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Antihyperlipidemic Activity of Tamarind (*Tamarindus indica*) Extract on the Lipid Profile of Albino Rats

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Abstract. Tamarind are widely used as antioxidants, anti-diabetes, anti-hyperlipidemia, and anti-cholesterolemia. The aim of this research is to analyze the anti-hyperlipidemic activity of extracts of tamarind fruit pulp, seeds and leaves on the lipid profile of rats.
Experimental research was designed with 20 male rats divided Control (C), Tamarind leaves (TL), Tamarind fruit pulp (TF), and Tamarind seeds (TS) extracts, each 500 mg/kg of body weight. All of the groups were given standard feed and induced with lard at 3 ml/rat/day for 14 days. On day 15, the rats were continued to be induced with lard and were given extracts according to each group for 2 weeks. The data obtained such as rats' body weight, level of cholesterol, HDL (high-density lipoprotein), LDL (low-density lipoprotein), triglycerides (TG) and Malondialdehyde (MDA) were analyzed using anova ($P<0.05$) and the Duncan Multiple Range Test (DMRT). The results showed that each extract had a significant effect on cholesterol, HDL and MDA levels but not on body weight, LDL and triglycerides. Levels of MDA in the group with the extract were significantly lower than the control. Tamarind pulp, seeds and leaf extracts show antihyperlipidemic activity by reducing cholesterol and HDL levels.
This can be the basis for using parts of the tamarind plant as a supplement for the community to maintain fat levels.

Keywords: anti-hyperlipidemia; lipid profile; malondialdehyde; tamarind

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INTRODUCTION

Cardiovascular disease is associated with various risk factors such as blood pressure, cholesterol levels, LDL levels, HDL levels, glucose intolerance, and smoking. Controlling high cholesterol conditions is becoming increasingly important to reduce the prevalence of cardiovascular and coronary heart diseases. Increased cholesterol in the body can cause plaque formation and buildup in blood vessels, causing atherosclerosis (Su et al., 2021). Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, LDL, and reduced HDL (Shattat, 2014).

The main goal of managing hyperlipidemia, especially reducing total cholesterol, LDL, triglycerides and increasing HDL, is to prevent complications from heart disease. (Lim et al., 2013). Epidemiological studies reveal that flavonoids from fruit and vegetables have a positive influence on human heart health (Nofianti et al., 2019). Flavonoids are one of the best phytochemicals that act as antioxidants and exhibit many biological properties that are beneficial to human health. Flavonoids neutralize the harmful effects of free radicals in the best way and help to prevent many diseases (Karak, 2019). Natural antioxidants, especially polyphenols, are found in almost every part of plants such as bark, fruit, leaves, nuts, roots and seeds. Flavonoids are the second most important natural antioxidant (Lu et al., 2011).

Tamarind (*Tamarindus indica*) contains fatty acids, arsenic, calcium, copper, iron, manganese, magnesium, zinc, phosphorus, calcium, vitamins A, B1, B2, B3, fiber, fat, protein, carbohydrates and calories (Arshad et al., 2019). Tamarind leaves contain isoorientin, orientin pipecolic acid, nicotinic acid, citric acid, essential oils (Akram, 2022). Tamarind seeds are rich in polymeric tannins and polyphenolic compounds such as catechin, procyanidin B2, epicatechin (Bandawane, 2013), which can be used as an important source of protein (Kuru, 2014). Tamarind fruit peel shows antioxidant properties and decreases lipid levels along with substantial increase in manifestation of Apo A1, LDL receptor gene which prevents accumulation of triglycerides in the liver and reduces oxidative damage as a major risk factor for atherosclerosis (Arshad et al., 2019). Therefore, this research aims to investigate the anti-hyperlipidemic activity of Tamarind leaves, fruit pulp, and seeds extracts.

