

Antihypercholesterolemic Activity of Tahongai Leaf infusion (*Kleinsovia hospita* L.) In Mice (*Mus musculus* L.)

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Abstract

Tahongai (*Kleinsovia hospita* L.) is an indigenous plant of East Kalimantan known for its health benefits, including antibacterial, anti-inflammatory, antioxidant, anticancer, and antidiabetic properties. This study evaluates the antihypercholesterolemic activity of Tahongai leaf infusion in hypercholesterolemic mice (*Mus musculus* L.), induced by egg yolk. The test groups included a positive control (simvastatin), a negative control (distilled water), and three treatment groups with Tahongai leaf infusion at concentrations of 15%, 30%, and 60%. The results indicated that the highest reduction in cholesterol levels was observed in the P1 group (15% infusion) with an average decrease of 30.84%, followed by P2 (30% infusion) at 24.79%, and P3 (60% infusion) at 9.31%. The positive control group showed an average reduction of 8.64%. Statistical analysis using One-Way ANOVA revealed a significant difference ($p < 0.05$) in cholesterol level reduction among the treatment groups.

Keywords: Anticholesterol; Tahongai Leaf; *Kleinsovia hospita* L; Hypercholesterolemia; Mice.

INTRODUCTION

The rapid advancement of technology has led to a shift in people's lifestyles, where individuals tend to choose instant solutions, including fast food consumption. Fast food is typically high in cholesterol but low in fiber (Bachmid et al., 2015). Cholesterol is a complex compound essential for regulating biochemical processes in the body. However, excessive cholesterol levels in the blood can be harmful, leading to hypercholesterolemia. This condition occurs when cholesterol levels exceed the normal range, increasing the risk of coronary heart disease (Muqowwiyah & Dewi, 2021).

According to the 2019 WHO report, heart disease is one of the leading causes of death worldwide, accounting for approximately 55% of 55.4 million global deaths. In 2000, coronary heart disease was the leading cause of death, with 2 million fatalities, which increased to 8.9 million in 2019 (Menkes RI, 2018). Changes in blood cholesterol levels play a crucial role in preventing the adverse effects of elevated cholesterol, such as coronary heart disease caused by atherosclerosis. Atherosclerosis leads to the narrowing of blood vessels, restricting blood flow, stimulating clot formation, and ultimately disrupting circulation (Mufida et al., 2018).

One of the primary strategies to mitigate the negative impacts of increased cholesterol levels is cholesterol reduction, a crucial healthcare approach to prevent heart disease risks. Cholesterol reduction is commonly achieved through hypolipidemic drugs or natural medicinal plants known for their cholesterol-lowering properties. Currently, extensive research is being conducted on medicinal plants with effects comparable to synthetic drugs but fewer side effects (Saryono et al., 2017). Traditional medicine has been found to have fewer side effects than synthetic drugs. Plants serve as a source of various bioactive compounds and have the potential to be used as raw materials for medicinal development. Thus, in-depth research on medicinal plants is essential. Traditional medicine often exerts multiple pharmacological effects, making it more suitable for treating metabolic and degenerative diseases (Tamuntuan et al., 2019).

Aviani et al. (2022) identified flavonoids, alkaloids, and tannins as bioactive compounds with cholesterol-lowering effects. Flavonoids inhibit the HMG-CoA reductase enzyme, which is crucial in cholesterol biosynthesis. By inhibiting this enzyme, the production of cholesterol in the liver is reduced. Alkaloids inhibit

lipase enzymes, thereby preventing cholesterol formation. Tannins, on the other hand, block fat absorption in the intestines and precipitate proteins, hindering cholesterol and fat uptake in the intestinal lining (Artha *et al.*, 2017).

One of the medicinal plants native to East Kalimantan with significant therapeutic potential is the Tahongai leaf (*Kleinhovia hospita* L.). Qualitative phytochemical analysis of Tahongai leaves has revealed the presence of alkaloids, tannins, flavonoids, triterpenoids, and steroids. Previous studies on laboratory mice (*Mus musculus* L.) have demonstrated the efficacy of Tahongai leaves in exhibiting antibacterial, anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. Based on literature studies and prior research, this study aims to determine the cholesterol-lowering effects of Tahongai leaf infusion in hypercholesterolemic mice at varying concentrations.

