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The effect of katuk leaf (*Sauropus androgynous* L. merr) extract administration on reducing blood triglyceride levels in male local rabbits induced with hypercholesterolemia

Idham Khalid[1*], Teuku Reza Ferasyi[2], Sugito[1], Nabila Latifa Hafizsha[3], Rasyida Ulfa[4], Rizky Fitriana[5], Ali Makmur[4]

[1] *Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[2] *Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[3] *Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[4] *Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[5] *Laboratory of Physiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
**Corresponding author: idhamkhalid157@usk.ac.id*

Abstract

This research aimed to study the effectiveness of katuk leaf extract in lowering blood triglyceride levels in male local rabbits treated with hypercholesterolemia. The research was done at the Laboratory of Veterinary Public Health and the Animal Experimental Unit of the Faculty of Veterinary Medicine, Universitas Syiah Kual. This experiment used the Split Plot design, comprising two factors. The first factor was treatment (P1, P2, and P3) that gives katuk leaf extract 2.5 cc, 5 cc, and 10 cc, whereas the second factor was the sampling time (I, II, III, IV, and V). The results of this research showed that the treatment did not significantly ($P<0.05$) on levels of triglycerides in the blood of local rabbits. Still, different sampling times were very significant ($P> 0.01$) on levels of triglycerides in the blood of local rabbits. After doing Duncan tests, the results test showed that the sampling time between a decrease of local rabbit blood triglyceride levels was not significant ($P<0.05$) by giving katuk leaf extract. The treatment of katuk leaf extracts of 2.5cc, 5cc, and 10cc has not been able to reduce levels of triglycerides in the blood of male local rabbits treated with hypercholesterolemia.

Keywords: Hypercholesterolemia, katuk leaf, rabbit, triglyceride level

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Introduction

Currently, in various parts of the world, deaths caused by non-communicable diseases such as cardiovascular diseases (including coronary heart disease), cancer, diabetes, and hypertension (high blood pressure) are on the rise. Cardiovascular diseases can be triggered by hypertension, triglycerides, and cholesterol (Tejayadi, 1991).

The continuous advancement of technology over time has led to an increase in activities, both individual and group, among the majority of the global population, including in Indonesia. This has also driven changes in social and cultural aspects, including lifestyle, particularly in urban communities, such as eating habits. Healthy eating patterns based on natural food sources are gradually replaced by unhealthy eating patterns that contain substances potentially disruptive to the body's physiological processes, such as foods high in saturated fats, including cholesterol, triglycerides, or both. This can serve as a trigger for the onset of coronary heart disease (CHD), which often leads to death (Sitepoe, 1993).

As risk factors continue to evolve, the incidence of CHD and cerebrovascular diseases (stroke) has been steadily increasing each year (Yahya, 2005). To date, both remain the leading causes of death in countries within the Asia-Pacific region (Soerjodibroto, 2005). Cerebrovascular diseases and CHD are clinical manifestations of atherosclerosis, resulting from disturbances in lipoprotein metabolism, which include increased levels of Low-Density Lipoprotein (LDL), triglycerides, total cholesterol, and a decrease in High-Density Lipoprotein (HDL) levels (Soerjodibroto, 2005).

In the United States, 50% of deaths are caused by CHD (Tejayadi, 1991). While CHD affects many people in developed countries, it is estimated that around 80% of global deaths caused by CHD occur in developing countries (WHO, 2010). In Indonesia, CHD ranks third, followed by diarrhea and respiratory diseases, in terms of diseases that cause death among the population (Wiryowidagdo & Sitanggang, 2002). Moreover, in the past 5 to 10 years, the number of deaths has increased, particularly among adults and the elderly (Tejayadi, 1991).

Triglycerides are fats produced by the body after food consumption. These substances are transported through the bloodstream to be used as an energy source for the body. Recently, triglycerides have been a topic of much discussion. Similar to excess cholesterol, which has long been recognized as a threat to human health due to its association with various fatal diseases, awareness of triglycerides is also growing within the medical community. Although there are many controversies surrounding this substance, its negative effects on health are becoming increasingly clear over time (Majid, 2010).

According to Abdullah (2007), the normal triglyceride level in cattle is <0.2 mmol/L, and in horses, it ranges between 0.1-0.5 mmol/L. According to Anonimus (2010), normal triglyceride levels in dogs range from 24-115 mg/dL, in cats from 30-125 mg/dL, and in cattle from 124-120 mg/dL. In laboratory animals such as rabbits, normal triglyceride levels are 124-156 mg/dL (Jenkins and Brown, 1994).