Based on research (Sandesh et al., 2014) which reported that methanol extract from tamarind seed has the potential to restore enzymatic antioxidant activity such as catalase and Superoxide dismutase (SOD) due to exposure to carbon tetrachloride. It is suspected that the flavonoid, phenol and tannin content, seed coat extract has a number of activities such as lipid peroxidation, antimicrobial, antihyperlipidemia, antidiabetic, anti-inflammatory (Soradech et al., 2016). Tamarind pulp extract showed anti-hyperlipidemia in mice fed a high cholesterol diet. A dose of 50 mg/kg tamarind pulp extract showed a significant effect on reducing body weight, serum cholesterol, triglycerides, and increasing HDL cholesterol in cafeteria diet and sulpiride-induced obese rats (Radha & Kusum, 2024).

Phytochemical components in seeds include flavonoids, saponins, phenolic compounds, pectin, organic acids and vitamin B. Flavonoids in the body act as reducers of LDL, triglycerides, increase the density of LDL receptors in the liver and bind apolipoprotein B, also increasing HDL (Purwaningsih et al., 2023). The antioxidant components in tamarind leaf extract are able to inhibit lipid peroxidation and free radical production, significantly regulate the expression of genes and proteins involved in the coagulation system, cholesterol biosynthesis (Razali et al., 2015). Overall, the research results are intended to analyze the anti-hyperlipidemic activity of extracts of tamarind fruit pulp, seeds and leaves on the lipid profile of rats. The results of this research are a basis for further studying the parts of the tamarind plant that have the potential to be antihyperlipidemic so that they can be developed as a supplement to maintain fat levels in the body.

METHODS

Extraction of plants parts

Extraction of Tamarind's leaves, fruit pulp, and seeds was conducted using maceration with 96% ethanol for 3-5 days. The macerate was filtered with filter paper (1) and the remains was remacerated with 96% ethanol and then filtered (2). The filtrate was evaporated using a rotary evaporator followed by drying to produce a thick extract (Abubakar & Haque,2020. ; Bandawane, 2013).

Treatment of Experimental Animals

This research was performed by experimental research with randomized posttest control group design. Maintenance and treatment of experimental animal was done in Biology Department Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. The Ethical Clearance for this research was obtained from the Health Research Ethics Commission (KEPK) Faculty of Sports Sciences, Universitas Negeri Semarang (No. 232/KEPK/EC/2023). The sample animal were 24 adult male Wistar rats aged more than 2 months with a body weight of 170-200 grams. Rats were kept in cages at a temperature of 25^0C, light-dark cycle 12:12 hours, standard feed in the form of pellets 20 grams/head/day and water ad libitum.

Rats in all groups received 3 ml lard/tail/day for 14 days to induce a high lipid profile (Darmawan et al., 2018; Ramadhani et al., 2017)). On the 15th day, control rats (C) were given placebo, the treatment group received extracts of Asam fruit flesh (TF), Asam seeds (TS), and Asam leaves (TL) each 500 mg/kg, and continued to receive lard. 1 hour before administering the extract. for 2 weeks. (Kuddus et al., 2020; Lim et al, 2013).

Variable Measurement

Rats' body weight was measured using a digital scale at 1st week (BW1), 3rd week (BW 3), and 5th week (BW 5). At the end of the study, blood was taken from the suborbital vein and then centrifuged at 3500 rpm for 10 minutes. The serum obtained was examined to measure levels of LDL, HDL, cholesterol with CHOD-PAP, triglycerides with GPO-PAP and the results of each examination are expressed in mg/dL (Aprilia et al., 2017). Measurement of Malondialdehyde (MDA) levels was performed using Thiobarbituric Acid Reactive Substances (TBARS) method with spectrophotometry at 532 nm (mg/ml) (Azman et al., 2012).

Statistic analysis

The data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS) version 18. After ensuring the normality and homogeneity of the data, differences between the control and treatment groups were carried out using one-way analysis of variance (ANOVA) followed by the DMRT post-hoc test at a test level of 95 %.

RESULTS AND DISCUSSION

Data in the form of cholesterol, HDL, LDL, triglyceride and MDA levels obtained from the end of treatment after 4 weeks are presented in Table 1. The results of statistical analysis show that administration of Tamarind fruit pulp, seeds, and leaves extracts did not exhibit a significant effect on LDL and TG levels, but had significant effect on HDL, cholesterol and MDA levels. Cholesterol and HDL levels in the control and TF groups were significantly different compared to TS and TL. This result showed that the seed and leaves extracts exerted effect on reducing cholesterol and HDL levels in hyperlipidemic rats. The MDA levels in the control group were significantly different from those in the treatment group with fruit pulp, seeds, and leaves extracts. The administration of Tamarind fruit pulp, seeds, and leaves extracts did not differ significantly.