MATERIALS AND METHODS

Research Location and Time

The study was conducted from February to March 2023 at STIKES Dirgahayu Samarinda Laboratory. The extraction process and phytochemical screening of the test material were carried out in the Phytochemistry Laboratory, while the *in vivo* tests on experimental animals were performed in the Pharmacology Laboratory at STIKES Dirgahayu Samarinda.

Research Equipment and Materials

The equipment used for testing the antihypercholesterolemic activity included: funnels, dropper pipettes, measuring cylinders, vial glasses, volumetric flasks, micropipettes, a stomach tube, a refrigerator, a rotary evaporator, mouse cages, an analytical balance, syringes, cholesterol test devices, and cholesterol test strips.

The materials used in the study included 25% ammonia (Mallinckrodt), hydrochloric acid (Merck), Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, ferric (III) chloride (Merck), sodium nitrite (Merck), aluminum chloride (Merck), sodium hydroxide (Merck), ether (Merck), acetic anhydride (Merck), concentrated H₂SO₄ (Merck), 70% ethanol (ASM), chloroform (Merck), carboxymethyl sodium, egg yolk, simvastatin 10 mg, mice weighing 20–40 g, and standard feed and water for the mice.

Procedures

Preparation of Tahongai Leaf Infusion

50 g of dried Tahongai leaves was infused using 50 mL of water as a solvent at 90°C for 15 minutes. Heating was performed on a hot plate while stirring continuously. The extract was filtered while still hot using filter paper. The resulting infusion was then prepared at different concentrations of 15%, 30%, and 60% (Aji *et al.*, 2021).

Phytochemical Screening

Alkaloid Identification

A total of 2 g of powdered sample was moistened with 5 mL of 25% ammonia in a beaker glass, followed by adding 20 mL of chloroform until the sample was fully submerged. The mixture was stirred, heated over a water bath, and filtered. The residue was placed into a test tube, and 3 drops of 2N hydrochloric acid were added, followed by shaking. The solution was left to form two layers, and the clear layer was divided into two separate test tubes. Mayer's and Bouchardat's reagents were then added. A positive alkaloid presence was indicated by the formation of a white precipitate in Mayer's reagent and a brown precipitate in Bouchardat's reagent (Satrana, 2017).

Flavonoid Identification

1 g of powdered sample was extracted with 100 mL of hot water and then filtered. A 5 mL aliquot of the filtrate was placed in a test tube. Next, 1 mL of 5% sodium nitrite solution and 1 mL of 10% aluminum chloride solution were added, followed by shaking. Then, 2 mL of 1N sodium hydroxide solution was added along the test tube wall. A color change to red or orange indicated the presence of flavonoids (Satrana, 2017).

Saponin Identification

1 g of powdered sample was extracted with 100 mL of hot water and then filtered. A 10 mL aliquot of the filtrate* was placed in a test tube and shaken vertically for 10 seconds. The presence of saponins was indicated by the formation of a stable foam measuring 1 to 10 cm in height, which did not disappear upon adding one drop of 2N hydrochloric acid (Satrana, 2017).

Tannin Identification

1 g of powdered sample was extracted with 100 mL of hot water and then filtered. A 5 mL aliquot of the filtrate was placed into a test tube, followed by adding a few drops of 1% ferric (III) chloride solution. A color change to green, purple, or black indicated the presence of tannins (Satrana, 2017).

Steroid and Triterpenoid Identification

A total of 2 g of powdered sample was macerated with 20 mL of ether for 2 hours, followed by filtration and evaporation to obtain a residue. The residue was then treated with 2 drops of acetic anhydride and 2 mL of chloroform and transferred into a test tube. A slow addition of 1 mL of concentrated H₂SO₄ (Liebermann-Burchard reagent) was performed along the test tube wall. A purple ring indicated the presence of terpenoids, while a green ring indicated the presence of steroids (Satrana, 2017).

Acclimatization of Experimental Animals

The experimental animals used in this study were male mice weighing 20–35 g. Before testing, the mice were

acclimatized in cages for one week to adjust to the new environment. They were provided food and water, and their general health and body weight were monitored. After acclimatization, all mice underwent fasting for 12–14 hours. According to Murray et al. (2003), fasting aims to significantly reduce HMG-CoA reductase activity and lower exogenous cholesterol synthesis.

