Anti-hypercholesterolemic Effect of Ethanol Fruit Extract of *Ficus racemosa* L.

Patimah and Dodi Irwandi*
Department of Pharmaceutical and Food Analyst, Poltekkes Kemenkes Jakarta II, Ministry of Health, REPUBLIC OF INDONESIA
*dodiirwandi@poltekkesjkt2.ac.id

Abstract

The objective of the study was to examine the impact of the ethanol extract of Ficus racemosa L *fruit on rats with hypercholesterolemia. The rats were divided into distinct groups and were administered varying doses of the extract or simvastatin. Hypercholesterolemia was induced in the rats, and the lipid profiles were measured at both the pre- and post-treatment stages. The findings demonstrated that the Ficus racemosa L fruit extract markedly reduced total cholesterol and triglyceride levels while elevating HDL levels in the hypercholesterolemic rats.*

The 500 mg/kg dose demonstrated the most promising outcomes, exhibiting a notable reduction in cholesterol and triglycerides and an increase in HDL. However, no impact on weight loss was observed. These findings indicate that Ficus racemosa L fruit extract may have the potential to be utilized in the management of hypercholesterolemia, although further investigation is necessary to elucidate the underlying mechanisms of action.

Keywords: *Ficus racemosa* L, hypercholesterolemia, ethanol extract, rats

Introduction

Hypercholesterolemia, a condition characterized by elevated blood cholesterol levels, is a significant risk factor for the development of cardiovascular diseases such as atherosclerosis, coronary artery disease, and stroke. This issue is increasingly widespread, particularly in developing countries, due to changes in lifestyle and diets high in saturated fat. While synthetic drugs like statins are effective in reducing cholesterol levels, they can also lead to long-term side effects such as muscle damage and liver dysfunction[1,4]. Consequently, there is an urgent need to explore safer alternative therapies. Research on the potential use of natural ingredients, such as plant extracts, as safer and more effective treatments for hypercholesterolemia is of paramount importance.

Ficus racemosa L., also known as the Loa tree in Indonesia, has a long history of use in traditional medicine across cultures for the treatment of a variety of health conditions. The plant's various parts including leaves, fruits, and bark, have been demonstrated to possess pharmacological activities including anti-inflammatory, antioxidant, antidiabetic, and hepatoprotective effects[6]. Furthermore, several studies have demonstrated that extracts of this plant can reduce cholesterol and blood lipid levels in animal models with experimentally induced hyperlipidaemia[13]. The ethanol extract of *Ficus racemosa* contains a variety of bioactive compounds including flavonoids, phenols, and terpenoids, which have been shown to play a role in reducing LDL (low-density lipoprotein) cholesterol and triglyceride levels while increasing HDL (high-density lipoprotein) cholesterol[1].

Previous studies in mouse models have shown that administering ethanolic extracts of Ficus racemosa plant parts can significantly lower lipid levels. This includes reducing total cholesterol, LDL, and triglycerides while increasing HDL levels. Additionally, the extract's high antioxidant content helps to reduce oxidative stress which contributes to the development of high cholesterol and related complications[6,13].

The potential of *Ficus racemosa* in reducing cholesterol levels has been extensively studied, but most of the research has focused on *in vitro* tests or other parts of the plant[13]. Limited information is available on the anti-hypercholesterolemic effects of the fruit extracts specifically, especially in animal models. This study aims to address this gap by examining the effects of ethanol extracts of *Ficus racemosa* fruit in rats with induced hypercholesterolemia *in vivo*. The study not only aims to gain a better understanding of the therapeutic potential of *Ficus racemosa* fruit but also hopes to contribute towards the development of safer herbal drugs for the treatment of high cholesterol levels[2].

Material and Methods

Sample Preparation: The Loa fruit was gathered from South Jakarta and then identified at the "Herbarium Bogoriense" within the Directorate of Scientific Collection Management at BRIN Cibinong. To prepare the Loa fruit simplicia, the fresh fruit was cleaned to remove impurities, cut into thin pieces, and air-dried without direct sunlight exposure. Once dried, the fruit was ground into powder and sieved using a 40-mesh sieve[11].

Preparation of Extracts: The extract was prepared by maceration using ethanol as the solvent. The ethanol extract was concentrated using a rotary evaporator until a thick methanol extract was obtained. The thick ethanol extract was then dried in an oven to produce a dry residue[11].

