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Potential of Ethanol Extract of Turmeric Leaves (*Curcuma longa*) on the Reduction of Cholesterol Levels in Mice (*Mus musculus*) Hypercholesterolemia Model

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ABSTRACT

Tests on the dosage and effectiveness of turmeric leaf ethanol extract in lowering cholesterol levels in animal models have never been conducted to explore its therapeutic potential. This study aims to determine the effect of turmeric leaf ethanol extract (*Curcuma longa*) in reducing hypercholesterolemia levels in mice. This study employed an experimental design, dividing 36 mice into six treatment groups. The treatment group consisted of a negative control, a positive control, a drug control, and three doses of turmeric leaf ethanol extract (14 mg/20 g body weight, 28 mg/20 g body weight, and 56 mg/20 g body weight). Cholesterol levels were measured using a spectrophotometer, and the results were analyzed with the ANOVA test. The results showed a significance value of 0.000, indicating a significant difference in the reduction of cholesterol levels between treatment groups ($p < 0.05$). Turmeric leaf ethanol extract at a dose of 56 mg/20 g body weight resulted in a reduction of 33.6 mg/dL in mouse cholesterol levels, which is higher than that observed with other doses. Turmeric leaf ethanol extract can lower cholesterol levels in mice, with a dose of 56 mg/20 g body weight providing the most significant reduction. This study demonstrates that turmeric leaf ethanol extract has the potential to serve as a natural therapeutic alternative for managing hypercholesterolemia.

Keyword: Turmeric Leaves (*Curcuma longa*), Hypercholesterolemia, Cholesterol, Mice (*Mus musculus*)

1. INTRODUCTION

Hypercholesterolemia is a condition that can cause narrowing of blood vessels and increase the risk of heart attack and stroke (Sari, 2014). High cholesterol levels, particularly LDL cholesterol, can contribute to atherosclerosis, which affects heart health (Yani, 2015). In Indonesia, the Riskesdas 2018 data show that 21.2% of the population over the age of 15 has abnormal cholesterol levels, with a higher prevalence in women (24.0%) than in men. As we age, deteriorating physical condition and increased risk of disease, especially coronary heart disease, become common. Coronary heart disease is the leading cause of death in Indonesia and the world, with hypercholesterolemia as the leading risk factor. Various factors, including age, gender, diet, stress, alcohol consumption, and physical activity levels, affect cholesterol levels in the body. Hypercholesterolemia, which is characterized by an increase in total cholesterol levels, is a global health problem with high prevalence, including in Southeast Asia and Indonesia, causing millions of deaths and disabilities each year (Dewi et al., 2025). Treatment of high cholesterol is usually carried out with pharmacological drugs that tend to

be expensive and have side effects. Therefore, research on natural medicine alternatives, such as turmeric leaves, is crucial for reducing the risk of cardiovascular disease more affordably and safely.

Turmeric leaves extracted using 80% methanol, 80% ethanol, and 80% acetone solvents contain significant amounts of phenolic compounds, flavonoids, and condensed tannins (Edi Suryanto, 2009). In another study, an ethanol extract from salak bark, known to contain flavonoids and tannins, can potentially lower cholesterol levels in individuals with dyslipidemia. These compounds function as inhibitors of HMG-CoA reductase, with the mechanism of action of simvastatin in lowering cholesterol levels being primarily through the inhibition of HMG-CoA reductase, the main pathway in cholesterol synthesis (Eisha Ira Maharani, 2025). Therefore, the flavonoids and tannins found in turmeric leaves may have a similar effect on lowering cholesterol levels.

Although numerous studies have investigated the potential of natural ingredients in reducing cholesterol levels, a research gap remains regarding the use of turmeric leaf ethanol extract as an antihyperlipidemic therapy, particularly in mice. Additionally, testing the optimal dose that can effectively lower cholesterol levels has not been conducted. Therefore, further testing of the dosage and effectiveness of turmeric leaf ethanol extract in lowering cholesterol levels in animal models is needed to explore its therapeutic potential.

