dan P3 diberikan MDTL selama 14 hari dilanjutkan dengan pemberian jus buah buni selama 14 hari. Hasil yang diperoleh berupa kadar kolesterol total dalam darah. Data dianalisis dengan uji One Way ANOVA dilanjutkan dengan uji Duncan pada tingkat kepercayaan $\alpha = 5\%$. Hasil penelitian menunjukkan bahwa pemberian jus buah buni pada konsentrasi 30% dapat menurunkan kadar kolesterol total mencit jantan yang hiperkolesterolemia.

Kata kunci: *Antidesma bunius* (L.) Spreng., hiperkolesterolemia, profil lipid

Abstract

*Hypercholesterolemia is influenced by excessive fat consumption. To lower blood cholesterol levels, both drug and non-drug therapy can be carried out. One plant that has the potential to lower cholesterol levels is the buni plant (*Antidesma bunius* (L.) Spreng.). This study aims to determine the effect of buni fruit juice and the most effective juice concentration in lowering total cholesterol levels in hypercholesterolemic male mice. This study used a completely randomized design consisting of 5 groups, each repeated 5 times. The experimental animals were given a High Fat Diet (MDTL) containing propylthiouracil (PTU) at a dose of 1.8 mg/200g BW suspended in 2% Pure Grain Alcohol (PGA) and 10 mL/KgBW of quail egg yolk. The treatments consisted of a negative control (standard feed); a positive control (MDTL and 0.52 mg simvastatin); P1 (MDTL and 1 ml of 20% buni fruit juice); P2 (MDTL and 1 ml of 25% berry juice); and P3 (MDTL and 1 ml of 30% berry juice). Treatments P1, P2, and P3 were administered MDTL for 14 days, followed by berry juice for another 14 days. The results were total blood cholesterol levels. Data were analyzed using a one-way ANOVA followed by Duncan's test at a confidence level of $\alpha = 5\%$. The results showed that administration of 30% berry juice reduced total cholesterol levels in male mice with hypercholesterolemia.*

Keywords: *Antidesma bunius* (L.) Spreng., hypercholesterolemia, lipid profile

INTRODUCTION

Advances in technology and modern lifestyles have led to unhealthy consumption patterns, including the increased consumption of junk food that is high in saturated fat and calories but low in essential nutrients such as protein, vitamins, and minerals [1]. Additionally, most Indonesians prefer to eat fried foods that contain saturated fat [2]. These unhealthy eating patterns can lead to diseases such as heart disease, diabetes, and stroke. Indonesia is one of the countries with the highest rates of cardiovascular disease [3]. One of the factors that causes cardiovascular disease is hypercholesterolemia [4]. Consuming excessive amounts of fat can contribute to hypercholesterolemia [5]. Saturated fats can cause blockages in blood flow to the heart and increase total cholesterol and LDL cholesterol levels [2].

In 2023, 37.4% of Indonesians aged 3 years and above regularly consumed fatty, high-cholesterol, and/or fried foods at least once a day [6]. In 2019, approximately 17.9 million people, or 32% of all deaths worldwide, were caused by cardiovascular disease [7]. Among people aged 25-34 years, 9.3% had hypercholesterolemia, while among those aged 55-64 years, the percentage reached 15.5% [8]. Based on gender, data on Indonesians aged 15 years and above showed LDL cholesterol levels above the standard limit. For women, the percentage is 4.4% of the total (17,438),

while for men it is 2.4% of the total (17,382) [9].

To lower blood cholesterol levels, treatment options can include pharmacological or non-pharmacological therapies [10]. Pharmacological therapy involves the use of antihypercholesterolemic drugs, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors, niacin, fibrates, bile acid sequestrants, and cholesterol absorption inhibitors [11]. Cholesterol absorption inhibitors, niacin, bile sequestrants, and fibrates are other commonly used pharmacological agents for treating hypercholesterolemia. However, these drugs have various adverse effects that can hinder treatment and quality of life [12]. Some of the adverse effects of these drugs include myopathy, loss of cognitive function, neuropathy, pancreatic and liver dysfunction, and sexual dysfunction [13]. Non-pharmacological treatments to lower LDL cholesterol levels include adopting a healthy lifestyle and following a balanced diet. In addition, consuming herbal plants can also help reduce hypercholesterolemia.