Increased blood triglyceride levels are associated with an elevated risk of coronary heart disease, especially in individuals with other health issues such as diabetes mellitus. Low HDL levels often appear alongside elevated triglycerides, creating a combination that raises the risk of coronary heart disease. This combination is also part of metabolic syndrome (Syndrome X). Syndrome X is a serious health issue affecting a quarter of the middle-aged population in the United States. It consists of symptoms, such as high triglycerides, low HDL cholesterol, high blood pressure, and elevated blood sugar levels (Murray et al., 2003).

The search for cholesterol-lowering medications, particularly those derived from natural sources, has been actively pursued. These natural-based drugs are not only affordable and easily accessible, but they also have fewer side effects, making them relatively safer compared to synthetic drugs. Plants are a source of chemical compounds, both known and unknown, many of which have the potential to serve as the basis for pharmaceutical products (Dachriyanus et al., 2007). One such plant with potential cholesterol-lowering properties is the katuk plant.

Several studies have demonstrated that phytosterols can reduce cholesterol in humans. Jones et al. (2000) stated that cholesterol reduction occurs due to the ability of phytosterols and phytostanols to decrease cholesterol absorption, while partially de-suppressing cholesterol biosynthesis. In poultry, the effects of adding katuk leaf powder to feed have also been studied. The use of 9% katuk leaf powder in the feed was shown to lower cholesterol in eggs, carcasses, and livers of local chickens (Subekti, 2003), laying hens (Saragih, 2005), and broilers (Nasution, 2005). This result is suspected to be due to the high papaverine content in katuk leaves (Suprayogi, 2000), which is believed to reduce cholesterol, though some experts did not find this compound in katuk (Pathmavati & Rao, 1990; Agusta et al., 1997). Therefore, the suspected cause of the cholesterol reduction is the high crude fiber content in katuk leaves.

Materials and Method

Research metodhology

This study is experimental research using a Split-Plot Design (SPD). The first factor is the treatment (P1, P2, and P3), which consists of the administration of 2.5 cc, 5 cc, and 10 cc of katuk leaf extract, while the second factor is the sample collection time (I, II, III, IV, and V).

Each treatment group (P0, P1, P2, and P3) consisted of four replications (four rabbits), all treated with hypercholesterolemia, including P0 as the control group. The hypercholesterolemia treatment is carried out by administering 2 g of egg yolk per rabbit orally for 7 days (Dachriyanus et al., 2007). After the hypercholesterolemia treatment, the P1, P2, and P3 groups were then given katuk leaf extract for five consecutive days at different doses, as follows: (a) Group P1: rabbits were given 2.5 ml of katuk leaf extract infusion, (b) Group P2: rabbits were given 5 ml of katuk leaf extract infusion, (c) Group P3: rabbits were given 10 ml of katuk leaf extract infusion.

Research Procedure

Preparation of Katuk Leaf Extract

The katuk leaves was obtained from a community garden in the Rukoh Village area, Darussalam, Banda Aceh. The infusion method, the leaves were placed in an infusion container with boiling water at a 1:1 ratio. The mixture was stirred and left for approximately 20 minutes. The result of decoction was then filtered to obtain a 100% katuk leaf extract.

Sample collection

Blood samples were taken from each rabbit in all groups before and after the hypercholesterolemia treatment, and on the 5th, 10th, and 15th days after the administration of katuk leaf extract. Blood was drawn through the jugular vein, collecting 3 ml, and transferred into a test tube. The test tubes were placed at an angle for 4-5 hours or until serum formation occurred. After the serum formed, it was transferred into a microtube and stored in a refrigerator before analysis.

Sample analysis

A 0.01 ml blood serum sample was pipetted using a micropipette and placed in a test tube. Then, 1 ml of triglyceride reagent solution was added. The mixture was vortexed and left for 20 minutes at room temperature. Absorbance was measured at a wavelength of 500 nm using a blank solution. The blank consisted of 1 ml of cholesterol reagent and 0.01 ml of aquades.

The standard absorbance measurement is the same as the sample absorbance measurement. Triglyceride levels were calculated using the formula (Artiss et al., 1997) as follows:

$$C = \frac{A\ Sampel}{A\ standar} \times C\ standar$$

information:
C = triglyceride concentration (mg/dL)
A = absorbance
C = standard triglyceride concentration (200 mg/dL)

Research parameters

The parameter observed in this study is the triglyceride concentration (mg/dL) in the blood of male local rabbits treated with hypercholesterolemia.

Data analysis

The data obtained were analyzed using Analysis of Variance (ANOVA), and/if there were significant differences (P<0.05) between the treatments and sampling times, further analysis was conducted using Duncan's multiple range test.

