Trop J Nat Prod Res, October 2024; 8(10): 8684 - 8689

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Mulberry Leaves Extract Ameliorates Lipid Profile, Oxidative Stress and Aortic Histopathological Features In Dyslipidemic Rats Induced by A High-Fat Diet

Yugi HC Purnama[1,2], Fifin L Rahmi[1], Hermawan Istiadi[3], Medina Sianturi[4], Banundari Rahmawati[1,5]*

[1] *Doctoral of Medical Science and Health Study Program, Faculty of Medicine, Universitas Diponegoro, Semarang 50275, Indonesia*
[2] *Department of Nursing, Faculty of Health Science, Universitas dr.Soebandi, Jember 68111, Indonesia*
[3] *Department of Anatomy Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang 50275, Indonesia*
[4] *Department of Nursing, STIKES Elisabeth Semarang, Semarang 50232, Indonesia*
[5] *Department of Clinical Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang 50275, Indonesia*

ARTICLE INFO

Article history:
Received 07 June 2024
Revised 19 August 2024
Accepted 30 September 2024
Published online 01 November 2024

ABSTRACT

Coronary heart disease (CHD) are regarded as the crucial factors associated with dyslipidemia. Mulberry leaves are composed of several bioactive chemicals, such as phenols, flavonoids, anthocyanins, alkaloids and terpenoids. The effects of mulberry leaves on dyslipidemia have been considered in scientific research. This study determined the effect of mulberry leaf extract on TG, LDL-C, ox-LDL serum levels, and aortic histopathological features. A randomized controlled study was carried out on rats at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. Twenty four *Sprague Dawley* (SD) rats were divided into normal control (n = 6) and dyslipidemia (Dys) (n = 18) groups. Rats in the Dys group were induced by a high-fat-diet (HFD). The Rats were categorized into 4 groups: the normal control group (N), negative control group (Dys), HFD+Simvastatin 0.18 mg/200gBW positive control (PC), and HFD+600 mg/kgBW mulberry leaf extract (MLE). Treatments were given daily for 8 weeks. The lipid profile parameters (TG, LDL, and HDL), ox-LDL levels and aortic histopathological features were evaluated using standard procedures. The results showed that MLE gave a significant differences in TG, LDL-C, HDL-C and ox-LDL levels in all groups (p<0,001). The mean changed TG, LDL-C, HDL-C and ox-LDL observed were 60.35 mg/dL, 49.43 mg/dL, 47.05 mg/dL, and 119.9 pg/mL, respectively. The aortic histopathological features indicated a statistically significant variance between the MLE group and the Dys group (p<0.001). The findings revealed that mulberry leaf extract significantly improved lipid profile, ox-LDL levels and aortic histopathological features.

***Keywords*:** Mulberry leaves, Antioxidant, Dyslipidemia, Lipid profile, Atherosclerosis.

Introduction

Cardiovascular disorders such as coronary heart disease (CHD) and stroke are caused by impaired heart and blood vessel function, which is directly linked to dyslipidemia and atherosclerosis.[1,2] Elevated of cholesterol levels contribute to approximately 3.9 million deaths globally. Based on *NCD Risk Factor Collaboration* (NCDRisC) report, the epicenter of elevated cholesterol levels has transitioned from Europe and North America to South and Southeast Asia.[3] In Indonesia, for instance, 11.7% of adults over the age of 15 have total cholesterol (TC) levels greater than 200 mg/dL; 8.5% of subjects had low density lipoprotein cholesterol (LDL-C) levels higher than 100 mg/dL, and 87% had had high density lipoprotein cholesterol (HDL-C) levels lower than 40 mg/dL. These data are from a sample of individuals in Southeast Asia. Moreover, triglyceride (TG) values beyond 150 mg/dL were raised in 22.8% of cases.[4]