Testing Antihypercholesterolemic Activity in Hypercholesterolemic Mice

Preparation of Experimental Animals

The mice were first fasted for approximately 14 hours without food but were provided with water. Their body weights were measured, and they were divided into five treatment groups: positive control, negative control, and three experimental groups receiving different doses (dose I, dose II, and dose III).

Determination of Simvastatin Dose (Positive Control)

Based on the dose conversion table, the dose conversion factor from a 70 kg human to a 20 g mouse is 0.0026. The human dose of simvastatin is 10 mg. The formula used for dose determination: Dose for humans \times 0.0026 = mg/mouse (20 g body weight). Thus, the simvastatin dose for mice was calculated as follows: $10\text{mg} \times 0.0026 = 0.026 \text{ mg}/20\text{g}$. For example, if a mouse weighed 30 g, the dose was determined as: $30/20 \times 0.026 = 0.039 \text{ mg}$.

Egg Yolk Administration for Hypercholesterolemia Induction

Herliana and Sitanggang (2009) state that high-cholesterol foods include brain and egg yolk. In this study, egg yolk was used for hypercholesterolemia induction. The calculation was as follows: Cholesterol content in 100 g of egg yolk = 1500 mg. Normal cholesterol level = $<200 \text{ mg/dL}$. Target cholesterol level = 300 mg/dL . Target cholesterol level/Normal cholesterol level \times Cholesterol content = required amount. Thus, the required egg yolk administration was: $300/200 \times 1500 = 2.25 \text{ g}$.

This study, induced mice with 2.25 g of egg yolk mixed with 100 g of standard feed for 2 weeks.

Preparation of Negative Control Solution

The negative control assessed substances without diuretic activity, enabling comparison with the test substance. The negative control group was administered 0.5 mL of distilled water orally.

Blood Sampling Technique

Blood samples were collected from the tail vein (vena lateralis caudae) of the mice. The mice were restrained in a suitable container, their tails were extended, and the vein was punctured with a lancet. The collected blood was applied to cholesterol test strips.

Experimental Procedure

Mice were fasted for approximately 14 hours while maintaining water intake. On the test day, body weights were recorded, and the mice were divided into five groups, each consisting of five individuals. Mice were assigned to groups based on weight to ensure uniform dosing. Hypercholesterolemia was induced using 2.25 g of egg yolk for 14 days, followed by cholesterol level measurements. After confirming hypercholesterolemia, the treatment was administered for 14 days.

RESULTS AND DISCUSSION

Table 1. Phytochemical screening of Tahongai Leaf (*Kleinhovia hospita* L.).

No	Test/Reagent	Result
1	Alkaloid (Dragendroff)	+
2	Alkaloid (Mayer)	+
3	Alkaloid (Bouchardat)	+
4	Flavonoid	+
5	Fenol	-
6	Saponin	+
7	Tanin	+
8	Steroid + Terpenoid	+

Note : (+) indicates the presence of secondary metabolites
(-) indicates the absence of secondary metabolites

Table 2. Cholesterol levels before and after treatment.

No	Hypercholesterolemia Induction (mg/dl)					Post treatment Infusion (mg/dl)				
	K-	K+	P1	P2	P3	K-	K+	P1	P2	P3
1	135	138	161	123	133	117	123	120	115	115
2	122	115	147	140	124	120	109	117	118	110
3	115	122	198	160	145	110	120	135	120	136
4	143	104	171	123	152	135	105	118	117	145
5	145	150	106	216	126	130	122	103	135	118
Mean \pm	132 \pm	125,8 \pm	156,6 \pm	152,4 \pm	136 \pm	122,4 \pm	115,8 \pm	118,6 \pm	121 \pm	124,8 \pm
STD	13,11	18,31	33,89	38,68	12,14	10,06	8,23	11,37	8,03	14,96

Note:

K- = Negative control (treatment with distilled water as solvent); K+ = Positive control (simvastatin at a dose of 0.026 mg/20 g body weight); P1 = Treatment with *Kleinhovia hospita* infusion at 15% concentration; P2 = Treatment with *Kleinhovia hospita* infusion at 30% concentration; P3 = Treatment with *Kleinhovia hospita* infusion at 60% concentration; STD = Standard deviation.