***In vivo* Evaluation of Anti-hypercholesterolemic Effects:** This study examined the effect of the ethanol extract of *Ficus*

racemosa fruit on hypercholesterolemia in male rats. The experiment followed the guidelines of the modified BPOM RI Regulation No. 20 Year 2023. A total of 24 rats were used and divided into different groups including a normal group, negative control, positive control with simvastatin, and treatment groups with different doses of *Ficus racemosa* fruit extract. Before treatment, the rats were acclimated for 7 days and hypercholesterolemia was induced using propylthiouracil (PTU) and high-fat feed. Cholesterol and LDL levels were measured to confirm induction success. Over a period of 14 days, the rats were treated with the fruit extract orally along with high-fat feed.

Body weight was monitored every 7 days, and at the end of treatment, blood samples were taken to measure lipid levels. Statistical analysis was carried out using ANOVA repeated measures and paired sample T-Test and One Way ANOVA for data analysis.

Results and Discussion

Body weight: The results of measuring the body weight of rats showed a significant difference between the normal group fed with standard feed and all groups fed with high-fat feed and induced hypercholesterolemia including the Loa fruit extract test preparation group in various doses of administration (Table 1). This shows that the preparation has not been able to withstand body weight gain due to excess fat intake. As the high-fat diet was fed for approximately 3 weeks, body weight continued to increase at a rapid rate, higher than the body weight of the normal group fed the standard diet ($P<0.05$).

This was most likely due to the high-fat diet fed for a total of 21 days. Feeding a high-fat diet with significantly higher total energy than the standard diet will lead to an increase in body weight over time[8], even though the amount of administration is the same as the standard diet. The effect of Loa fruit extract on hypercholesterolemic rats was further observed through lipid profile.

Lipid Profile: In this study, hypercholesterolemia animal models were created by feeding the animals a high-fat diet and administering propylthiouracil orally. To confirm the success of the induction, it was necessary to compare the values of the tested parameters before and after induction. The results of total cholesterol and LDL levels in rat serum before and after induction are presented in table 2. Before induction, total cholesterol and LDL levels were similar and not significantly different between treatment groups. However, after the induction of a high-fat diet and propylthiouracil, significant differences were observed between the induction group and the normal group ($P<0.05$), with the mean values of the two parameters exceeding 1.5 times the values of the normal group.

The higher levels of total cholesterol and LDL after induction were also significantly different from those before induction ($P<0.05$), confirming the successful induction of hypercholesterolemia. Animals with confirmed hypercholesterolemia were then treated with Loa fruit extract, and the treatment results can be observed in table 3.

Testing of lipid profiles including total cholesterol, triglycerides, LDL, and HDL was carried out to see the effect of Loa fruit preparations on hypercholesterolemic rats. Excess fat consumption from high-fat feed will affect the blood lipid profile, namely an increase in total cholesterol, triglycerides, low density liporpotein (LDL), and a decrease in high density lipoprotein (HDL) levels[9]. This is because the high-fat feed consumed is then metabolised in the liver and accelerates de novo lipogenesis and lipoprotein levels which ultimately form these lipid components[5].

The results showed that the administration of Loa fruit extract at various doses could reduce total cholesterol and triglyceride levels, and will increase HDL levels statistically significantly compared to the negative control group (Table 3). The 500 mg/kg bw administration dose was observed to have the best effect in general compared to other administration doses because the total cholesterol, triglyceride and LDL values were the lowest, and the HDL value was still relatively good compared to other hypercholesterolemia groups. However, the doses of 250 and 750 mg/kg bw also showed a good effect with lipid profile values that were still lower than the negative control lipid profile values.

Table 1
Body weight of test rats during treatment

Group	Body weight (gram)			
	Before induction	**After induction**	**H7 treatment**	**H14 treatment**
Normal	242.00 ± 4.24^{a}	258.75 ± 10.52^{a}	275.50 ± 10.24^{a}	285.75 ± 13.12^{a}
Negative control	239.75 ± 1.47^{a}	295.00 ± 15.58^{b}	318.25 ± 9.64^{b}	331.75 ± 8.88^{b}
Positive control (simvastatin 20 mg/kg)	241.25 ± 6.90^{a}	294.00 ± 8.04^{b}	308.50 ± 21.39^{b}	320.50 ± 23.81^{b}
Loa fruit extract dose 250 mg/kg	243.75 ± 8.64^{a}	297.25 ± 13.52^{b}	306.50 ± 7.14^{b}	316.75 ± 7.89^{b}
Loa fruit extract dose of 750 mg/kg	242.50 ± 8.74^{a}	290.50 ± 9.04^{b}	310.50 ± 18.09^{b}	326.50 ± 19.18^{b}
Loa fruit extract dose of 500 mg/kg	240.00 ±12.91^{a}	294.00 ± 12.30^{b}	311.50 ± 12.23^{b}	326.75 ± 12.92^{b}
Total	240.00 ± 4.04^{a}	294.00 ± 6.37^{b}	311.50 ± 7.02^{c}	326.75 ± 7.67^{d}