*Corresponding author. E mail: banundaridr@yahoo.com
Tel: +628122875120

Citation: Purnama YHC, Rahmi FL, Istiadi H, Sianturi M, Rahmawati B. Mulberry Leaves Extract Ameliorates Lipid Profile, Oxidative Stress and Aortic Histopathological Features In Dyslipidemic Rats Induced by A High-Fat Diet. Trop J Nat Prod Res. 2024; 8(10): 8684 – 8689 https://doi.org/10.26538/tjnpr/v8i10.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Reactive oxygen species (ROS) are produced when leftover very low density lipoprotein (VLDL) and atherogenic lipoproteins accumulate in the subendothelial region as a result of elevated TG, LDL-C, and decreased HDL-C. This process can cause oxidative stress.[5,6] The disparity in ROS and antioxidants within the body leads to alterations in lipoproteins that carry apoB-100. It results the transformation of LDL cholesterol into oxidized-LDL (ox-LDL) via the lipid peroxidation process. Oxidized LDL is highly immunogenic and readily identified by macrophages via scavenger receptors, leading to macrophage activation and infiltration into atherosclerotic lesions for the phagocytosis of ox-LDL through scavenger receptors.[5,7,8] This process causes lipids to build up in macrophages, which in turn triggers the formation of foam cells. The foam cells are responsible for producing growth factors and cytokines, including TNF-α and interleukin-1 (IL-1), which draw more macrophages to the lesion site. Plaque development advances more quickly as a consequence.[8,9] The LDL modification can trigger the activation of *nuclear factor-kappa β* (NF-*κβ*), leading to the upregulation of adhesion molecules, leukocytes, and chemokines.[8,9] The activation of NF-*κβ* in regions susceptible to atherosclerosis triggers the activation of endothelial cells through elevated expression of monocyte adhesion proteins, pro-inflammatory receptors, cytokines, and chemokines.[5,10] This process ultimately influences the migration of monocytes into the intimal space. Thus, it leads to the narrowing of the aortic lumen.

Atherosclerosis prevention involves managing dyslipidemia through lifestyle modifications such as controlling cholesterol levels through dietary adjustments and the use of statins.[11,12] On the other hand, prolonged statin treatment might lead to elevated levels of liver and muscle enzymes.[13] Fruits and vegetables are able to treat and manage

diseases because they contain bioactive compounds. Their ability to display anti-inflammatory and antioxidant properties is made possible by these compounds, which helps to improve the lipid profile.[14] Phenolic compounds, flavonoids, and anthocyanins are bioactive compounds found in vegetables and fruits, demonstrating powerful antioxidant properties. They are currently being studied as potential interventions to mitigate cardiovascular diseases.[15]

Mulberry leaves contain high levels of phenols, flavonoids, anthocyanins, and various antioxidants.[16] They are utilized in the food and pharmaceutical industries due to their pharmacological properties, including their hypolipidemic effects.[17,18] Several investigations have demonstrated that hyperlipidemic rats and given mulberry leaf extract were able to reduce TG, LDL cholesterol and increase HDL cholesterol levels.[19,20] In order to prevent the morphology change of the Aorta intima caused by an atherogenic diet, Sprague Dawley rats fed mulberry leaf extract 100 mg/kg/day can significantly reduce adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin.[21] Mulberry leaf extract is known to influence lipid and ox-LDL levels, according to the majority of study. As a result, the current study's goals included assessing lipid profiles and ox-LDL levels as well as the histopathology of blood vessels using native Indonesian mulberry leaves, particularly in Malang, East Java, using rats that were both dyslipidemia-treated and in good condition.

Materials and Methods

Collection, identification, and extraction of the plant sample

Fresh mulberry leaves were harvested at Lawang Mulberry Farm, Malang, East Java, Indonesia in December 2022. Mulberry plants were identified by the Taxonomist at Ahmad Dahlan University's Biology Learning Laboratory in Bantul, Yogyakarta with reference number 472/Lab.bio/B/XII/2022. It was shown that the sample utilized was a mulberry leaf plant with the Latin name (*Morus alba* L) from the *Moracea* family. Mulberry leaves that had just been collected were cleaned, chopped into smaller pieces, and allowed to air dry at room temperature until they reached a constant dry weight. After that, a grinder was used to turn the dried samples into a coarse powder.

Extraction of Plant Material

Samples of coarsely crushed plant material weighing 974 g were macerated in a sterile, dry container with 70% ethanol at a ratio of 1:4 and covered with foil. Replace one 24-hour marinade with the same solvent and let the simplicias sit for three times, then stirring occasionally. The first, second, and third filtrates were combined, evaporated, and concentrated using a waterbath, with the extract yield percentage being measured. The extract was then stored appropriately. The rotary vacuum evaporator (Heidolph, Germany) was used to achieve this thick extract.[22]

Animals

Male *Sprague Dawley* (SD) rats in healthy conditions, weighing between 150 and 200 g, were acquired from the Animals Laboratory at the Center for Food and Nutrition Studies, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. These rats were housed in individual stainless steel cages within an animal room that maintained a 12-hour light-dark cycle, a temperature range of 24°C ± 2°C, and a relative humidity between 50% and 60%. The rats were acclimatized for one week and fed with Standard Comfeed-Par S (Japfa Comfeed, Indonesia) and water *ad libitum.* The animal experimental procedures were approved by Health Research Ethics Committee of the Medicine Faculty of Diponegoro University (23/EC-H/KEPK/FK-UNDIP/II/2023). All rats were cared according to the Animal Laboratory Guidelines of the Center for Food and Nutrition Studies.

