Research article

Ethanolic extract of *Muntingia calabura L.* as an antihypercholesterolemic by reducing malondialdehyde (MDA) levels in white mice (*Mus musculus*)

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ABSTRACT

Introduction and Aim: A diet high in cholesterol causes hypercholesterolemia by elevating plasma cholesterol levels. Hypercholesterolemia causes an increase in cholesterol concentration within cells, resulting in membrane-altering lipid peroxidation. Malondialdehyde (MDA) is produced during lipid peroxidation to form peroxides and other free radicals. The present study aims to evaluate the potential of *Muntingia calabura* L. ethanolic extracts as a hypercholesterolemia agent by reducing MDA levels in hypercholesterolemic white mice (*Mus musculus*).

Materials and Methods: The present study has a true experimental design with a control group consisting only of post-test samples. The research utilized approximately 25 white mice *(Mus musculus)* randomly. These white mice were separated into five groups, consists of negative control (C1), positive control (C2), *M. calabura* L. ethanolic extracts at doses of 13 mg/20g/BW (C3), 26 mg/20g/BW (C4), and 52mg/20g/BW (C5). The concentration of MDA was measured on the 21st day of treatment using the TBARS method.

Results: The results indicated that the *M. calabura* L. extracts significantly ($p<0.05$) reduce the total cholesterol and MDA levels in the blood of white mice (*Mus musculus*). The evidence supporting this conclusion is based on the data obtained from mice that received various doses of *M. calabura* L leaf extract, specifically 13mg/20g/BW, 26mg/20g/BW, and 52mg/20g/BW. These doses already demonstrated a substantial reduction in MDA levels following the treatment.

Conclusion: In this research, it was found that the ethanolic extract of *M. calabura* L leaves effectively acted as an anti-hypercholesterolemic agent in mice with hypercholesterolemia. The assessment of its anti-hypercholesterolemic properties was based on the observation of reduced MDA levels. It is suggested that the leaf extract of *M. calabura* L contains a compound known as phytol, which is believed to have the capacity to inhibit hypercholesterolemia in white mice *(Mus musculus)*.

Keywords: *Muntingia calabura*; Hypercholesterolemia; malondialdehyde.

INTRODUCTION

High levels of cholesterol in the bloodstream have long been identified as a significant factor contributing to the onset of atherosclerosis, a condition linked to coronary heart disease (CHD; 1,2). An established connection exists between increased serum cholesterol and the development of CHD, with dietary adjustments capable of diminishing this risk. Low-density lipoprotein (LDL), the primary transporter of cholesterol in the bloodstream, seems to play a part in the progression of multiple degenerative ailments, including atherosclerosis, cancer formation, the aging process, and diabetes. Some researchers have proposed that the oxidative alteration of LDL plays a pivotal role in the advancement of atherosclerotic changes. This oxidative transformation of LDL changes its structure, making it susceptible to uptake by scavenger receptors found on macrophages, endothelial cells, and smooth muscle cells. This process leads to the creation of foam cells that accumulate lipids, a distinctive feature in the early stages of atherosclerotic lesions (3,4). Persistent elevation in blood cholesterol levels leads to an increase in oxidative stress and the generation of reactive oxygen species (ROS). ROS triggers the oxidation of cell membranes, giving rise to the production of Malondialdehyde (MDA). MDA is the end product of lipid peroxidation and can exist freely or bind to tissues. Additionally, MDA can be seen as a breakdown product of amino acids, carbohydrate complexes, pentose, and hexose. Plasma MDA levels can serve as an indicator of free radical activity (5,6). Lipid peroxidation encompasses a process in which free radicals and polyunsaturated fatty acids engage in a chain reaction. These reactions lead to changes in the structure of double bonds in conjugated dienes, the formation of hydroperoxides, and the degradation of lipids into smaller molecular fragments like ketones, alcohols, hydrocarbons, acids, and epoxides. Furthermore, these reactions result in chemical alterations in the Apo-B protein. The extent of lipid peroxidation can be assessed by measuring thiobarbituric reactive substances (TBARS). Malondialdehyde, a by-product of lipid hydroperoxide

degradation, is associated with thiobarbituric acid (TBA) levels (6-8).

There has been a growing fascination with natural products and their potential to maintain and improve health and well-being. The cholesterol-lowering impact of various plant-based foods has been thoroughly investigated, and several plants have been proven to effectively lower plasma cholesterol levels while maintaining favorable safety profiles. Therefore, consuming plants is an effective way to prevent diseases such as atherosclerosis (9,10). It requires no special care to cultivate *M. calabura* L in Indonesia, which has a variety of soil conditions. The presence of flavonoids, tannins, triterpenes, saponins, and polyphenols in *M. calabura* L indicates antioxidative activity. The inhibition of lipid peroxides caused by free radicals is decreased, so the function of the cell membrane is maintained. Recent research has shown that various species of *M. calabura* L contain antioxidant-active compounds such as flavonoids, xanthones, and phenolics (11). This study aims to determine the potential antihypercholesterolemic activity of *M. calabura* L leaf in hypercholesterolemia white mice (*Mus musculus*) by decreasing MDA levels.