One plant that has the potential to lower cholesterol levels is the buni plant (*Antidesma bunius* (L.) Spreng.). Buni fruit contains flavonoids and organic acids. It also contains catechin, procyanidin B1, and procyanidin B2, which are basic forms of antioxidants. In addition, buni fruit also contains various organic acids, including tartaric acid, ascorbic acid, citric acid,

benzoic acid, malic acid, oxalic acid, lactic acid, and acetic acid [14]. Ascorbic acid and anthocyanin compounds play a role in lowering blood cholesterol levels. Ascorbic acid can help the hydroxylation process in the formation of bile salts. With increased bile salts, cholesterol excretion also increases, thereby reducing blood cholesterol levels [15].

The primary issue in this study is high total cholesterol levels in the blood, which are a key clinical risk factor for various cardiovascular diseases. Increased total cholesterol levels, especially those of the Low-Density Lipoprotein (LDL) fraction, can lead to the formation of atherosclerotic plaque on the walls of blood vessels, narrowing the lumen of the arteries and impairing blood flow to vital organs, such as the heart and brain. In response to the background information, researchers examined the effect of administering Buni Fruit (*Antidesma bunius* (L.) Spreng.) on total cholesterol levels in hypercholesterolemic mice (*Mus musculus* Linnaeus, 1758). This study aims to investigate the effect of administering buni fruit juice on the blood lipid profile of mice with hypercholesterolemia induced by a high-fat diet. Through the use of an animal model, this study aims to generate meaningful data regarding the effectiveness of buni fruit as a dietary intervention against hypercholesterolemia.

METHODS

The research team conducted this study from February to March 2025 at the Zoology Laboratory of Lampung University.

Material and Equipment

The equipment used in this study consisted of plastic containers measuring 30 x 25 x 10 cm, used as mouse cages, equipped with wire mesh to cover the containers, ensuring the mice could not escape. The researchers also equipped the cages with food and water containers, husks as bedding, and syringes for drawing blood from the mice. In addition, the researcher used a 1 ml syringe and a gastric tube to administer MDTL to the mice, along with a stirring rod, Erlenmeyer flasks, cannulas, mixers, measuring cups, scissors, mortars and pestles to make buni fruit juice, digital devices (Nesco Lipid) to measure total cholesterol levels, markers, cholesterol strips to detect cholesterol levels in the blood using the Nesco Lipid device, jars, analytical scales, and containers.

The materials used in this study were buni fruit (*Antidesma bunius* Linn) and distilled water, 25 male mice weighing 20-40 g, simvastatin, standard pellet feed, High-Fat Diet (HFD) consisting of propylthiouracil (PTU) 1.8 mg/200 g mouse body weight, Pure Grain Alcohol (PGA) 2%, and egg yolk 10 mL/kg body weight, tissue for cleaning mouse wounds, and alcohol as a material for cleaning wounds on mice.

Procedure

This study employed a completely randomized design (CRD), divided into five treatment groups with five replicates each. Researcher fed the test animals a high-fat diet (HFD) consisting of propylthiouracil (PTU) 1.8 mg/200 g mouse body weight suspended in 2% pure grain alcohol (PGA) and 10 mL/kg body weight quail egg yolk. The treatments consisted of control - (standard feed), control + (HFDF and simvastatin 0.52 mg), P1 (HFDF and 1 mL of 20% buni fruit juice), P2 (HFDF and 1 mL of 25% buni fruit juice), and P3 (HFDF and 1 mL of 30% buni fruit juice). Treatments P1, P2, and P3 were administered MDTL for 14

days and followed by buni fruit juice for an additional 14 days. The results obtained were the total cholesterol levels in the blood, which were then analyzed using a One-Way ANOVA test at a confidence level of $\alpha = 0.05$ and followed by a Duncan test at a confidence level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The results showed that buni fruit juice was able to reduce total cholesterol levels in the blood of mice with hypercholesterolemia induced by a high-fat diet (HFD). Table 1 presents the results of the analysis of average total cholesterol levels in mice.