SD Rats were fed a high-fat diet (HFD) containing Comfeed-Par S (Japfa Comfeed Indonesia, Indonesia; 60%), flour (27.80%), cholesterol (2%), colic acid (0.20%), and lard (10%) given *ad libitum* for 4 weeks. Increased serum levels of TG (67.03–147.03 mg/dL), LDL-C (41.33–97.30 mg/dL), and decreased HDL-C (>50 mg/dL) were the assessments used to evaluate the dyslipidemia criteria.[23] Induced dyslipidemic rats with a HFD was successful, with serum levels TG, LDL-C, ox-LDL and lower serum HDL-C than the control group of rats (Table.2)

Experimental design

This randomized controlled study was conducted in rats at the Center for Food and Nutrition Studies, UGM, Yogyakarta, Indonesia. Twenty four SD rats were randomly divided into four groups (n = 6 per groups), normal control (N); dyslipidemia rats untreated (Dys); Dyslipidemia rats were given simvastatin 0.18mg/200g BW/day positive control (PC); Dyslipidemia rats were given mulberry leaf extract 600 mg/kg/day (MLE). Rats were given different treatments for 8 weeks.

Biochemical Analyses

The blood samples were collected using retroorbital bleeding technic before and after the intervention. Rats did 12-hour fasting period before measurement. Examination of TG, LDL-C and HDL-C levels was conducted using an enzymatic method with photometric principles according to the manual procedure (FineTest, Fine Tech Co., Ltd., China), whereas the ox-LDL examination was measured using the Sandwich ELISA Method using the Rat Ox-LDL ELISA kit (Elabscience, China).

Preparation of Aorta samples for histological examination

Rats were anaesthetised after 8 weeks treatment, Aorta were immediately removed, and histological preparations were made using paraffin. The samples were fixed in a solution of 10% buffered neutral formalin, followed by tissue processing steps as follows: dehydration step using graded alcohol (80%, 90%, and 95%), clearing step using xylol, and impregnation step using paraffin liquid. The tissue was embedded into paraffin block, followed by microsectioning using microtome in 4 µm of thickness. *Hematoxylin-Eosin* (HE) staining was applied into all slides.[24]

Examination of Aorta histology

All histopathological slides of the aorta were examined using light microscope (Olympus BX51, Japan) in five distinct fields with high power field, 400X magnification, for assesement of endotelial and tunica media. The assesement of atherosclerosis grade was carried out by pathologist using Sakamoto parameters as outlined in Table 1.[25]

Table 1: Assessment of atherosclerosis parameters

Type		Parameters
I (*Initial lesion*)	:	Isolated macrophage foam cells
II (*Fatty streak*)	:	Mainly intracellular lipid accumulation
III (*Intermediate lesion*)	:	Type II changes and small extracellular lipid pools
IV (*Atheroma lesion*)	:	Type II changes and core of extracellular lipid
V (*Fibroatheroma lesion*)	:	Lipid core and fibrotic layer, or multiple lipid cores and fibrotic layers, or mainly calcific, or mainly fibrotic
VI (*Complicated lesion*)	:	Surface defect, hematoma-hemorrhage, trombus

Table 2: The effect of mulberry leaf extract on TG, LDL-C, HDL-C and Ox-LDL levels of Dyslipidemic SD rats (n=24)

Parameter	Groups				*p value*
	N	Dys	PC	MLE	
TG (mg/dL)					
Pre-test	67.03 ±2.25	142.3 ±2.69	139.7 ±1.59	139.7 ±2.58	<0.001*
Post-test	67.5 ±2.58	143.8 ±2.80#	82.1 ±3.64#	79.37 ±3.87#	
LDL-C (mg/dL)					
Pre-test	23.2 ±1.63	79.1 ±1.32	79.5 ±2.25	80.8 ±2.17	<0.001**
Post-test	24.1 ±1.97	80.1 ±1.43#	35.3 ±2.39#	31.4 ±1.67#	
HDL-C (mg/dL)					
Pre-test	81.2 ±1.75	24.1 ±1.60	24.7 ±2.46	23.5 ±1.38	<0.001**
Post-test	81.1 ±1.77	23.2 ±1.33#	63 ±2.18#	70.6 ±1.65#	
Ox-LDL (pg/mL)					
Pre-test	28.3 ±1.36	159.2 ±3.61	160.3 ±3.94	159.4 ±4.39	<0.001**
Post-test	28.4 ±1.40	160.3 ±3.54#	50.4 ±2.39#	39.5 ±1.62#	