Table 1. Results of Lipid Profile Measurement of Rats on a High Fat Diet Which Received Tamarind Fruit Pulp Extract, Seeds, and Leaves Extracts for 4 Weeks.

Variable	C	TF (500 mg/kg)	TS (500 mg/kg)	TL (500 mg/kg)
Cholesterol (mg/dL)	74.74 ± 13.0^{a}	63.76 ± 9.7^{a}	57.82 ± 10.2^{b}	54.24 ± 10.2^{b}
HDL (mg/dL)	38.0 ± 8.15^{a}	31.8 ± 7.69^{a}	26.8 ± 3.70^{b}	24.8 ± 4.81^{b}
LDL (mg/dL)^{ns}	12.09 ± 10.13	13.02 ± 8.06	8.57 ± 5.48	10.43 ± 5.48
Triglyceride (mg/dL)^{ns}	163.39 ± 44.45	131.64 ± 41.35	112.26 ± 53.26	93.44 ± 54.76
MDA (mg/ml)	0.46 ± 0.05^{a}	0.29 ± 0.13^{b}	0.26 ± 0.08^{b}	0.20 ± 0.09^{b}

Information: *significant difference, ^{ns} not significantly different, *superscript* is not significantly different and different ones indicate significant differences. Control group (C), Tamarind Fruit (TF), Tamarind Seeds (TS), Tamarind leaves (TL).

Providing high-fat feed results in changes in the lipid profile as illustrated by increased levels of HDL, cholesterol and triglycerides. These results are in accordance with Sripradha et al., 2016 who reported the effect of changes in dyslipidemia.

Table 2. Result of Rats' Body Weight Measurement

Variable	K	TF (500 mg/kg)	TS (500 mg/kg)	TL (500 mg/kg)
BW 1^{ns}	199.2 ± 17.81	175.4 ± 13.63	192.2 ± 23.94	185.4 ± 14.81
BW 3^{ns}	206.4 ± 25.12	178.6 ± 15.53	209.2 ± 47.87	180.2 ± 31.90
BW 5^{ns}	205.80 ± 26.46	170.2 ± 19.52	206.2 ± 40.87	169.6 ± 35.64

The body weight of rats in all groups was obtained from weighing at the 1st, 3rd, and 5th weeks as shown in Figure 2. Providing a high-fat diet supplement in the form of lard as much as 3 ml/rat for 14 days (BW 3) had no significant effect on increasing the body weight of all groups. Tamarind fruit pulp, seeds, and leaves extract also had no effect on weight loss after being given for 2 weeks. However, based on the data obtained in Table 2, it was found that there was a tendency for weight loss in the group of mice given extracts from the meat, seeds and leaves of tamarind.

