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The impact of Kersen leaves (*Muntingia calabura* L.) extract on Mus musculus with hypercholesterolemia

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ABSTRACT

Background: Hyperlipidemia, a condition marked by elevated levels of total cholesterol and related lipids, poses significant health risks. The potential cholesterol-lowering effects of kersen leaves (*Muntingia calabura* L.), which are rich in flavonoids, saponins, and tannins, warrant investigation. Despite the recognized benefits of kersen leaves, its potential, particularly from the East Nusa Tenggara (NTT) region, still needs to be explored.

Objective: This study aimed to assess the effect of kersen leaves extract, sourced from arid regions of NTT, on hypercholesterolemia in *Mus musculus*.

Methods: Healthy male white mice (*Mus musculus*) were used in the experiment and divided into five groups: a normal group, a negative control group, and three treatment groups receiving a high-cholesterol diet supplemented with kersen leaves extract at doses of 75, 150, and 300 mg/kg body weight, respectively. Total cholesterol levels were measured using the Easy Touch GCU monitoring system. Statistical analysis was conducted using the paired T-test to compare the pre and post data in each group.

Results: A high-cholesterol diet for 14 days significantly increased body weight and total cholesterol levels in male white mice. Meanwhile, treatment with kersen leaves extract at 300 mg/kg body weight effectively mitigated the elevation in cholesterol levels.

Conclusion: Kersen leaves extract significantly reduced hypercholesterolemia in Mus musculus, suggesting its potential as a natural therapeutic agent for cholesterol management. Further research is necessary to elucidate the underlying mechanisms of action and evaluate its clinical applicability.

Keywords: hypercholesterolemia, kersen leaves extract, *Mus musculus*

Introduction

Hyperlipidemia is a pathological condition characterized by elevated levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL), along with reduced levels of high-density lipoprotein (HDL) [1]. Numerous studies have demonstrated that antioxidant compounds derived from herbal extracts can effectively lower cholesterol levels. However, the geographical conditions in which these plants are cultivated significantly influence their bioactive efficacy [2]. Environmental factors, such as dry land climates, can induce adaptive responses in plants, resulting in variations that may impact their therapeutic potential [3,4]. Nusa Tenggara Timur (NTT), an area characterized by a dry climate, presents such conditions that could affect the quality and cholesterol-lowering effectiveness of herbal plants grown there.

According to the 2010 National Workshop on Indonesia Medicinal Plants, conducted by the Ministry of Forestry of Indonesia, the country is home to a diverse range of medicinal plants with health benefits. Among these is the kersen leaves (*Muntingia calabura* L.), which is well-

adapted to dry land regions. In NTT, the local population traditionally utilizes kersen leaves to treat various ailments by boiling the leaves and consuming the resulting decoction. This practice underscores the potential medicinal value of kersen leaves, particularly in arid climates where the plant thrives in abundance [5].

Research has further highlighted the antihyperlipidemic properties of various plant extracts. For example, Khera and Bhatia demonstrated that *Woodfordia fruticosa* extract significantly improved blood lipid profiles in hypercholesterolemic mice, showcasing its potential in managing dyslipidemia [5]. Similarly, Purpasari's study found that kersen leaves extract effectively reduced lipid levels in *Mus musculus* subjected to a high-fat diet, emphasizing the bioactive compounds like flavonoids, saponins, and tannins in kersen leaves that contribute to their lipid-lowering effects [6,7].

This study investigates whether kersen leaves traditionally consumed by NTT people and grown in dryland areas, exhibit similar efficacy in reducing total blood cholesterol levels. The primary objective is to evaluate the effects of kersen leaves extract on *Mus musculus* with experimentally induced hypercholesterolemia.

Methods

Experimental design

Kersen leaves used for the maceration process were collected from the Maulafa Village in Kupang City. The evaporation process was carried out at the Bioscience Laboratory of the University of Nusa Cendana. After that, the phytochemical analysis of the kersen leaves extract was conducted at the Widya Mandira University Chemical Laboratory. The adaptation and treatment of *Mus musculus* were performed at the Laboratory of the Faculty of Medicine, University of Nusa Cendana. The study, conducted from October 20, 2021, to November 22, 2021, received ethical approval from the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nusa Cendana, under the approval number 061/KEH/SK/XI/2021.