This study aims to investigate the potential of turmeric leaf ethanol extract (*Curcuma longa*) in reducing cholesterol levels in mice (*Mus musculus*) using a hypercholesterolemia model. This research is essential for filling the gap in the literature regarding the use of turmeric leaves as a natural remedy for overcoming hypercholesterolemia and testing effective doses. The results of this study are expected to provide a more affordable and safer alternative to natural therapies for lowering cholesterol levels.

2. RESEARCH METHOD

Tools and Materials

The tools used are a spectrophotometer, a 1 ml/1 cc syringe, a measuring flask, a stirring rod, an analytical scale, a mouse cage, *a handscoon*, *a beaker glass*, a probe, and scissors. The ingredients used are turmeric leaves, ethanol (96% v/v), mice (*Mus musculus*) *of the Deutschland Denken Yoken* (DDY) strain, aged 2-3 months with a weight of 20-30 g, cotton, simvastatin tablets, alcohol for disinfection, propylthiouracil, and aquades.

Extraction

Five hundred grams of turmeric leaf powder is placed into a maceration container, typically a glass bottle. Then 1,250 ml of 96% ethanol solvent is added until the simplicia is fully submerged. The maceration container is closed and stored for 24 hours, stirring occasionally. After that, the mixture is filtered to separate the pulp and filtrate. The obtained pulp is re-macerated with a new 96% ethanol solvent. The extraction process continued up to four times of filtration within 4 x 24 hours, with a simplicia and solvent ratio of 1:10. The filtrate obtained as much as 5 liters is then evaporated for 40 minutes and evaporated until a thick 96% ethanol extract is obtained (ANDI TENRI PARAMITHA, 2017).

Test Animal Preparation

The UNUSA Health Research Ethics Committee approved the animal testing protocol with approval number 0945/EC/KEPK/UNUSA/2024. The test animals used in this study were 36 male mice (Mus musculus), which were divided into six groups. The mice used weighed approximately 20–30 grams and were in good health before the study's commencement. Before treatment, mice were acclimated for 7 days to minimize stress during the study. The first group was a negative control group that contained only healthy mice without any treatment. The second group was a positive control group who were given the drug propylthiouracil, which had been discontinued. The third group was the control group of the drug that received the drug simvastatin. The fourth group

was given turmeric leaf extract at a dose of 14 mg/20 g BW. The fifth group was given turmeric leaf extract at a dose of 28 mg/20 g body weight (BW). The sixth group was given turmeric leaf extract at a dose of 56 mg per 20 g of body weight (BW). All treatments will be examined at the Veterinary Laboratory, Faculty of Health Sciences, Maarif Hasyim Latif University, Sidoarjo.

Test Animal Blood Sampling

Mouse blood was taken on day 0, before any induction, day 16 after administration of fat-boosting drugs, and day 20 after administration of turmeric leaf extract. Blood collection is carried out by cutting the tail of the mice to a length of approximately 1-2 mm, which has been previously fixed with a 70% ethanol swab. Next, the tip of the tail is gently massaged until blood appears and is collected in a tube, allowing it to reach the desired volume of approximately 1.5 ml. The obtained blood was then centrifuged at 1500 rpm, and the resulting serum was used to measure cholesterol levels using the COD-PAP method. Blood collection is carried out after the test animal has been given sufficient food and water to ensure that the volume of blood collected reaches the desired limit.

Implementation of Test Animal Research

Each test animal was adapted for 14 days to avoid ontro, divided into six groups: group 1 (control), group 2 (positive control), group 3 (drug control), group 4 (dose 14 mg/20 g BW), group 5 (dose 28 mg/20 g BW), and group 6 (dose 56 mg/20 g BW), which were fed CP511 concentrate. In Group 1, blood was taken first for an initial cholesterol level examination before treatment was administered. On the following day, groups 2 to 6 were fed CP511 concentrate and propylthiouracil control as a cholesterol enhancer for 14 days, with cholesterol level measurement in group 2 carried out on the 15th day as a positive control. Group 3 was fed and propylthiouracil control for 14 days, plus simvastatin for 3 days as a drug control; group 4 was given turmeric leaf ethanol extract at a dose of 14 mg/20 g BW after 14 days of propylthiouracil; group 5 was given turmeric leaf ethanol extract at a dose of 28 mg/20 g BW after 14 days of propylthiouracil; and group 6 was given turmeric leaf ethanol extract at a dose of 56 mg/20 g BW after 14 days of propylthiouracil. Cholesterol levels for groups 3, 4, 5, and 6 were measured on the 15th day after fasting.