Results and Discussion

The average triglyceride levels in the blood of male local rabbits can be seen in Table 1. Statistical analysis results indicate that the treatments (administration of katuk leaf extract in various doses: 2.5 cc, 5 cc, and 10 cc) did not have a significant effect (p<0.05) on the triglyceride levels in the blood of male local rabbits. However, the time of blood serum sample collection had a highly significant effect ($p>0.01$) on the triglyceride levels in the blood of male local rabbits.

Table 1. Mean (±SD) triglyceride levels in the blood of male local rabbits after treatment

Observation time	Treatments			
	P0	P1	P2	P3
W1	72.25 ± 12.07^{d}	63.5 ± 24.80^{c}	56.25 ± 7.27^{c}	70.25 ± 13.96^{c}
W2	204.75 ± 32.66^{c}	174.25 ± 40.31^{b}	204.5 ± 115.40^{b}	208 ± 24.51^{b}
W3	466.5 ± 210.84^{b}	580 ± 138.58^{a}	607 ± 25.42^{a}	657.5 ± 49.17^{a}
W4	629 ± 27.28^{a}	633.75 ± 33.38^{a}	696.25 ± 106.69^{a}	672 ± 20.70^{a}
W5	665.75 ± 62.08^{a}	610 ± 15.68^{a}	594.75 ± 31.48^{a}	592.25 ± 33.95^{a}

Note: Different lowercase superscript letters in the same column indicate significant differences ($p>0.05$). P0: Control; P1: 2.5 cc katuk leaf extract; P2: 5 cc katuk leaf extract; P3: 10 cc katuk leaf extract; Examination = Time = W; W1: Examination I (Day 1) before hypercholesterolemia treatment (chicken egg yolk administration for 7 consecutive days); W2: Examination II (Day 7) after hypercholesterolemia treatment and before katuk leaf extract treatment (for 5 consecutive days); W3: Examination III (Day 12) after katuk leaf extract treatment (5 days after the first dose of katuk leaf extract or 1 day after the 5-day consecutive katuk leaf extract administration process); W4: Examination IV (Day 17) after katuk leaf extract treatment (10 days after the first dose of Katuk leaf extract or 5 days after the 10-day consecutive katuk leaf extract administration process); W5: Examination V (Day 22) after katuk leaf extract treatment (15 days after the first dose of katuk leaf extract or 10 days after the 15-day consecutive katuk leaf extract administration process).

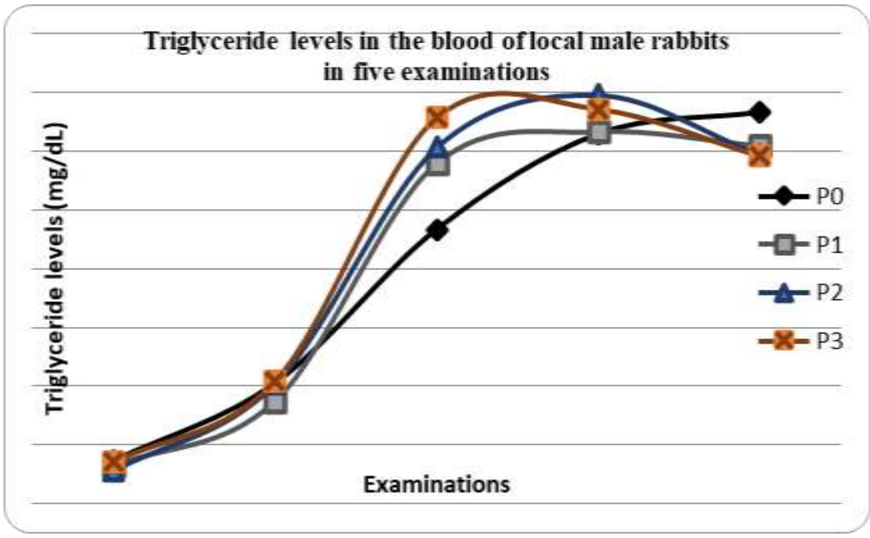

Figure 1. Triglyceride levels in the blood of local rabbits at five examinations. Note: Different lowercase superscript letters in the same column indicate significant differences ($p>0.05$). P0: Control; P1: 2.5 cc katuk leaf extract; P2: 5 cc katuk leaf extract; P3: 10 cc katuk leaf extract; Examination = Time = W; W1: Examination I (Day 1) before hypercholesterolemia treatment (chicken egg yolk administration for 7 consecutive days); W2: Examination II (Day 7) after hypercholesterolemia treatment and before katuk leaf extract treatment (for 5 consecutive days); W3: Examination III (Day 12) after katuk leaf extract treatment (5 days after the first dose of katuk leaf extract or 1 day after the 5-day consecutive katuk leaf extract administration process); W4: Examination IV (Day 17) after katuk leaf extract treatment (10 days after the first dose of katuk leaf extract or 5 days after the 10-day consecutive katuk leaf extract administration process); W5: Examination V (Day 22) after katuk leaf extract treatment (15 days after the first dose of katuk leaf extract or 10 days after the 15-day consecutive katuk leaf extract administration process).