Values were expressed in terms of the mean ± SD. Statistically significant data and parameters are bold, #paired t-test p<0.05, *ANOVA p<0.05, **_Kruscall-Wallis_ p<0.05. Dys: Dyslipidemia group, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, MLE: Mulberry leaf extract 600 mg/kg+HFD, N: Normal group, ox-LDL: Oxidized low density lipoprotein, PC: Positif control Simvastatin 0.18 g/200 g BW+HFD, and TG: Triglyseride.

Statistical analysis

The *Shapiro-Wilk* normality test was used to initially examine lipid profile values, ox-LDL levels, and aortic histopathology features. The one way *Analysis of Variance* (ANOVA) test was used for parametric analysis, which was followed by the *Tukey* post hoc test. For non-parametric data, *Kruskal-Wallis* and *Mann-Whitney* tests was conducted for the analysis. Statistical analyses were performed using SPSS software version 22.0 (IBM Corp., USA).

Results and Discussion

Studies have shown that mulberry leaves possess natural hypolipidemic. it was found in this study that MLE improved lipid profile, ox-LDL and impact on the histopathological features of the aorta.

The study of lipid profiles and ox-LDL levels in the intervention group during an 8-week period was presented in Table 2. The results of the analysis showed, there was a higher and significant reduction in TG, LDL-C, ox-LDL levels and a higher and significant increase in HDL-C levels in the intervention group compared to the control group. Figure 1 showed the serum of TG, LDL-C, HDL-C and ox-LDL after 8 weeks of treatment. There were significant differences in TG, LDL-C, HDL-C and ox-LDL levels between all groups (p<0.001). The MLE group exhibited the biggest changes. MLE group exhibited significantly higher changes in TG, LDL-C, HDL-C, and ox-LDL levels compared to the Dys group. The mean changed observed were 60.35 mg/dL, 49.43 mg/dL, 47.05 mg/dL, and 119.9 pg/mL, respectively.

Mulberry leaves containe flavonoid and phenolic compounds. The flavonoids in mulberry leaves show the effect of decreasing TG, LDL-C levels and increasing HDL cholesterol levels.[26–28] *In vivo* study showed that MLE can effectively decrease lipids using multiple mechanisms. Liangyu, *et al* (2022) stated that MLE controls lipid metabolism through downregulating the major protein of sterol regulatory element-binding protein 1 (SREBP1), controlling the Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) center, and thus encouraging fatty acid oxidation and inhibiting fatty acid synthesis.[29] Typically, the flavonoids found in mulberry leaves have shown to greatly reduce lipid levels in animal models with dyslipidemia by lowering TG, LDL-C, and free fatty acid levels while boosting HDL-C levels.[30] This was in linear with the findings of research conducted in the MLE group, which demonstrated significant changes in rats that were fed a HFD ($\rho < 0.001$). Moreover, mulberry leaves were rich in flavonoids, which can directly reduce ox-LDL levels.[30]

The *Mann-Whitney* test (Figure 2) results showed that the group that received intervention had significantly better aortic type compared to the dyslipidemia group or those who only received HFD. These results were supported by the histopathological features of the aorta in dyslipidemic rats as shown in Figure 3.

Rats given a high-fat diet (HFD) plus cholic acid supplementation may develop foam cells by the eighth week and have markedly elevated blood cholesterol levels. Figure 3a depicted the normal histomorphology of the aorta in rats that were part of the normal control group. Dyslipidemic rats exhibited changes in the morphology of the aortic wall due to the formation of foam cells and the proliferation of smooth muscle cells. They also accumulated extracellular lipids from the smooth muscle, which lead to the formation of atheroma plaque and the development of atherosclerosis, as demonstrated in the Dys group (Figure 3b). The changes in aortic lesion morphology were shown by the aorta histomorphology of the positive control (PC) HFD-induced rats treated with simvastatin 0.18 mg/200gBW/day (Figure 3c) and the HFD-induced rats treated with 600 mg/kgBW/day of MLE (Figures 3d). Foam cells and intracellular lipid buildup in smooth muscle were noted in the PC group. But for the MLE group, all they had were foam cells. When compared to the Dys group, the MLE treatment had a substantial impact (p<0,001).