MATERIALS AND METHODS

This research employs a genuine experimental framework involving both pre and post-tests along with a control group. The study involved the use of 25 five-week-old *Mus musculus* from the Faculty of Veterinary Medicine Laboratory in Indonesia, with a weight ranging from 30 to 40 grams (five animals in each group). In the initial week of the adaptation period, the mice were relocated to new cages with rice husks as the bedding material. During this acclimatization phase, they were provided with water and food pellets as their regular diet, amounting to 3-4 grams of food per day. *Muntingia calabura* L plants were gathered from Kupang Regency in East Nusa Tenggara, Indonesia. The extraction process started with the sorting of *Muntingia calabura* L. leaves, followed by weighing and thorough cleansing. The leaves were then dried for 24 hours, cut into small pieces, and baked at 70°C for a further 24 hours. After drying, the leaves were weighed and ground to a powdery consistency. The powdered leaves were then macerated three times in 1 litre of 96% ethanol for 24 hours. The macerate was filtered using a Buchner funnel to obtain an ethanol filtrate. This filtrate was then concentrated at 50°C using a rotary evaporator to yield a thick extract. To complete the process, the mixture was shaped and thickened with a 1% solution of sodium carboxymethyl cellulose. The mice were randomly divided into five groups: a negative control (C1), a positive control (C2) and three treatment groups (C3, C4 and C5), each of which received *Muntingia calabura* L. ethanolic extracts at different doses. The concentration of MDA was measured on day 21 using the TBARS method. The treatment was administered to male white mice (*Mus musculus*) fed standard diets over a 21-day period. The negative control group (C1), consisting of male white mice with hypercholesterolemia, received standard food for 21 days. The positive control group (C2), composed of hypercholesterolemic male mice, was given atorvastatin at a dose of 5 mg/kg/BW per day for 14 days, starting on day 7 and ending on day 21. For group C3, male white mice with hypercholesterolemia received ethanolic extracts of *Muntingia calabura* L at a dose of 13mg/20g/BW per day from day 7 to day 21. Similarly, the treatment groups C4 and C5, comprising hypercholesterolemic male mice, were administered ethanolic extracts of *Muntingia calabura* L at doses of 26 mg/20g/BW and 52 mg/20g/BW daily from day 7 to day 21. On the 28th day, ten hours after the last dose, the mice were anesthetized subcutaneously with 0.50 ml of ketamine and 0.40 ml of diazepam. This involved the taking of both blood and tissue samples. The TBARS method was used to determine MDA concentrations in a series of steps. Initially, blood was obtained from the retro-orbital sinus for the determination of MDA levels. In a polypropylene container, 0.75 ml of folic acid was combined with 0.25 ml of a solution containing thiobarbituric acid (TBA). To this mixture 0.05 ml of blood serum was added, followed by an infusion of 0.45 ml of water. The contents were stirred vigorously for two minutes and then heated in a water bath set at 100°C for one hour. After allowing the mixture to cool for 1-2 hours to a temperature of 30°C, it was passed through a Sep-pak C-18 column and thoroughly washed with 5 ml of a solution of methanol and water. Finally, to facilitate the determination of the MDA concentration by the TBARS method, 4 ml of methanol was transferred to a special cuvette and the intensity of the color produced was measured at a specific wavelength of 532 nm using a spectrophotometer.

Ethical clearance

The research in this study has received ethical approval from the Health Research Ethics Committee at the Faculty of Medicine, Universitas Nusa Cendana, under the reference number 016/KEH/SK/II/2022.

Statistical analysis

The statistical analysis was conducted using ANOVA (Analysis of Variance). The ANOVA examination demonstrated statistically significant findings, indicating notable distinctions between the groups, as indicated by a p-value of 0.05. Post-hoc tests, in particular the Least Significant Difference (LSD), were then used to assess the extent of the variation between the groups.

RESULTS

Total cholesterol values represent the average measurements taken over a span of 14 days for each group, all of which were subjected to a high-

cholesterol diet for seven days. Tables 1 and 2 contain the detailed data regarding total cholesterol levels and MDA levels that were collected throughout the study.

Table 1: Mean cholesterol levels of mice

Group	Mean cholesterol level (mg/dL)
Negative control	122.8*
Positive control	120.2*
Group 1	116.2*
Group 2	112.4*
Group 3	116.2*

Normal value: 10-54 mg/dL ***High value***

Table 1 summarizes the mean cholesterol levels (mg/dL) in various groups of mice, with an emphasis on the notable elevation of these values compared to the normal range for mice, typically between 10-54 mg/dL. Every group, including the positive and negative control groups showed elevated cholesterol levels, implying the influence of a high fat diet given to the mice prior to treatments, were significant.