Animal model

Healthy male white mice (*Mus musculus*) weighing approximately 20–25 grams were selected for the study. The mice were acclimated to the laboratory environment for one week prior to the commencement of the experiment. The subjects were then randomly assigned to five groups (five mice in each group): a normal group, a negative control group, and three treatment groups. The normal group received a standard diet, while the negative control group was fed a high-cholesterol diet for 14 days without any treatment. The three treatment groups were also fed a high-cholesterol diet but were administered kersen leaves extract at 75, 150, and 300 mg/kg body weight, respectively.

Preparation of kersen leaves extract

Kersen leaves were collected from the vicinity of Kupang City, East Nusa Tenggara, with a total initial weight of 5 kilograms. The leaves were dried and ground, producing 3 kilograms of dry kersen leaves powder. This powder was then combined with 5 liters of 70% ethanol and allowed to macerate for three days in a dark environment, with agitation every 8 hours. After the maceration process, the mixture was filtered using filter paper, yielding 2 liters of kersen leaves extract. This extract was subsequently evaporated at the Undana Bioscience Laboratory using a rotary evaporator, resulting in a final extract weight of 50.87 grams. A phytochemical analysis was then conducted on the extract to determine its compound composition.

Total cholesterol level measurement

Blood samples were collected from male white mice (*Mus musculus*) following the induction of hypercholesterolemia and subsequent treatment with kersen leaves extract. Total cholesterol levels were measured using the Easy Touch GCU monitoring system, a portable device designed for rapid and accurate blood cholesterol testing.

Table 1. Phytochemical test result of kersen leaves extract

No	Compounds	Intepretation
1	Alkaloid	+
2	Flavonoid	+
3	Tanin	+
4	Terpenoid	+
5	Saponin	+

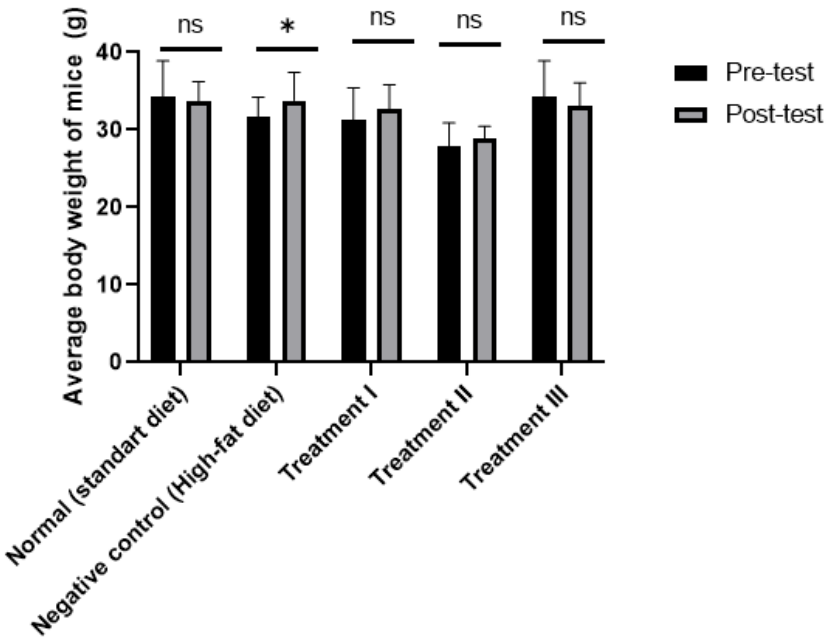

Figure 1. Mean weight of white mice before and after treatment. Data are expressed as mean ± standard error of the mean (SEM). Asterisk denotes a statistically significant (P ≤ 0.05) difference between means of weight between pre- and post-treatment; ns: not significant

Stastical Analysis

Statistical analysis was conducted using SPSS version 28. A paired samples t-test was employed to compare weights and cholesterol levels between groups, following normality testing using the Shapiro-Wilk test and homogeneity testing using Levene's test. The significance level was set at $p < 0.05$.