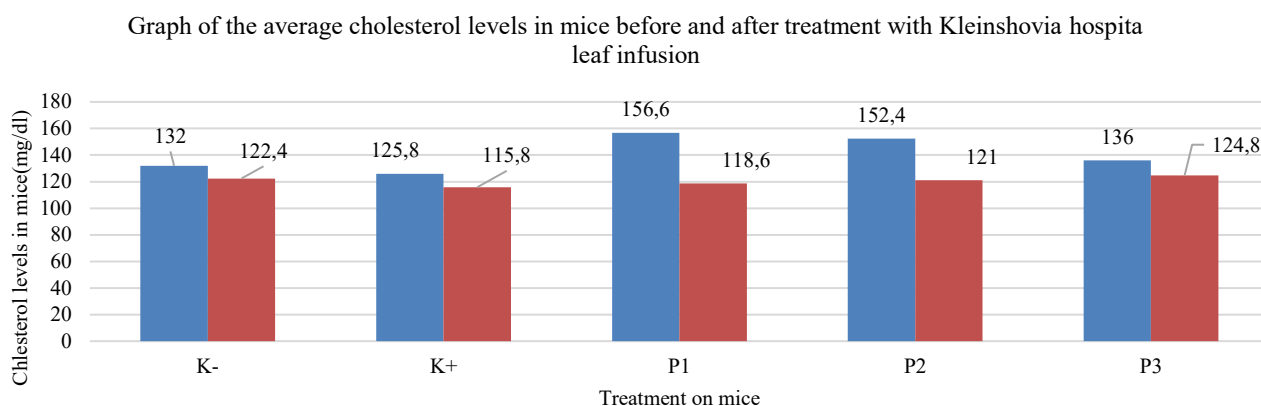


Figure 1. Graph of the average cholesterol levels in mice before and after treatment with *Kleinshovia hospita* leaf infusion.

Table 3. Percentage reduction in cholesterol levels in mice after *Kleinshovia hospita* infusion treatment.

No	K-	K+	P1	P2	P3
1	15,38	12,19	34,16	6,9	15,65
2	1,66	5,5	25,64	18,64	12,72
3	4,54	1,66	46,6	33,3	6,61
4	5,92	0,95	44,9	5,13	4,82
5	11,53	22,9	2,9	60	6,78
Mean (%)	7,81	8,64	30,84	24,79	9,31

$$\% \text{ cholesterol reduction} = \frac{\text{initial cholesterol} - \text{post treatment cholesterol}}{\text{initial cholesterol}} \times 100\%$$

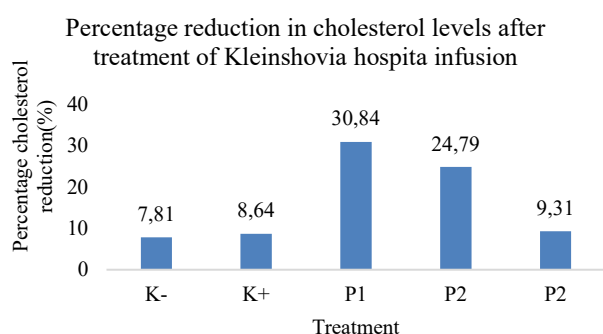


Figure 2. Graph percentage reduction in cholesterol levels after *Kleinshovia hospita* treatment

Note:

K-= Negative control (treatment with distilled water as solvent)
 K+= Positive control (simvastatin at a dose of 0.026 mg/20 g body weight)
 P1= Treatment with *Kleinshovia hospita* infusion at 15% concentration
 P2= Treatment with *Kleinshovia hospita* infusion at 30% concentration
 P3= Treatment with *Kleinshovia hospita* infusion at 60% concentration

Based on the results shown in Table 1, the metabolite compounds found in tahongai leaves include alkaloids, flavonoids, saponins, and tannins. These findings align with the research conducted by Budiarti and Joko (2020), which qualitatively confirmed the presence of alkaloids, tannins, saponins, flavonoids, and steroids in tahongai

leaves. Quantitatively, these leaves contain 2.83% alkaloids, 19.78% flavonoids, and 14.23% saponins (Yunita *et al.*, 2019).