Table 1. Mean Total of Blood Cholesterol Levels in Mice (Mean \pm SEM)

Treatment Group	Total Cholesterol Level (mg/dl)	
	Day 15	Day 30
K	104,20 \pm 27,59 ^a	81,6 \pm 25,43 ^c
K-	150,2 \pm 21,87 ^b	152 \pm 23,19 ^d
K+	150,4 \pm 13,84 ^b	71,6 \pm 2,51 ^{bc}
P1	148,4 \pm 20,40 ^b	52,6 \pm 5,59 ^{ab}
P2	147,2 \pm 15,36 ^b	39,4 \pm 9,52 ^a
P3	157 \pm 16,26 ^b	33 \pm 4,63 ^a

Note: Numbers followed by different supercript letters indicate significant differences at the 5% confidence level ($P < 0,05$).

- K = Control (fed only standard feed)
- K- = Negative Control (fed a high-fat diet)
- K+ = Positive Control (fed a simvastatin)
- P1 = Treatment Group 1 (given 20% concentrated buni fruit juice)
- P2 = Treatment Group 2 (given 25% concentrated buni fruit juice)
- P3 = Treatment Group 3 (given 30% concentrated buni fruit juice)

Based on ANOVA analysis with a significance level of $\alpha = 5\%$, the mean total cholesterol level in the blood of mice on day 15 after being fed a high-fat diet (HFD) showed a

significant difference between the control group and the treatment group. The control group (K) had an average cholesterol level of 104.20 mg/dl with a standard deviation of

27.59. Meanwhile, treatment group 3 (P3) recorded the highest average, namely 157 mg/dl. On day 30, the negative control group (K-) had the highest average cholesterol level, at 152 mg/dL, while treatment group 3 (P3) recorded the lowest level, at 33 mg/dL. In the negative control group, the average total blood cholesterol level of male mice on day 30 increased from 150.2 mg/dl to 152 mg/dl. Results demonstrate that the negative control group's cholesterol level increased but stayed higher than the control group's level of 81.6 mg/dL. On the other hand, the P1, P2, and P3 treatment groups exhibited a significant decrease in cholesterol levels, with P2 recording the lowest level at 39.4 mg/dL.

Based on the post hoc test results, it was found that total cholesterol levels on day 15 after MDTL administration differed significantly between the negative control (K-), positive control (K+), P1, P2, and P3 groups and the control group. The results indicate that MDTL administration can increase total cholesterol levels in male mice to the point of hypercholesterolemia. The presence of PTU caused an increase in total cholesterol levels in the blood of male mice after they received a high-fat diet consisting of 1.8 mg/200 g BW propylthiouracil (PTU) suspended in 2% Pure Grain Alcohol (PGA) and 10 mL/kg BW quail egg yolk. This antithyroid substance can damage the thyroid gland, thereby inhibiting the production of thyroid hormones. Thyroid

hormones play a role in lowering blood cholesterol levels by increasing LDL synthesis in the liver, which ultimately promotes increased cholesterol excretion from the circulation. When thyroid hormone levels decrease, the cholesterol catabolism process also decreases, causing an increase in blood cholesterol levels [16].