Results

This experimental study evaluates the effects of kersen leaves extract on hypercholesterolemia in male white mice (*Mus musculus*). The research focuses on kersen leaves, which are well-adapted to dry land regions such as Nusa Tenggara Timur (NTT). A qualitative phytochemical analysis was performed to identify the compounds present in the kersen leaves extract. The analysis revealed that the extract contained positive indicators for various classes of compounds, including alkaloids, flavonoids, tannins, terpenoids, and saponins (Table 1).

There were differences in the weights of the mice measured before and after treatment across the groups. The negative control group, which was fed a high-fat diet supplemented with quail egg yolk, exhibited significant weight gain. In contrast, the remaining groups showed varying changes in body weight, with some groups experiencing increases and others decreases (Figure 1).

Figure 2 illustrates a significant increase in total cholesterol levels in the negative control

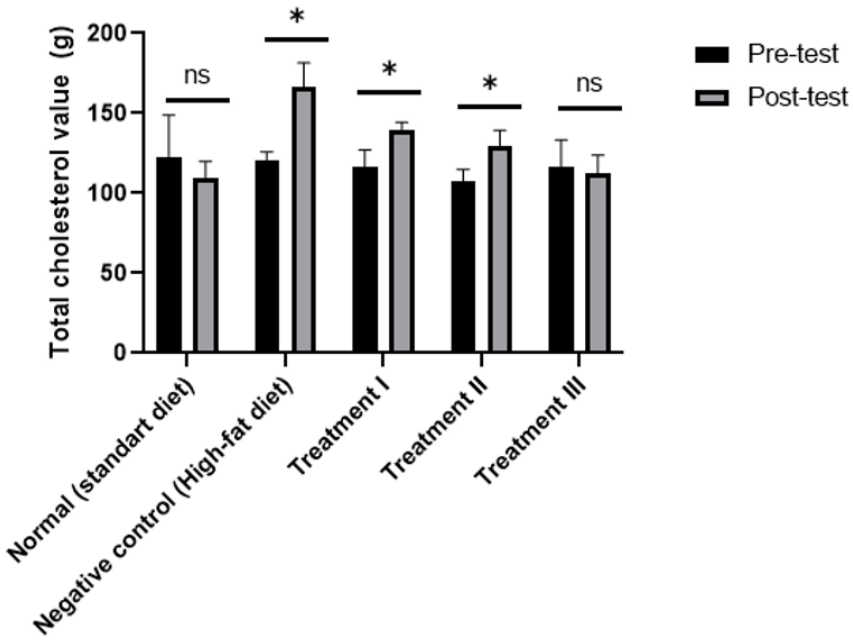

Figure 2. Total cholesterol value before and after treatment. Data are expressed as mean ± standard error of the mean (SEM). Asterisk denotes a statistically significant (P ≤ 0.05) difference between means of weight between pre- and post-treatment; ns: not significant

group, as well as in treatment groups 1 and 2, compared to the normal group. The statistical test comparing total cholesterol levels in the negative control group before and after a high-cholesterol diet showed a significance level of $p < 0.05$. This result confirms a significant increase in total cholesterol attributable to the high-cholesterol diet induced by quail egg yolk. Additionally, this study reveals varying degrees of change in average total cholesterol across each treatment group. All three treatment groups demonstrated decreases in total cholesterol levels relative to the negative control group, with the magnitude of decrease corresponding to the increasing doses of the treatment. In the 75 mg/kg and 150 mg/kg dose groups, although a reduction in total cholesterol levels was observed compared to the negative control group, the changes remained statistically significant between pre-and post-treatment measurements. In contrast, at the 300 mg/kg dose, total cholesterol levels remained relatively unchanged between pre-and post-treatment, indicating that kersen leaves extract at this dose provided the most effective reduction in total cholesterol in this study.