The results of this study showed that MLE can improve the histopathological features aortic atherosclerosis by improving the lipid profile and reducing oxidative stress by lowering the levels of ox-LDL in dyslipidemic rats. Simvastatin was selected as the statin therapy for positive control due to its continued prevalence in Indonesia for effectively reducing LDL cholesterol levels by 18-55%.[31] In conventional medicine, statins are advised as the primary treatment for management of high LDL level however they possess considerable side effects.[32] The selection of a single dose in this study was based on previous research. In a study conducted by Huang *et al*, a dose of 0.6 g/kg was stated to be one of the optimum doses.[19] Previous studies have shown that mulberry leaf extract enhances serum lipid profiles and inhibits the progression of atherosclerosis in rabbits on a high-fat diet[33], which was consistent with the findings of this study. Li *et al*, studied the efficacy and mechanism of alkaloids, flavonoids, phenolics, polysaccharides, and other bioactive components in mulberry leaves alone in the treatment of metabolic syndrome.[34] Several studies have

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

reported that by increasing LDL resistance to oxidative modification, flavonoids found in mulberry leaves can prevent the development of atherosclerotic lesions in experimental animal models lacking LDL receptors.[15] In addition to flavonoids, 1-*deoxynojirimycin* (DNJ), one of the alkaloids found in mulberry leaves, was also effective in improving lipid profiles and lowering atherogenic index values in rats with SD dyslipidemia.[11] Treatment with mulberry leaves normalized circulating endothelial dysfunction markers such as soluble VCAM-1, fibrinogen and nitric oxide. Mulberry leaves were also efficient against plaques of atherosclerotic that had already formed.[35] The results of this study can support the effectiveness of mulberry plants in treating dyslipidemic rats, particularly in relation to their hypolipidemic and antioxidant characteristics. Determining the primary component responsible for the antidyslipidemia and antiatherosclerotic effects would be an interesting area for further research.