On day 30 (after administration of buni fruit juice), it was found that group K differed significantly from group K-. The results indicate that MDTL administration can increase total cholesterol levels in male mice. Meanwhile, group K also differed considerably from group K+, suggesting that simvastatin administration in group K+ can lower total cholesterol levels in male mice. The K+ treatment group did not differ significantly from the P1 group, indicating that the effect of buni fruit juice administration was equivalent to that of simvastatin administration. These findings suggest that buni fruit juice produces effects comparable to statin drugs, which are commonly used to reduce cholesterol levels. The P2 treatment group did not differ significantly from P3, indicating that there was no difference between administering 25% and 30% concentrations of buni fruit juice.

Buni fruit juice is known to contain ascorbic acid, organic acids, carbohydrates, flavonoids, phenolic acids, vitamins, minerals, and anthocyanins. The secondary metabolites found in buni fruit are thought

to have the ability and potential to lower total cholesterol levels in the blood of male mice used in the study. Ascorbic acid itself plays a role in reducing total blood cholesterol levels. These findings are consistent with previous studies examining the effects of mandai vinegar—a substance rich in ascorbic acid—on total cholesterol levels in rats. In those studies, an average decrease in total cholesterol levels in rat blood of 3.9 mg/dL was observed on day 14, which was attributed to ascorbic acid's ability to inhibit the increase in cholesterol derived from egg yolks [17].

Vitamin C, or ascorbic acid, has long been known as one of the antioxidants involved in the hydroxylation process to produce bile salts. If bile salt production increases, cholesterol excretion also increases, thereby reducing blood cholesterol levels. Cholesterol produced by the liver reacts with VLDL, then changes into Intermediate Density Lipoprotein (IDL). Next, IDL enters cells outside the liver through endocytosis and binds to cell membranes. Vesicles containing IDL then merge with lysosomes, where lysosomal enzymes break down the cholesterol esters in the LDL core. Cholesterol in the cell membrane is then transferred to HDL, converted into cholesterol esters, and then transferred to the HDL core, leaving unbound lipoproteins that are eventually converted into bile acids.

Flavonoids found in buni fruit are known to significantly increase the activity of

antioxidant enzymes such as Superoxide Dismutase (SOD) and catalase, while reducing lipid peroxidase levels, which leads to a decrease in cholesterol levels. Additionally, flavonoids can inhibit cholesterol production by reducing the activity of the enzyme acyl-CoA cholesterol acyltransferase (ACAT) in HepG2 cells, which is involved in the cholesterol esterification process in the intestines and liver. Flavonoids are also able to slow down the action of the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which is the primary enzyme in the cholesterol formation process. By inhibiting this enzyme, mevalonic acid production is reduced, thereby decreasing cholesterol synthesis in the liver [16]. Previous studies have shown that flavonoids can reduce the risk of hypercholesterolemia by increasing Apo A1 production, thereby increasing HDL levels. This increase accelerates the process of transferring excess cholesterol from tissues to the liver, thereby helping to prevent high blood cholesterol levels [18].

In general, cholesterol balance in the body is influenced by synthesis in the liver, absorption in the intestines, and excretion through bile. Physiologically, the liver plays a crucial role in regulating lipid homeostasis by synthesizing, storing, and distributing cholesterol through lipoproteins, including VLDL, LDL, and HDL. When HMG-CoA reductase enzyme activity increases, endogenous cholesterol synthesis in the

liver also increases, causing total cholesterol levels in the blood to rise. Conversely, bioactive compounds such as flavonoids and ascorbic acid found in buni fruit can inhibit the activity of this enzyme, which causes a decrease in the formation of new cholesterol. In addition, increased activity of antioxidant enzymes such as Superoxide Dismutase (SOD) and catalase helps reduce oxidative stress that can damage liver cells and trigger increased cholesterol synthesis.

CONCLUSION

Based on the research conducted, it was concluded that administering buni fruit juice (*Antidesma bunius* (L.) Spreng.) to male hypercholesterolemic mice can reduce total cholesterol levels by 30%.

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CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in the writing of this scientific paper.

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