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THE EFFECT OF MALAKA LEAF EXTRACT (*Phyllanthus emblica L.*) ADMINISTRATION ON TOTAL CHOLESTEROL LEVELS IN FEMALE MICE (*Mus musculus*) ON A HIGH-SATURATED-FAT DIET

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Abstract

Background: cholesterol is a fatty substance circulating in the blood, yellowish in color and in the form of wax, which is produced by the liver and is needed by the body. High cholesterol increases a person's risk of developing narrowing of the arteries or atherosclerosis, blood clots in certain parts of the body, strokes and heart attacks. One plant that is thought to have antihypercholesterol activity is Malacca leaf (Phyllanthus emblica L.). Malacca leaf extract works as an inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoAR) so that cholesterol synthesis decreases.

Objective: to determine the effect of Malacca leaf extract (Phyllanthus emblica L.) on total cholesterol levels in mice (Mus musculus) females with a diet high in saturated fat.

Methods: this study is experimental laboratory using the design method of Randomized control group pretest & post test design. Samples in this study using mice (Mus musculus) female sex. Mice are divided into 4 groups, namely P0, P1, P2 and P3. Group P0 was given standard feed, Group P1 was given standard feed and quail egg yolk, group P2 was given standard feed, quail egg yolk and Malacca leaf extract, group P3 was given standard feed, quail egg yolk and orlistat drug.

Results: the average percentage of increase in total cholesterol levels in the group given Malacca leaf extract has a percentage of increase in total cholesterol levels is smaller than the other groups of 3%.

Conclusion: the administration of Malacca Leaf Extract can prevent the increase in total cholesterol levels in mice.

Keywords: **Cholesterol, Malacca leaf extract, Quail egg yolk, Orlistat**

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INTRODUCTION

Cholesterol is a type of fat found in the blood; it is yellowish in color and has a waxy texture. It is produced by the liver and plays a vital role in the body.[1] Blood cholesterol originates from two main sources: approximately 15% is obtained through dietary intake, while the remaining 85% is synthesized in the liver from acetyl-CoA. As it circulates in the blood, cholesterol is transported by molecules known as lipoproteins. In humans, there are six distinguishable types of lipoproteins: *High-Density Lipoprotein* (HDL or α-lipoprotein), *Very Low-Density Lipoprotein* (VLDL or pre-β-lipoprotein), *Intermediate-Density Lipoprotein* (IDL), *Low-Density Lipoprotein* (LDL or β-lipoprotein), and small lipoprotein a [Lp(a)].[2] Hypercholesterolemia is a condition characterized by elevated cholesterol levels in the blood.[3] Hypercholesterolemia is a condition in which total blood cholesterol levels exceed 240 mg/dl.[4]

According to estimates by the World Health Organization (WHO), the global prevalence of hypercholesterolemia is approximately 45%. Meanwhile, in Southeast Asia, the figure is around 30%, while in Indonesia it is estimated to be 35%.[5] In Indonesia, the proportion of individuals aged 25–34 with hypercholesterolemia is 9.3% and increases with age, reaching 15.5% in the 55–64 age group. Based on the results of the 2018 Riskesdas survey, the prevalence of high cholesterol among Indonesians is higher in women, at 20.7%, compared to men at only 6.8%.[6]

High cholesterol levels can increase an individual's risk of developing narrowed arteries (atherosclerosis), blood clots in certain areas, and the risk of stroke and heart attack. In addition, high cholesterol can also cause pain in the front of the chest or arms, especially when a person is under stress or engaging in physical activity.[7]

Currently, lowering cholesterol levels is generally achieved by using statin medications, such as atorvastatin and simvastatin, or bile acid-binding agents, such as cholestyramine and colesevelam. These medications fall under the category of synthetic drugs. Based on clinical research, the use of statins is known to cause various side effects, including cognitive impairment, neuropathy, pancreatic, dysfunction, liver damage, and sexual dysfunction.[8] This is why people today tend to opt for natural remedies, as they are considered safer, more affordable, and made from ingredients that are more readily available in their local environment compared to synthetic drugs.[9,10]

An example of a plant believed to have anti-hypercholesterolemic effects is the malaka leaf (Phyllanthus emblica L.). This plant is frequently used by the general public in traditional medicine. In India, this plant has long been used to treat various diseases, such as cancer, diabetes, liver disorders, heart problems, and anemia.[11] Malaka leaves contain various chemical compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, benzenoids, phenols, lignin, furanolactones, teriterpenes, and carbohydrates.[11] Flavonoids are natural polyphenolic compounds that act as antioxidants and can be found in various plants, fruits, and beverages such as tea and wine. These compounds help reduce cholesterol and triglyceride levels in the blood, protect arteries from damage, and decrease cholesterol buildup in the endothelial lining of arterial blood vessels. Research in experimental animals, particularly mice, has demonstrated that

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flavonoids can inhibit lipid peroxidation. Meanwhile, in vitro studies have shown that flavonoids act as inhibitors of the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoAR), which contributes to a reduction in cholesterol synthesis. Some theories suggest that saponins form insoluble complexes with dietary cholesterol, interact with bile acids to form micelles, and increase cholesterol binding to dietary fiber, thereby preventing its absorption in the intestine. In addition, the tannins found in Malaka leaves are believed to inhibit fat absorption in the intestine by interacting with mucosal proteins and intestinal epithelial cells.[12]

Based on the preceding explanation, this study aims to investigate the anticholesterol activity of Malaka leaf extract (Phyllanthus emblica L.) in female mice (Mus musculus) fed a diet high in saturated fat.