Cholesterol Level Measurement

The method employed is the enzymatic colorimetric method (*Cholesterol Oxidase Method*/*CHOD-PAP*), which adheres to WHO/IFCC standards and utilizes a spectrophotometer as a device for detecting gas or vapor compounds. The spectrophotometer features an LED array or LED series as the source of light radiation, which is captured by the photodiode sensor. The sample used was mouse blood serum with total cholesterol levels, which was examined using the CHOD-PAP reagent kit. A total of 5 µL of mouse serum was mixed with 500 µL of reagent in a test tube, then homogenized and incubated for 10 minutes at room temperature (37 °C). Total cholesterol levels were measured using a spectrophotometer with a wavelength of 546 nanometers (nm).

3. RESULTS AND ANALYSIS

Extraction

Table 1 presents the yield of ethanol extract from turmeric leaf simplicia. Yield is a comparison between the weight of the extract produced with the initial weight of the simplicia powder before the extraction process, expressed as a percentage. From 500 g of simplicia powder, turmeric leaf extract yields 65 g, corresponding to a yield of 13%.

Table 1. Turmeric leaf extract yield

Simplisia	Powder Weight(g)	Extract Weight (g)	Rendemen (%)
Turmeric leaves	500	65	13

Test of the Hypocholesterolemic Effect of Turmeric Leaf Extract

Table 2 presents data on the cholesterol levels of male mice treated with turmeric leaf extract at various doses. This test was conducted by comparing cholesterol levels in several groups: the negative control group, the positive control, the drug control, and the group that received turmeric leaf treatment at doses of 14 mg/g body weight (BW), 28 mg/g BW, and 56 mg/g BW. The negative control group had an average cholesterol level of 113.2 mg/dL, indicating normal conditions without treatment. Meanwhile, the positive control group that experienced hypercholesterolemia without treatment had higher cholesterol levels, namely 189.3 mg/dL. The control group experienced a decrease in cholesterol levels of up to 117.7 mg/dL, suggesting that the treatment was effective in lowering cholesterol levels. In the treated group, the group given turmeric leaf extract also experienced a decrease in cholesterol levels with levels of 168.8 mg/dL at a dose of 14 mg/g BW, 163.2 mg/dL at a dose of 28 mg/g BW, and 155.8 mg/dL at a dose of 56 mg/g BW.

Table 2. Cholesterol level test in male mice given turmeric leaf extract

Animal Test	Mouse Cholesterol Level (mg/dl)					
	Negative Control	Positive Control	Drug Control	Dose 1 (14 mg/g BW)	Dose 2 (28 mg/g BW)	Dose 3 (56 mg/g BW)
1	120.9	191.2	130.6	168.3	175.6	156.9
2	128.5	189.3	128.5	156.9	163.9	157.2
3	102.4	196.5	120.6	179.1	159.7	145.7
4	99.3	194.7	117.5	165.8	164.2	169.3
5	116.8	183.7	96.2	172.4	154.7	147.3
6	111.5	180.4	112.5	170.2	160.8	158.1
Average	113.2	189.3	117.7	168.8	163.2	155.8

The results of cholesterol levels are shown in **Table 2**, which shows an increase in cholesterol levels in the positive control group. This indicates that the administration of propylthiouracil successfully raises cholesterol levels in male mice. The average normal cholesterol level of male mice is between 40–130 mg/dL (Rumtal et al., 2019). The administration of propylthiouracil can increase blood cholesterol levels and induce hypercholesterolemia in mice. Hypercholesterolemia can also be caused by several factors, including body weight, gender, age, lack of exercise, emotional stress, metabolic disorders, genetic disorders, as well as the consumption of foods containing carbohydrates, fats, and proteins (Jempormase et al., 2016).