Note: Data are presented as mean ± SD. Different superscript in the same column and row indicates significant difference. $P<0.05$

Table 2
Total cholesterol and LDL levels of test rats before and after induction

Group	Total cholesterol (mg/dL)		LDL (mg/dL)	
	Before induction	After induction	Before induction	After induction
Normal	70.18 ± 1.22[a]	64.24 ± 6.49[a]	18.67 ± 1.43[a]	20.61 ± 3.04[a]
Negative control	65.97 ± 7.02[a]	114.46 ± 6.78[b]	16.87 ± 3.38[a]	36.32 ± 7.22[b]
Positive control (simvastatin 20 mg/kg)	65.90 ± 6.14[a]	113.38 ± 14.26[b]	18.28 ± 2.33[a]	36.55 ± 7.45[b]
Loa fruit extract dose 250 mg/kg	67.78 ± 2.56[a]	112.92 ± 9.49[b]	15.20 ± 2.33[a]	35.10 ± 3.76[b]
Loa fruit extract dose of 750 mg/kg	70.92 ± 5.61[a]	114.93 ± 7.56[b]	15.41 ± 1.42[a]	39.01 ± 2.57[b]
Loa fruit extract dose of 500 mg/kg	69.92 ± 3.58[a]	114.29 ± 5.82[b]	15.47 ± 5.00[a]	38.11 ± 2.15[b]
Sig. 2-tailed (Paired t-test)		0.000		0.000

Note: Data are presented as mean ± SD. Different superscript in the same column indicates significant difference. P<0.05

Table 3
Lipid profile of rats at the end of treatment

Group	Profil lipid (mg/dL)			
	Total cholesterol	Triglycerides	LDL	HDL
Normal	56.22 ± 2.25[a]	92.22 ± 20.48[a]	26.41 ± 5.11[a]	47.92 ± 12.68[b]
Negative control	102.76 ± 26.91[b]	178.10± 74.03[ab]	22.71 ± 5.25a	28.81 ± 6.60[a]
Positive control (simvastatin 20 mg/kg)	76.54 ± 15.67[a]	76.54 ± 15.67[a]	208.65 ± 33.28[b]	19.44 ± 2.18[ab]
Loa fruit extract dose 250 mg/kg	64.91 ± 21.04[a]	64.91 ± 21.04[a]	132.60 ± 33.68[ab]	53.51 ± 13.68[b]
Loa fruit extract dose of 750 mg/kg	54.36 ± 12.93[a]	99.13 ± 12.49[a]	19.21 ± 2.28[a]	41.34 ± 2.02[ab]
Loa fruit extract dose of 500 mg/kg	73.74 ± 7.78[a]	98.98 ± 37.22[a]	20.14 ± 1.18[a]	53.92 ± 1.12[b]

Note: Data are presented as mean ± SD. Different superscript in the same column indicates significant difference. P<0.05

The findings align with prior research indicating that administering Loa fruit stem bark extract for 45 days can lower triglyceride, LDL, and VLDL levels while increasing HDL levels in alloxan-induced diabetic rats[13]. Additionally, administering tannin metabolite compounds isolated from Loa fruit stem extract for 30 days can reduce total cholesterol and LDL levels while boosting HDL levels[13]. Although our study utilized extracts from Loa fruit for a shorter duration of 2 weeks, we observed an impact on the lipid profile.

The potential mechanism behind the reduction in lipid profile may be attributed to the antioxidant properties of Loa fruit extract. Excessive free radicals or oxidants are known to be present in conditions of dyslipidemia and obesity[10]. Consumption of a high-fat diet can lead to an increase in reactive oxygen species (ROS) and a decrease in antioxidant enzymes[7]. *In vitro* studies have demonstrated that tannins from Loa extract can reduce nitric oxide (NO) free radical levels compared to quercetin. Moreover, further assessments of the antioxidant function of tannins from Loa stem extracts have confirmed that these extracts can elevate the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the heart, liver, and kidney organs, thereby safeguarding the organs from free radicals[13].

The presence of antioxidants in this extract can enhance the liver's function in metabolizing fats and fatty acids, and it can also help to regulate lipoprotein hydrolysis[13]. Although this study did not investigate the specific mechanism of Loa fruit extract in reducing lipid levels, it was observed that despite an increase in body weight, the rats' lipid levels decreased after being treated with the extract. This finding aligns with other studies that have shown that increased blood lipid levels are not always linked to increased body weight[13].