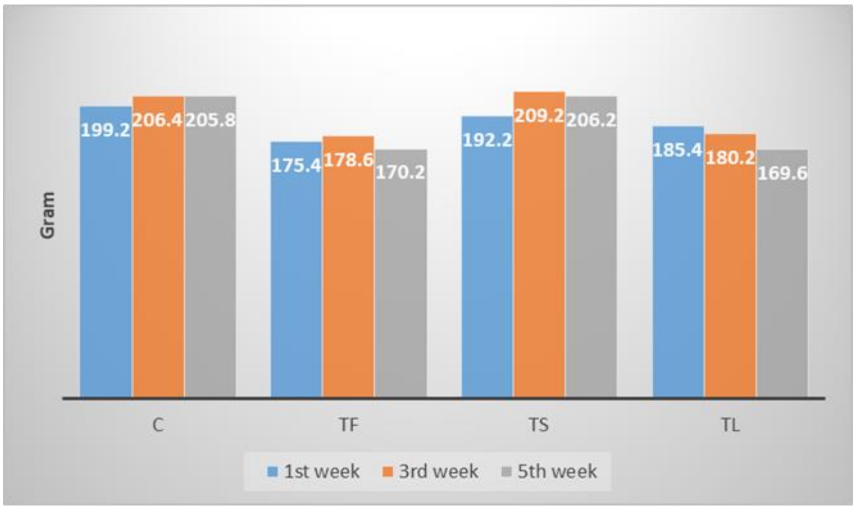

Figure 1. Body Weight of Control Group Rats and those Treated with Tamarind Fruit Extract, Seeds and Leaves. Control group (C), Tamarind Fruits (TF), Tamarind Seeds (TS), Tamarind leaves (TL).

Figure 1 showed that the body weight of rats in control group (C) tended to increase every week, which was 199.2, 206.4 and 205.8 grams respectively, and was higher than the TS group (192.2, 209.2, and 206.2 grams), TL group (185.4, 180.2, and 169.6 grams) and TF group (175.4, 178.6 and 170.2 grams). Weight loss in the treatment group was not significantly different from the control group.

Hyperlipidemia indicates an abnormal increase in lipid or lipoprotein levels in the blood due to abnormal fat metabolism or function, and is caused by eating disorders, obesity, genetic diseases (Yao et al., 2020). Hyperlipidemia is divided into two, namely the primary hyperlipidemia which is caused by genetic disorders and the secondary hyperlipidemia which is caused by alternative etiologies, such as unhealthy diet, drugs, hypothyroidism, diabetes, and heart disease. Apart from excessive consumption of animal fats, common causes of hypercholesterolemia and/or increased triglycerides are diabetes, chronic renal failure, nephrotic syndrome, hypothyroidism, age, and sedentary lifestyle (Hill & Bordoni,2023).

The hyperlipidemia disorder can manifest in the increase of total serum cholesterol, LDL, triglyceride, and HDL levels. Food fat that has undergone digestion will be absorbed into the intestinal lymphatic vessels in chylomicrons. These lipoproteins will be hydrolyzed by lipoprotein lipase which converts triglycerides into glycerol and non-esterified fatty acids. Furthermore, the remaining chylomicrons are absorbed in the liver and packaged with cholesterol, cholesterol esters to form VLDL (Very Low Density Lipoprotein) (Shattat, 2014). Lipids are organic compounds that are insoluble in water and cannot be transported in plasma, so they are transported in plasma as macromolecular complexes or lipoproteins. Some examples of lipoproteins include chylomicrons, VLDL, LDL, and HDL (Karam et al., 2017).

Elevated plasma total and LDL cholesterol concentrations are associated with increased risk of coronary heart disease and increased plasma triglycerides. Elevated VLDL is associated with a greater prevalence of atherosclerotic heart disease (Ahmed, 2023). This can trigger the formation and buildup of plaque in blood vessels and cause atherosclerosis (Su et al., 2021).

Elevated serum total cholesterol and LDL have been reported as major risk factors for cardiovascular disease. The potential of herbal medicines is very significant and has fewer side effects than synthetic hypolipidemic drugs (Madhekar, 2020). Some medicinal plants have been studied for their hypolipidemic effects. The reported advantages of herbal medicines are effectiveness, safety, affordability and acceptability. Tamarind ethanol extract is known to exhibit anti-hyperlipidemic activity through antioxidant mechanism and the inhibition of lipid oxidation. Phytochemical components such as alkaloids exerts lipase enzyme inhibitory activity, while flavonoids and polyphenols can reduce lipoprotein secretion in the liver and intestines and increase bile acid secretion which will increase the rate of lipid excretion (Nofianti et al., 2019).

The research results for the TF group were not significant compared to the control group for all lipid profiles, such as cholesterol, HDL, LD and triglycerides as well as body weight. These results are in accordance with Ekpe *et al.* (2021)'s research which stated that weight loss requires a higher dose of extracts and longer time to produce significant weight loss. Tamarind fruit pulp can be beneficial in the long term to help managing weight which associated with hypolipidemic activity (Azman et al., 2012).