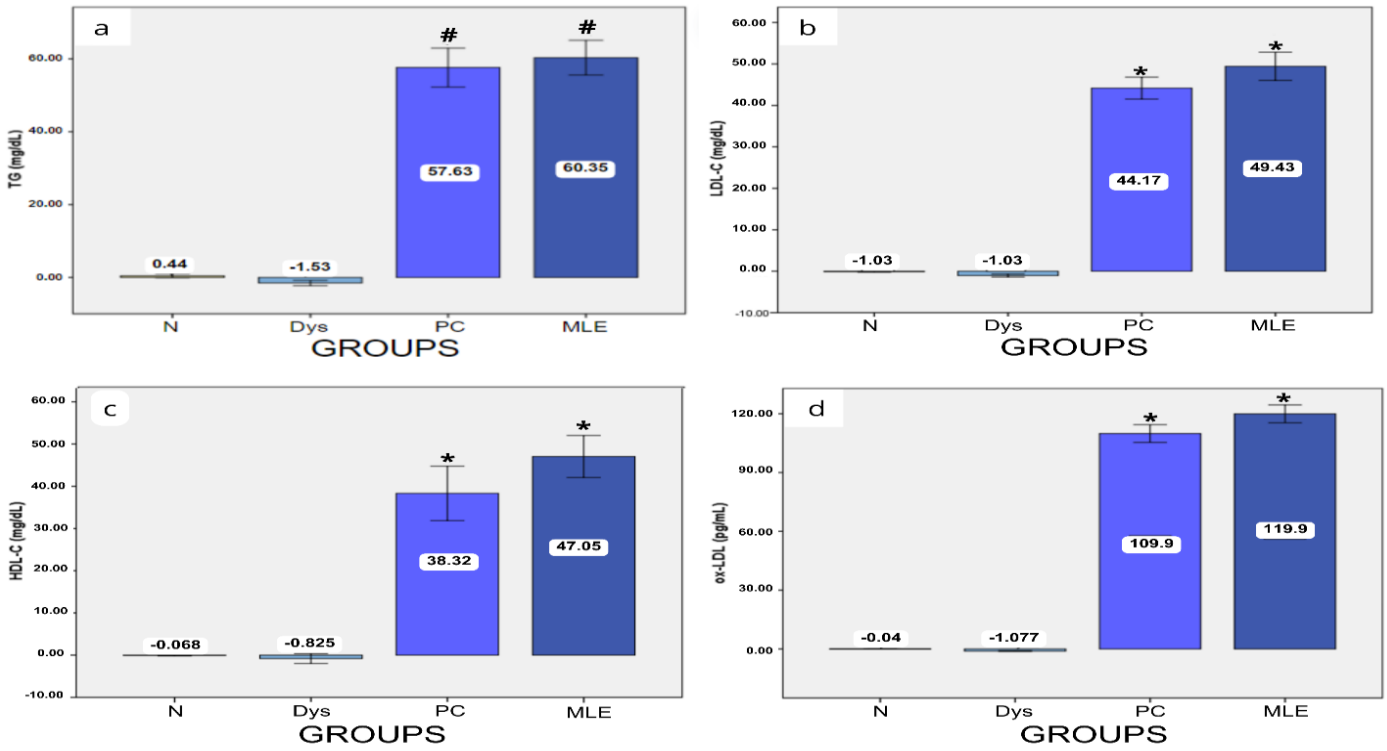

Figure 1: Effect of MLE on TG (1a), LDL-C (1b), HDL-C (1c) and ox-LDL (1d) in SD rats with dyslipidemia. Values were expressed in terms of the mean ± SD. Differences in TG levels between groups were analyzed using ANOVA followed by the post hoc *Tukey* test: #p<0.05 vs Dys Group. Difference in LDL-C, HDL-C and ox-LDL levels between groups were analyzed using *Kruskal-Wallis* followed by the *Mann-Whitney* test: *p<0.05 vs Dys Group. Dys: Dyslipidemia group, MLE: Mulberry leaf extract 600 mg/kg BW+HFD, N: Normal groups, and PC: Positif control Simvastatin 0.18 mg/200 g BW+HFD.

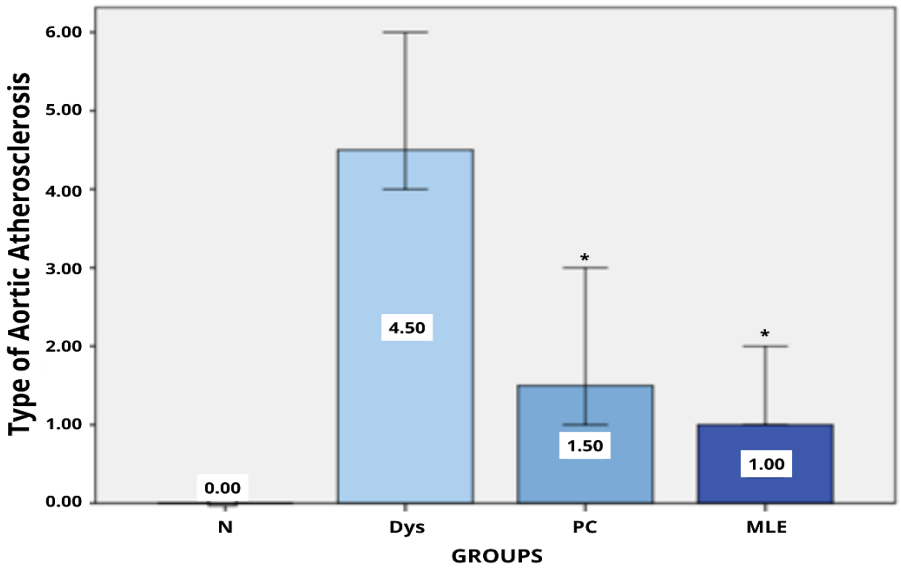

Figure2: Differences in type of Aortic atherosclerosis lesion in SD rats between groups were analyzed using *Kruskal-Wallis* test followed by the *Mann–Whitney* test; *p<0.05 vs Dys Group. Dys: Dyslipidemia, MLE: Mulberry leaf extract 600 mg/kg BW+HFD, N: Normal groups, and PC: Positif control Simvastatin 0.18 mg/200 g BW+HFD.