Table 2: Mean MDA levels of mice

Group of animal tests	Average MDA levels of mice (µM)	P value
Negative control	0.0146	
Positive control	0.0636	0.812
Group 1	0.0524	
Group 2	0.0448	
Group 3	0.0222	

*one-way ANOVA: $p<0,05$. [a] post-hoc LSD : $p<0,05$

Table 2 provides insights into the mean malondialdehyde (MDA) levels in different groups of mice. Notably, the positive control group stands out with a significantly higher average MDA level due to the Atorvastatin given in the treatment. In addition, Group 1, Group 2, and Group 3 all exhibit higher MDA levels in comparison to the negative control. Table 2 also indicates that a One-Way Analysis of Variance (ANOVA) test did not find any statistically significant differences in MDA levels among the various groups. The p-value for this test was greater than 0.05, indicating that there is no significant distinction in MDA levels between the groups. Consequently, there was no need for a post hoc least significant difference (LSD) analysis since the initial ANOVA did not reveal any significant differences. The intriguing outcome observed in the negative control group, where MDA levels were notably lower than both the positive control and treatment groups, can be attributed to a multitude of potential factors including human error, environmental conditions, and the precision of calibrated tools used in the experiment. However, this does not alter the observation that *M. calabura* L. displayed potential in mitigating hypercholesterolemia.

DISCUSSION

This study demonstrated that *M. calabura* L leaves extract inhibited hypercholesterolemia in male white mice (*Mus musculus*) fed with a high-cholesterol diet. The MDA levels in the treatment group, which received different doses of *M. calabura* L (13 mg/ 20g/BW, 26mg/20g/BW, and 52mg/20g/BW), showed a consistent and notable reduction in MDA levels after a 14-day treatment period. This indicates a potentially beneficial impact of *M. calabura* L on lowering oxidative stress markers over time. Indeed, this is likely due to the antioxidant properties of the leaf extract of *M. calabura* L, which mitigate the effect of free radicals on white mice consuming a high-cholesterol diet.

The antioxidant system in the body protects against free radicals by attempting to maintain a balance with the oxidation reaction. The input of antioxidant exogen from the leaf extract of *M. calabura* L will protect against free radical reaction. Therefore, there was no further lipid peroxidation, which will reduce the formation of MDA (12-15). Triglyceride was produced by activating acyl-CoA from fatty acids. These fatty acids are derived from triglyceride fatty acids synthesized in the liver that are transported in VLDL (lipoprotein) and glycolysis-derived lipogenesis in fatty tissue. Consequently, there are always two contributing factors to this condition. The first metabolic pathway, lipolysis, yields fatty acid and glycerol. Triglyceride is formed by activating and re-esterifying this fatty acid. Due to lipolysis, Glycerol could not be utilized effectively in the absence of glycerol kinase. Due to excessive triglyceride accumulation in fatty tissue, the second pathway is esterification, which leads to obesity (16).

In certain instances, lipolysis is greater than esterification, causing fatty acids to accumulate in blood fatty tissue (15). Blood fatty acid accumulation is transported as a complex of fatty acid and albumin. This complex chemical will influence the metabolic processes of all tissues. Like palmitic acid, n-hexadecanoic acid is a saturated fatty acid compound. It has no effect on the hypercholesterolemia process because it has no effect on the lipolysis process (14). In contrast, the phytol content of *M. calabura L* leaf extract is hypocholesterolemic and decreases LDL cholesterol. However, the mechanism underlying this activity is poorly understood. Probably because the leaf extract inhibits cholesterol micellization during digestion in the small intestine (16,17). Consequently, this will reduce the amount of cholesterol absorbed by enterocyte cells. The leaf extract of *M. calabura* L likely inhibited cholesterol absorption in mice, reduced bile acid reabsorption, and synthesized cholesterol. This is the result of the interaction between saponin and bile acid, which produces a large mixture of stable micelles in mice that cannot be absorbed by the small intestine, thus cholesterol is eliminated in the feces.

Inhibition of bile acid reabsorption from the small intestine stimulates the metabolism of cholesterol in

the liver, which is then converted to bile acid (17). Inhibition of reabsorption of bile acids from the intestine spurs cholesterol metabolism in the liver then converts them into bile acids. Indirectly or directly, phytol inhibits cholesterol absorption in general. Direct inhibition of absorption occurs in the small intestine, and inhibition of bile acid reabsorption through enterohepatic circulation results in indirect inhibition of absorption.

CONCLUSION

In this research, it was found that the ethanolic extract of *M. calabura* L leaves effectively acted as an anti-hypercholesterolemic agent in mice with hypercholesterolemia. The assessment of its anti-hypercholesterolemic properties was based on the observation of reduced MDA levels. It is suggested that the leaf extract of *M. calabura* L contains a compound known as phytol, which is believed to have the capacity to inhibit hypercholesterolemia in white mice (*Mus musculus*).

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CONFLICT OF INTEREST

There is no conflict of interest found during this study.

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