Other research shows that Tamarind fruit pulp does not have a significant lipid-lowering and hypoglycemic effects. However, when given for 10 weeks, Tamarind fruit pulp extract was able to reduce serum levels of total cholesterol (50%), non-HDL cholesterol (73%), TG (60%) and increase HDL cholesterol. Therefore it is suggested that the use of higher doses over a longer period may have a positive effect on the serum lipid profile (Kiakalayeh et al., 2017). Epicatechin in fruit shows hypolipidemic effects by reducing triglyceride levels by increasing LDL receptor gene expression, decreasing gene expression of microsomal triglyceride protein (MTP) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in the liver (Lim, 2013).

The cholesterol level of the TS group received 500 mg/kg of seed extract was 57.82 ± 10.2 mg/dl, significantly lower than the control group which was 74.74 ± 13.0 mg/dl. These results confirm the research by Uchenna *et al*. (2018) which stated that 2% extract of Tamarind seeds can significantly reduce blood cholesterol. Tamarind seeds contain phenolic compounds which are known to reduce plasma lipoprotein and cholesterol levels by reducing the solubility and absorption of cholesterol in the intestine. Furthermore Sun et al.(2021) stated that flavonoids reduce cholesterol absorption, regulate LDL-R expression through increasing the processing of sterol regulatory element binding protein, and facilitate the biosynthesis and excretion of bile acids. Epigallocatechin-3-gallate (EGCG), a form of flavonoid, can significantly reduce plasma LDL-C levels. Huang et al. (2018) found that 0.32% EGCG reduced LDL-C by 28% in mice fed a high-fat diet by increasing fecal excretion of bile acids and cholesterol and decreasing reabsorption of cholesterol and bile acids.

Tamarind leaves extract at a dose of 500 mg/kg was able to reduce cholesterol, HDL, and MDA levels but had no significant effect on LDL and triglycerides levels. These results support the research by (Aprilia et al., 2017) that Tamarind leaves extract with phytochemical components such as saponin, flavonoids, epicatechin, polyphenols can act as a hypolipidemic agent. Other research shows that antioxidant-rich Tamarind leaves extracts can reduce blood lipid profiles through downregulating the expression of proadipogenic genes, transcription factors, and lipogenic enzymes by decreasing gene expression for lipolytic enzymes and hormone-sensitive lipase (Kuddus et al., 2020). Another research stated that Tamarind leaves extracts causes improvements in lipid and carbohydrate profiles as evidenced by a decrease in plasma lipid and glucose levels, lipid peroxidation levels, hexokinase enzyme activity, and cholesterol excretion. (Vasant & Narasimhacharya, 2012).

Phenol is the most dominant compound as a natural antioxidant. This compound acts as a hydrogen donor or metal ion chelator by inhibiting LDL oxidation. Phenolic compounds inactivate free radicals by transferring hydrogen molecules from their hydroxyl groups (Menezes et al., 2016). Flavonoids in plants with their lipophilic properties are able to inhibit LDL and Very Low Density Lipoprotein (VLDL) in blood serum. Based on these properties, it is necessary to consider consuming fat followed by foods containing flavonoids in the daily diet (Iswari et al., 2020). Vitamin E is a potential fat-soluble antioxidant found in tamarind. This compound functions to break the lipid peroxidation chain in the membrane. Tocopherol reacts with lipid peroxyl radicals and forms stable tocopheroxyl radical products that are not reactive enough to initiate membrane lipid peroxidation by themselves (Nimse & Pal, 2015). The results showed that extracts from parts of the tamarind plant such as fruit flesh, seeds and leaves had the same effect in reducing the lipid profile of rats induced by high-fat diet. Therefore, further research is needed to analyze the effect of these plants on more complete blood biochemical variables, especially as antioxidants. This can be developed further as a herbal alternative to maintain health.

CONCLUSION

This research showed that parts of the Tamarind plant have the potential to act as anti-hyperlipidemia. The Tamarind leaves extract is better at lowering the lipid profile and the fruit pulp extracts plays a greater role as an antioxidant by reducing MDA levels. Further research is needed to be developed with broader variables because hyperlipidemia is a major risk factor for atherosclerosis, which is characterized by the accumulation of lipids, cholesterol and the development of plaque in blood vessels.

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