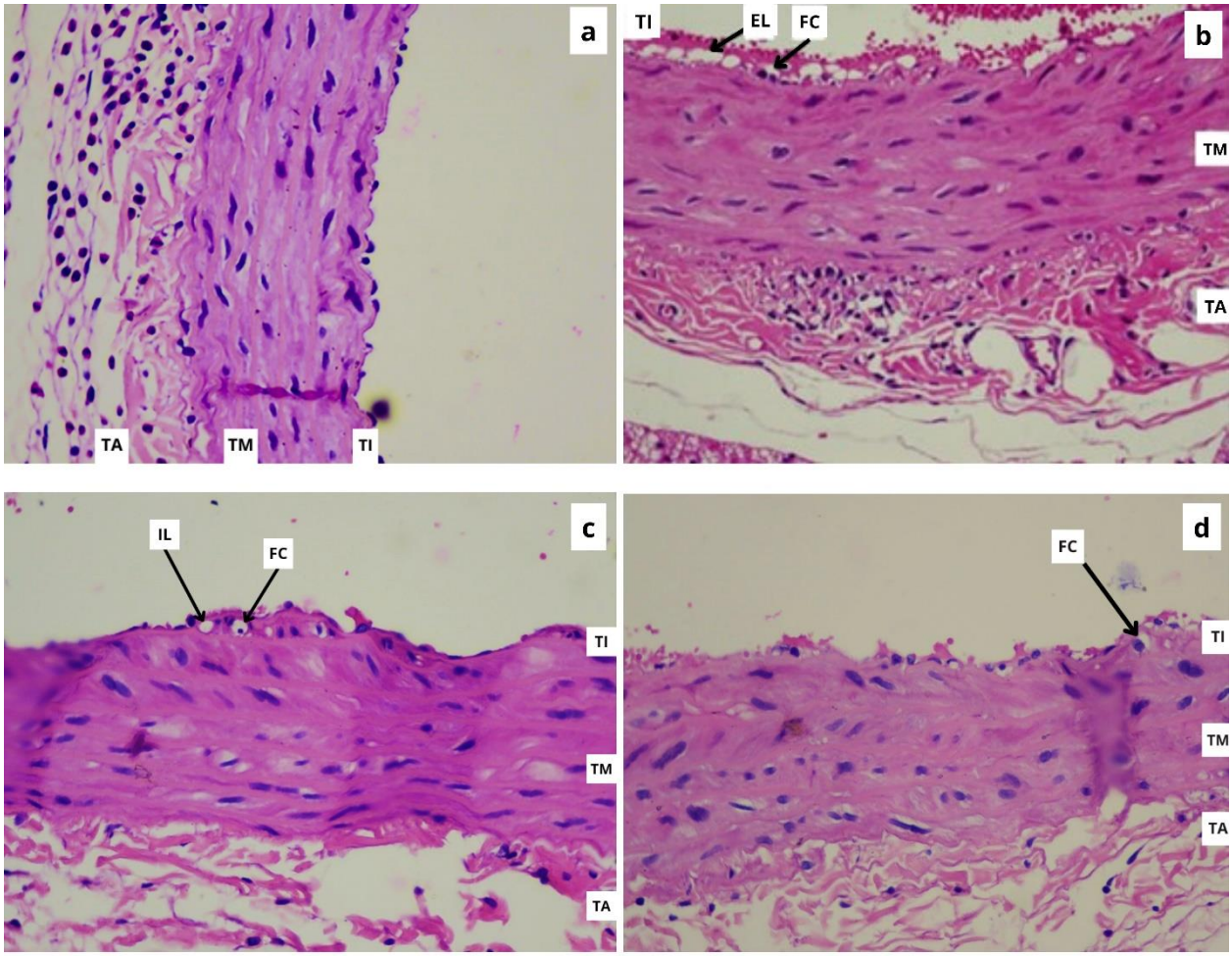

Figure 3: Description of the histological structure of the aorta of SD dyslipidemia rats using HE staining, (3a) Normal groups (N), (3b) Dyslipidemia groups (Dys), (3c) HFD+Simvastatin 0.18 mg/200 g BW (PC), (3d) HFD+Mulberry leaf extract 600 mg/kgBW (MLE) (zoom 400x). EL: Extracelluler Lipid, FC: Foam cell, IL: Intracellular Lipid TA: Tunica Adventitia, TI: Tunica Intima, and TM: Tunica Media.

Conclusion

Mulberry leaf extract has been demonstrated to benefit the aorta with atherosclerotic lesions in dyslipidemic rats by lowering ox-LDL levels and improving the lipid profile that might shield blood vessels from damage by free radicals and inflammation, delaying the development of atherosclerosis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

We would like to thank the Ministry of Education, Culture, Research, and Technology No. 1924/E4/DT.04.02/2023 and Universitas dr.Soebandi Jember, East Java, Indonesia 0143/UDS/K/II/2023 for providing funding for this study.