RESEARCH METHODS

This study is a laboratory experiment using a randomized controlled pretest-posttest design. The samples used in this study were female mice (Mus musculus).

The sample size for this study was calculated using Federer's formula for experimental sample size as follows:

$p(n-1) \geq 15$

p = total number of treatments; n = total number of replicates for each treatment

In this study, p = 4, so the total number of replicates is:

$4(n-1) \geq 15$

$4n - 4 \geq 15$

$4n \geq 19$; since $n \geq 4.75$, this is rounded up to $n \geq 5$.

Each treatment requires a sample of 5 mice. As a precaution against possible mouse deaths during the adaptation and treatment periods, 1 spare mouse is added to each treatment. Thus, the total number of mice required is 6 per treatment multiplied by 4, for a total of 24 mice.

Equipment

This study utilized various equipment, including an analytical balance, a rotary evaporator, a probe, 1-ml syringes, a blood cholesterol measurement kit (Easy Touch), and commonly used laboratory glassware.

Materials

Malaka leaf powder from PT. MUSTIKA FAMILY NUSANTARA, 70% ethanol for maceration, female mice, a high-saturated-fat diet consisting of quail egg yolks, standard feed, and 120 mg of Orlistat.

Preparation of Malaka Leaf Extract

The Malaka leaf powder was placed in a sealed container and extracted through a maceration process using 70% ethanol at a 1:3 ratio for three days. The solvent was replaced every 24 hours. The maceration process was repeated until a clear solution was obtained. The resulting extract solution was then filtered using a vacuum pump with filter paper. The resulting liquid extract was then evaporated or concentrated using a rotary evaporator equipped with a water heater and a vacuum pump at a temperature of 30 to 40°C until a thick or coarse extract was obtained.

Research Procedure

Before undergoing the treatment phase, the mice were first acclimated for 7 days. On the 8th day, the mice were fasted for 10–12 hours, after which their total cholesterol levels were measured using the Easy Touch GCU device.

On the 9th day, the mice began receiving

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the following treatments:
- Group P0: standard diet
- Group P1: quail egg yolk
- Group P2: quail egg yolk and 4.2 mg/20 g body weight of malak leaf extract
- Group P3: quail egg yolk and 0.31 mg of Orlistat per 20 g of mouse body weight

The experiment lasted for 12 days. On day 21, the mice fasted for 10 hours before total cholesterol levels were measured.

RESULTS AND DISCUSSION

Table 1. Mean Total Cholesterol Levels in the Control Group (P0) Before and After Treatment

Treatment Group	Code	Total Cholesterol Level After Acclimatization (mg/dL)	Total Cholesterol Level After Treatment (mg/dL)	Percentage Increase in Total Cholesterol
P0	A1	105	109	4%
	A2	112	118	5%
	A3	110	114	4%
	A4	108	111	3%
	A5	106	111	5%

The data from the control group (P0) showed that total cholesterol levels increased after treatment in all samples. Total cholesterol levels before treatment ranged from 105 to 112 mg/dL, whereas after treatment they ranged from 109 to 118 mg/dL. The mean total cholesterol level increased from 108.2 mg/dL to 112.6 mg/dL, representing an average increase of 4%. These findings indicate that the control group experienced a relatively small increase in total cholesterol levels following treatment.

Table 2. Mean Total Cholesterol Levels in Treatment Group 1 (P1) Before and After Treatment

Treatment Group	Code	Total Cholesterol Level After Acclimatization (mg/dL)	Total Cholesterol Level After Treatment (mg/dL)	Percentage Increase in Total Cholesterol
P1	B1	107	119	11%
	B2	105	120	14%
	B3	109	123	13%
	B4	103	119	16%
	B5	111	126	14%
Mean Total Cholesterol Level		**107**	**121,4**	**13%**

In Treatment Group 1 (P1), all samples demonstrated an increase in total cholesterol levels following treatment. Baseline total cholesterol levels ranged from 103 to 111 mg/dL and increased to 119–126 mg/dL after treatment. The mean total cholesterol level increased from 107.0 mg/dL to 121.4 mg/dL, corresponding to an average increase of 13%**.** Compared with the other groups, P1 exhibited the greatest increase in total cholesterol levels, suggesting that this treatment had the strongest effect on elevating total cholesterol.