Based on Table 1, the average triglyceride levels in male local rabbits, in both treatments with katuk leaf extract at doses of 2.5 cc, 5 cc, or 10 cc, tended to increase significantly from the first to the third examination. At the fourth examination, there was an increase, though not significant, and at the fifth examination, there was a tendency for a decrease, although this decrease was not significant. The average triglyceride levels in the blood of male local rabbits from the first to the fourth examination increased from 63.5 ± 24.80 to 633.75 ± 33.38 in treatment I, 56.25 ± 7.27 to 696.25 ± 106.69 in treatment II, and 70.25 ± 13.96 to 672 ± 20.70 in treatment III. Meanwhile, the average decrease in triglyceride levels from the fourth to the fifth examination was as follows: 633.75 ± 33.38 to 610 ± 15.68 in treatment I, 696.25 ± 106.69 to 594.75 ± 31.48 in treatment II, and 672 ± 20.70 to 592.25 ± 33.95 in treatment III.

Compared to the control group (positive control subjected to hypercholesterolemia, similar to the treatment groups), all three treatment groups showed a similar average increase in triglyceride levels in the blood, comparable to the control group, from the first to the fourth examination. However, at the fifth examination, the control group tended to continue increasing, while the three treatment groups (treatments I, II, and III) showed a tendency for a decrease, although statistical tests indicated no significant difference ($p<0.05$). To observe the trend of triglyceride, increase and decrease in the blood of male local rabbits from the first to the fifth examination in each control and treatment group, please refer to Figure 1.

Based on the various results above, in general, the average triglyceride levels in the blood of local male rabbits in the three treatment groups increased from examination I to examination IV and decreased in examination V. However, the decrease in examination V was not significant in examination IV.

The administration of katuk leaf extract in various doses did not significantly reduce triglyceride levels in the blood of local male rabbits after hypercholesterolemia induction from day 12 to day 22 (from examination III to examination IV). This may be due to several factors, such as the possibility that the Katuk leaf extract doses were still insufficient, the active compounds in katuk leaves potentially being ineffective against the chemical structure of triglycerides, the relatively short duration of the examinations, and other factors.

The insufficient Katuk leaf extract (2.5–10 cc) in this study may be due to the extraction method used, which could affect the concentration of active compounds in the extract. Several studies conducted by Santoso et al. (2001; 2001, and 2002) showed that adding katuk leaf extract to the feed of laying hens could reduce fat accumulation in the body, stomach, and liver. Serum triglyceride concentrations are influenced by the extraction method used, such as hot water extraction of katuk leaves, which can reduce serum triglycerides, ethanol extraction at a level of 1.8 g/kg, which can increase serum triglycerides, and methanol extraction, which does not affect egg triglyceride levels.

In addition to the dosage factor, the effectiveness of the active compounds in the katuk leaf extract, which may be less sensitive to the chemical structure of triglycerides, could also be a contributing factor. The variation in the dosage of katuk leaf extract likely relates to differences in the concentration of active compounds produced by each extraction method, as previously explained. Another factor is the relatively short duration of the examination in this study, which led to the inability to observe further decreases after day 22 (examination V), as shown in the table and graph above. However, there is a possibility of a more significant decrease in triglyceride levels if the examination period is extended.

Based on Suprayogi's (2005) study on the effects of katuk leaves on volatile fatty acids (VFAs), it was shown that VFA levels increased individually on day 14 and significantly decreased by day 35. Triglycerides are a type of fatty acid with a structure similar to VFAs and are also synthesized from carbohydrates. Therefore, it is likely that the decrease in triglycerides due to the administration of katuk leaves could be observed between days 14 and 35. This statement supports the findings of the current study, where a decrease in triglycerides was already observed on day 15 after the first dose of katuk leaf extract in a hypercholesterolemic condition.

Conclusion

Based on the results of this study, it can be concluded that the administration of katuk leaf extract at doses of 2.5 cc, 5 cc, and 10 cc does not affect the triglyceride levels in the blood of male local rabbits. It is recommended to explore higher doses or different durations of katuk leaf extract administration to determine if there is a potential effect on triglyceride levels, as the current doses may not have been sufficient to induce significant changes.

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