The data in Tables 2 and 3 indicate reduced cholesterol levels in the control and treatment groups using tahongai leaf infusion. For the negative control group, before treatment, the average cholesterol level was 135 mg/dl and after treatment with tahongai leaf infusion, it averaged 122.4 mg/dl, which calculates to an average cholesterol reduction percentage of 7.81% as shown in Table 3. Furthermore, for the positive control group, the average cholesterol level decreased from 125.8 mg/dl before treatment to 115.8 mg/dl after treatment. For the P1 treatment group, which received a 15% tahongai leaf infusion, the data showed a decrease from an initial average cholesterol level of 156.6 mg/dl to 118.6 mg/dl. The P2 treatment group, using a 30% infusion concentration, started with an average cholesterol level of 152.4 mg/dl and experienced a reduction to 121 mg/dl. For the P3 group with a 60% infusion concentration, the initial average cholesterol level was 136 mg/dl, which decreased to 124.8 mg/dl.

According to Table 3, the average percentage reduction in cholesterol levels for all treatments showed that the positive control group had an average reduction of 7.81%, while the 15% tahongai leaf infusion group showed a significant reduction of 30.84%, the 30% concentration group showed 24.79%, and the 60% concentration group showed 9.31%. This result is further supported by statistical analysis data from the LSD test in Table 4.6, indicating significant differences ($p < 0.05$) between the control and treatment groups, specifically between the positive control group and the P1 treatment group.

The treatment with a 15% tahongai leaf infusion showed the most effective anti-cholesterol activity in reducing cholesterol levels in hypercholesterolemic mice. The anti-cholesterol effect observed in this study is attributed to metabolite compounds such as flavonoids, alkaloids, and tannins in the leaves. The flavonoid in

kemuning leaves, derived from a subgroup called apigenin, which is light yellow and crystal-like in shape (Adfa, 2007), works by inhibiting the HMG-CoA reductase enzyme, which aids in cholesterol formation. By inhibiting this enzyme, cholesterol production by the liver decreases (Artha et al., 2017). Flavonoids also help dissolve lipid clusters attached to the walls of coronary vessels, improving blood flow (Anggraini & Nabillah, 2018). Alkaloids, organic compounds containing nitrogen and commonly found in plants, inhibit the activity of the pancreatic lipase enzyme, which is converts fats into glycerol and fatty acids. By inhibiting lipase, fat absorption by the liver is hindered, preventing cholesterol synthesis (Artha et al., 2017). Tannins in kemuning leaves, serve multiple medicinal purposes such as anti-diarrheal, antibacterial, and antioxidant. Their mechanism in reducing cholesterol levels involves inhibiting fat absorption in the intestines and precipitating protein tissues, thereby obstructing cholesterol and fat absorption at the intestinal surface (Artha et al., 2017).

Plant steroids, or phytosterols, also help lower total cholesterol levels. Research by Jones et al. (2000) and Nguyen (1999) suggests that the consumption of 2-3 g/day of phytosterols can reduce total and LDL cholesterol in humans, while HDL serum concentrations do not change significantly. Additionally, phytosterols are believed to inhibit the absorption of exogenous cholesterol and the reabsorption of endogenous cholesterol in the digestive tract, enhance the excretion of absorbed cholesterol, and cause a reduction in serum cholesterol levels due to competition between cholesterol and phytosterols for absorption in the intestines (Bonsdorff-Nikander, 2005). Alkaloids also have a cholesterol-lowering effect, as indicated by Kou et al. (2016) who concluded that a combination of five primary alkaloids (berberine, coptisine, palmatine, epiberberine, and jatrorrhizine) shows synergistic effects in reducing cholesterol levels.

CONCLUSIONS

The conclusions from this research are as follows:

- Tahongai leaf infusion has an anti-cholesterol effect on hypercholesterolemic mice (*Mus musculus* L.).
- The percentage reduction in cholesterol levels in mice through tahongai leaf infusion is most effective at the 15% concentration, with an average percentage reduction of 30.84%, followed by 24.79% at the 30% concentration and 9.31% at the 60% concentration. The positive control group showed an average reduction of 8.64%.

Authors' Contributions: The contributions of the authors include the first author conducting animal tests and analyzing cholesterol reduction data, while other

members contributed to the chemical testing of plant compounds by conducting phytochemical screening and assisting in the statistical analysis of data obtained. All authors collectively participated in reviewing the article.

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Competing Interests: We declare that there are no competing interests in conducting this research.

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