Table 3. Mean Total Cholesterol Levels in Treatment Group 2 (P2) Before and After Treatment

Treatm	Co	Total	Total	Percent

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ent Group	de	Cholesterol Level After Acclimatization (mg/dL)	Cholesterol Level After Treatment (mg/dL)	age Increase in Total Cholesterol
P2	C1	107	110	3%
	C2	112	116	4%
	C3	106	109	3%
	C4	104	108	4%
	C5	105	107	2%
Mean Total Cholesterol Level		**107**	**110**	**3%**

The results for Treatment Group 2 (P2) showed that total cholesterol levels increased in all samples after treatment; however, the increase was relatively small. Total cholesterol levels before treatment ranged from 104 to 112 mg/dL, while post-treatment levels ranged from 107 to 116 mg/dL. The mean total cholesterol level increased from 107.0 mg/dL to 110.0 mg/dL, representing an average increase of 3%. Among all study groups, P2 demonstrated the lowest increase in total cholesterol levels.

Table 4. Mean Total Cholesterol Levels in Treatment Group 3 (P3) Before and After Treatment

Treatment Group	Code	Total Cholesterol Level After Acclimatization (mg/dL)	Total Cholesterol Level After Treatment (mg/dL)	Percentage Increase in Total Cholesterol
P3	D1	108	114	6%
	D2	105	109	4%
	D3	114	117	3%
	D4	111	115	4%
	D5	106	111	5%
Mean Total Cholesterol Level		**108,8**	**112,8**	**4%**

Treatment Group 3 (P3) also showed an increase in total cholesterol levels in all samples following treatment. Baseline total cholesterol levels ranged from 105 to 114 mg/dL, increasing to 109–117 mg/dL after treatment. The mean total cholesterol level increased from 108.8 mg/dL to 112.8 mg/dL, with an average increase of 4%. The increase observed in P3 was comparable to that of the control group (P0), indicating that the effect of the treatment in this group was not substantially different from that observed in the control group.

The highest percentage increase in total cholesterol levels was 16% in the group given quail egg yolk (P1), and the lowest percentage increase was 2% in the group given quail egg yolk and Malaka leaf extract. The average percentage increase in total cholesterol levels in group P2 was lower than in the other treatment groups due to the presence of bioactive compounds in the Malaka leaf extract, namely flavonoids. Flavonoids in Phyllanthus emblica act as hypolipidemic agents by inhibiting HMG-CoA reductase activity while simultaneously increasing the activity of plasma lecithin cholesterol acyltransferase (LCAT).[13] Another chemical compound found in Malaka leaf

extract is saponin. Saponins work by inhibiting the biosynthesis of endogenous cholesterol; they also regulate lipid metabolism, redistribution, transport, and excretion.[14] This study aligns with research conducted by Seno et al., which found that Malaka leaf extract (Phyllanthus emblica L.) contains chemical compounds namely flavonoids and saponins that affect total cholesterol levels in Wistar rats compared to the control group.[11] Another study by Santos Kumar et al. demonstrated an anti-hyperlipidemic effect, evidenced by statistically significant differences compared to the control group. This effect can be attributed to the flavonoid content found in Phyllanthus emblica, which exhibits a hypolipidemic effect in atherogenic albino rats.[15]

The control group in this study consisted of quail egg yolks and the drug Orlistat (P3). Based on Table 4, the average percentage increase in total cholesterol levels in the P3 group was higher. However, the average percentage increase in total cholesterol levels in the P3 group was lower compared to the P1 group. Orlistat works by reversibly inhibiting the lipase enzyme in the stomach and pancreas, an enzyme that plays a crucial role in digesting dietary fat. Lipase breaks down triglycerides into monoglycerides and free fatty acids that can be absorbed by the body. Orlistat covalently binds to the serine residue at the active site of lipase, causing the enzyme to lose its function. As a result, triglycerides cannot be hydrolyzed, which in turn reduces the absorption of free fatty acids.[16]

The increase in total cholesterol levels in the mice was likely caused by the daily administration of quail egg yolks in conjunction with the administration of Malaka leaf extract and Orlistat. Quail egg yolks contain a relatively high amount of fat, namely 11 grams.[17] In this study, a double dose was administered; however, as shown in Table 1, Malacca leaf extract and Orlistat were able to reduce the increase in total cholesterol levels in mice compared to the P0 and P1 groups.

CONCLUSION AND RECOMMENDATIONS

Conclusion

1. Feeding quail egg yolks increases total cholesterol levels in mice.
2. Administration of Malaka leaf extract can prevent an increase in total cholesterol levels in mice.

Recommendations

1. Further research is needed to study the effects of Malaka leaf extract (Phyllanthus Emblica L.) over a longer period of time.
2. Further research is needed to investigate the side effects of administering Malaka leaf extract.
3. Further research is needed on Malaka leaf extract using different extraction techniques.

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