Conclusion

The extract from Loa fruit has shown potential in improving hypercholesterolemia by lowering total cholesterol and triglyceride levels, and raising HDL levels to nearly normal. Although administering Loa fruit extract did not lead to a reduction in body weight, it did result in an improved lipid profile. However, the mechanism behind the reduction in lipid levels with Loa fruit extract remains unknown, indicating the need for further testing.

Acknowledgement

This research was funded by the Jakarta II Health Polytechnic of the Ministry of Health in 2024 with the Basic Higher Education Excellence Research Scheme.

References

1. Agnello F., Ingala S., Laterra G., Scalia L. and Barbanti M., Novel and Emerging LDL-C Lowering Strategies: A New Era of Dyslipidemia Management, *Journal of Clinical Medicine*, **13(5)**, https://doi.org/10.3390/jcm13051251 **(2024)**

2. Ariff A.M., Omar E., Muid S., Kasim N.A.M., Ismail N.H. and Nawawi H., Ficus deltoidea var. kunstleri Extract Administration in Hypercholesterolaemic, Atherosclerotic Rabbits: Effects on Organ Function, Morphology, and Atherosclerosis Development, *Malaysian Applied Biology*, **52(1)**, 73–85 **(2023)**

3. Duan L., Zhao G., Ji B., Cao Y. and Chen X., Effect of crude-herb moxibustion on blood lipids in rats with dyslipidemia, *Journal of Traditional Chinese Medical Sciences*, **1(2)**, 140–147 **(2014)**

4. Gaita L., Timar B., Timar R., Fras Z., Gaita D. and Banach M., Lipid Disorders Management Strategies in Prediabetic and Diabetic Patients, *Pharmaceuticals*, **17(2)**, https://doi.org/10.3390/ph17020219. **(2024)**

5. Harfi Maulana A.R., High-fat diets-induced metabolic disorders to study molecular mechanism of hyperlipidemia in rats, *Heart Disease (CHD)*, **3**, 18 **(2021)**

6. Kosankar K.V. and Aher A.N., The Phytoconstituents and pharmacological actions of Ficus racemosa Linn (Family: Moraceae)-An updated review, *Pharma Tutor*, **6(12)**, 55–63 **(2018)**

7. Miah P., Mohona S.B.S., Rahman M.M., Subhan N., Khan F., Hossain H., Sharker S.M. and Alam M.A., Supplementation of cumin seed powder prevents oxidative stress, hyperlipidemia and non-alcoholic fatty liver in high fat diet fed rats, *Biomedicine & Pharmacotherapy*, **141**, 111908 **(2021)**

8. Moreno-Fernández S., Garcés-Rimón M., Vera G., Astier J., Landrier J.F. and Miguel M., High fat/high glucose diet induces metabolic syndrome in an experimental rat model, *Nutrients*, **10(10)**, 1502 **(2018)**

9. Munshi R.P., Joshi S.G. and Rane B.N., Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease, *Indian Journal of Pharmacology*, **46(3)**, 270–276 **(2014)**

10. Pastori D., Carnevale R. and Pignatelli P., Is there a clinical role for oxidative stress biomarkers in atherosclerotic diseases?, *Internal and Emergency Medicine*, **9(2)**, 123–131 **(2014)**

11. Patimah and Irwandi D., Characterization and In Vitro Anti-cholesterol Activity Test of Ethanol Extract of Fruit Ficus racemosa L., *IJSES*, **7(12)**, 73-75 **(2023)**

12. Sophia D. and Manoharan S., Hypolipidemic activities of Ficus racemosa Linn. bark in alloxan induced diabetic rats, *African Journal of Traditional, Complementary and Alternative Medicines*, **4(3)**, 279–288 **(2007)**

13. Sukowati Y.K., Johan A. and Murwani R., Ethanol extracts of Ficus carica fruit and leaf normalize high serum lipid profile, TNF-α, and MDA due to high fat diet in Sprague Dawley rat, *Current Research in Nutrition and Food Science Journal*, **7(3)**, 772–782 **(2019)**

14. Velayutham R., Sankaradoss N. and Ahamed K.F.H.N., Protective effect of tannins from Ficus racemosa in hypercholesterolemia and diabetes induced vascular tissue damage in rats, *Asian Pacific Journal of Tropical Medicine*, **5(5)**, 367–373 **(2012)**.

(Received 10th October 2024, accepted 14th November 2024)