In this study, the control group received the drug simvastatin. Simvastatin is a generic drug used to treat hyperlipidemia, lower cholesterol and bad fats (such as LDL and triglycerides), and increase good cholesterol (HDL) in the blood. This drug was chosen as a comparator because simvastatin belongs to the statin group, which is one of the first drugs often used in the therapy of hyperlipidemia (Setiawan et al., 2023). Simvastatin works by inhibiting the synthesis of endogenous cholesterol in the liver through the inhibition of the HMG-CoA enzyme, leading to a significant decrease in total cholesterol levels (Dea Ayu Sri Wulansari, 2025; Iqbaal Fauzi Arfani, 2025; Rara Aditya Santoso, 2025). This drug control is used to compare and provide a clear picture of the

decrease in blood cholesterol levels in mice. Additionally, simvastatin is readily available, inexpensive, and easily accessible.

Based on statistical test data, starting with the normality and homogeneity test methods, it is evident that the significance value in the cholesterol level results indicates the data is in regular and homogeneous conditions, as the p-value is greater than 0.05. Furthermore, to determine the effect of turmeric leaf ethanol extract on reducing hypercholesterolemia levels in mice, a statistical analysis was performed using the ANOVA test. The results of the analysis showed a significance value of 0.000, indicating a significant difference in reducing hypercholesterolemia levels in mice after administration of turmeric leaf extract, as the p-value was less than 0.05.

The effect of turmeric leaf ethanol extract (*Curcuma longa*) in reducing hypercholesterolemia levels is attributed to the compounds contained in turmeric leaves, particularly flavonoids, which possess antioxidant properties and help ward off free radicals in the body. This compound is suspected to have antihypercholesterolemic activity, as well as a role in improving the endothelial function of blood vessels and lowering LDL levels in the blood. A decrease in total cholesterol levels can occur due to the inhibition of cholesterol synthesis in the liver, caused by the inhibition of the HMG-CoA enzyme, which is also the mechanism of action of simvastatin (SUWAIBAH, 2021).

Table 3. Comparison of mouse cholesterol levels (mg/dl) after administration of turmeric leaf extract

Treatment	Reduction of Cholesterol Levels in Mice Turmeric Leaf Extract (mg/dl)
Simvastatin Drug Control	71.7
Dosis 1 (14 mg / 20 g BW)	20.5
Dosis 2 (28 mg / 20 g BW)	26.2
Dosis 3 (56 mg / 20 g BW)	33.6

Table 3 presents a comparison of the reduction in mouse cholesterol levels (mg/dL) after administration of turmeric leaf extract at various doses and the control drug simvastatin. From the table, it can be seen that the highest reduction in cholesterol levels occurred in the control group treated with simvastatin, with a decrease of 71.7 mg/dL. Meanwhile, in the group given turmeric leaf extract, dose 3 (56 mg/20 g BW) showed a decrease of 33.6 mg/dl, followed by dose 2 (28 mg/20 g BW) with a decrease of 26.2 mg/dl, and dose 1 (14 mg/20 g BW) which provided a decrease of 20.5 mg/dl. This data pattern suggests that the higher the dose of turmeric leaf extract administered, the greater the decrease in cholesterol levels in mice. However, none exceeded the effectiveness of simvastatin as a drug control.

4. CONCLUSION

This study aims to investigate the potential of turmeric leaf ethanol extract (*Curcuma longa*) in reducing cholesterol levels in mice (*Mus musculus*) using a hypercholesterolemia model. The results showed that turmeric leaf ethanol extract at a dose of 56 mg/20 g BW showed a reduction in mouse cholesterol levels by 33.6 mg/dl, which is higher than other doses. The study demonstrated that the dose of turmeric leaf ethanol extract could significantly reduce cholesterol levels in mice, potentially serving as a natural therapeutic alternative for hypercholesterolemia.

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