Discussion

This experiment demonstrated the anti-cholesterol effects of kersen leaves extract on total cholesterol levels in male white mice (*Mus musculus*), showing that kersen leaves extract can effectively reduce cholesterol levels. Kersen leaves were selected for their high content of active compounds—saponins, tannins, and flavonoids—known for their cholesterol-lowering properties through distinct mechanisms. Phytochemical tests confirmed the presence of alkaloids, saponins, tannins, flavonoids, and terpenoids in the extract, consistent with findings from Widjaya's research on kersen leaves, which identified similar active compounds [7]. These results suggest that kersen leaves from dry land areas possess the same beneficial compounds as those from other regions, demonstrating potential effects on mice's total cholesterol levels and body weight [8-10].

The experiment demonstrated an increase in body weight among mice in the negative control and treatment groups, consistent with the findings of Nastiti [11], who reported significant differences in body weight and adipose tissue weight between hypercholesterolemic and control rats. The observed

weight gain in hypercholesterolemic mice is likely attributed to the high-calorie intake from quail egg yolks, as excess energy is stored in adipose tissue as fat [12]. In this study, the increase in body weight in the positive control group paralleled the rise in cholesterol levels, further indicating the development of hypercholesterolemia. White mice are considered hypercholesterolemic if their total blood cholesterol level exceeds 130 mg/dl. In this study, total cholesterol levels were measured before and after administering quail egg yolk for 14 days in all groups except the negative control. The significant increase in cholesterol levels observed in the negative control group ($p < 0.05$) confirmed the effectiveness of quail egg yolk in inducing hypercholesterolemia, aligning with findings from previous studies on the relationship between egg yolk consumption and elevated cholesterol levels [13].

After 14 days of treatment, an increase in total cholesterol levels was observed in all groups compared to the normal group. Although treatment groups one, two, and three exhibited elevated cholesterol levels, a reduction in total cholesterol was noted with increasing doses of kersen leaves extract. The Bonferroni post hoc test confirmed significant differences between the treatment groups and the negative control group. The finding is consistent with the studies conducted by Puspasari on the impact of different doses of kersen leaves extract on cholesterol levels [6]. Therefore, higher doses of kersen leaves extract have a significant impact in lowering the overall levels of cholesterol in mice.

The decrease in cholesterol levels observed in the treatment groups might be affected by the antioxidant compounds in kersen leaves [7]. Flavonoids contribute to cholesterol reduction by inhibiting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase [14]. Saponins may indirectly affect cholesterol absorption and excretion through their interactions with the gut microbiota [15]. Tannins may reduce cholesterol levels by altering the gut microbiota diversity and modulating cholesterol metabolism pathways [16,17]. However, the precise mechanism by which kersen leaves extract reduces cholesterol levels remains to be fully elucidated. While the antioxidant compounds in kersen leaves—such as flavonoids, saponins, and tannins—have been shown to influence cholesterol reduction through various pathways, further research is needed to comprehensively understand the underlying mechanisms.

Conclusion

This study identified significant differences in total cholesterol levels in mice both before and after administration of a high-cholesterol diet and between groups before and after treatment with kersen leaves extract. Among the treatment groups, the 300 mg/kg body weight dosage was the most effective, demonstrating significantly reduced cholesterol levels compared to the negative control group. It is advisable to explore further research to confirm kersen leaves extract's optimal dosage and long-term efficacy in more extensive and diverse animal models. Additionally, investigating the underlying mechanisms by which kersen leaves extract reduces cholesterol levels could provide valuable insights for potential therapeutic applications in managing hypercholesterolemia. Future studies should also consider assessing the safety and effectiveness of kersen leaves extract in human clinical trials to validate its potential as a natural remedy for cholesterol reduction.

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Authors Contributions

Conceptualization: SB and ALSA; Methodology: SB and ALSA; Data curation: SB; Formal analysis: SB, AT, and RLN; Writing-original draft: SB; Supervision: ALSA, AT, and RLN.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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