References

1. Nugraha G. Lipids; Classification, Metabolism, Atherosclerosis and Laboratory Analysis. 1st ed. Jakarta: TIM; 2017. 68–82 p.
2. Aman AM, Soewondo P, Soelistijo SA, Arsana PM, Wismandari, Zufry H, Rosandi R. Guidelines for Dyslipidemia Management in Indonesia in 2019. 1st ed. Jakarta: PB PERKENI; 2019.
3. NCD Risk Factor Collaboration (NCD-RisC). Repositioning of the global epicentre of non-optimal cholesterol. Nature. 2020; 582(7810):73–77. https://www.nature.com/articles/s41586-020-2338-1
4. BKPK Kemenkes. Indonesian Health Survey in Numbers [Internet]. Indonesian Health Survey. Jakarta; 2023. https://www.badankebijakan.kemkes.go.id/hasil-ski-2023/
5. Wengrofsky P, Lee J, Makaryus AN. Dyslipidemia and Its Role in the Pathogenesis of Atherosclerotic Cardiovascular Disease: Implications for Evaluation and Targets for Treatment of Dyslipidemia Based on Recent Guidelines. In: Samy I. McFarlane, editor. Dyslipidemia. 1st ed. London: IntechOpen; 2019. p. 180.
6. Ullah Z, Malik SE, Ghaffar T, Kanwal S, Noor A, Aamir AH. Frequency of hypertriglyceridemia in type 2 diabetic patients receiving statin therapy and its correlation with glycemic control. J Postgrad Med Inst. 2020; 34(4):253–257. https://jpmi.org.pk/index.php/jpmi/article/view/2779

7. Linton MF, Yancey PG, Davies SS, Jerome WG (Jay), Linton EF, Song WL, Doran AC, Vickers, KC. The Role of Lipids and Lipoproteins in Atherosclerosis. Feingold, Anawalt, Boyce, editors. Nashville: Endotext.org; 2019. https://www.endotext.org/chapter/the-role-of-lipids-and-lipoproteins-in-atherosclerosis/
8. Sargowo D. Endothel Dysfunction. 1st ed. Sargowo D, editor. Malang: Universitas Brawijaya Press; 2015. 168 p.
9. Sargowo D. Pathogenesis of Atherosclerosis. 1st ed. Sargowo D, editor. Malang: Universitas Brawijaya Press; 2015. 298 p.
10. Sarbini D, Sargowo D, Rohman MS. *Hibiscus sabdariffa Linn* tea extract against NF-κ*β*, TNF-α and ICAM-1 in Human Umbilical Vein Endothelial Cells (HUVECs) Cultured exposed to Oxidized Low Density Lipoprotein (LDL). J Exp Life Sci. 2011; 1(2):102–110. https://doi.org/10.21776/ub.jels.2011.001.02.07
11. Susanti E, Sulchan M, Mahati E. Anti-atherogenic Effect of Mulberry Leaf Tea in Atherogenic Index on Rats Dyslipidemia. J Aisyah J Ilmu Kesehat. 2022; 7(3):669–674. https://doi.org/10.30604/jika.v7i3.1013
12. Khavandi M, Duarte F, Ginsberg HN, Reyes-Soffer G. Treatment of Dyslipidemias to Prevent Cardiovascular Disease in Patients with Type 2 Diabetes. Curr Cardiol Rep. 2017; 19(1):1-10. https://doi.org/10.1007/s11886-017-0818-1
13. Cheon DY, Jo S-H. Adverse effects of statin therapy and their treatment. Cardiovasc Prev Pharmacother. 2022; 4(1):1–6. https://doi.org/10.36011/cpp.2022.4.e4
14. Fakayode AE, Emma-Okon BO, Morakinyo AE, Fajobi AO, Akinyele KN, Oyedapo OO. Investigations of Ameliorative Potentials Extract of *P. pellucida* on Salt-Fructose Induced Dyslipidemia in Wistar Rats. Trop J Nat Prod Res. 2023; 7(9):4112–4116. https://doi.org/10.26538/tjnpr/v7i9.40
15. Lee G-H, Hoang T-H, Jung E-S, Jung S-J, Chae S-W, Chae H-J. Mulberry Extract Attenuates Endothelial Dysfunction through the Regulation of Uncoupling Endothelial Nitric Oxide Synthase in High Fat Diet Rats. Nutrients. 2019; 11(5):1-16. https://doi.org/10.3390/nu11050978
16. Purnama YHC. Identification of Chemical Compounds in Mulberry Leaf Extract (*Morus alba. L*). Sci Proc Islam Complement Med. 2022; 1(1):135–138. https://doi.org/10.55116/spicm.v1i1.16
17. Lee Y, Lee E, Lee M-S, Lee S, Kim C, Kim Y. Hypolipidemic Effect of Mulberry Leaf Extract in Rats Fed a High-cholesterol Diet (P06-014-19). Curr Dev Nutr. 2019; 3:14-19. https://doi.org/10.1093/cdn/nzz031.P06-014-19
18. Zhang H, Ma ZF, Luo X, Li X. Effects of mulberry fruit (*Morus alba L*.) consumption on health outcomes: A mini-review. Antioxidant. 2018 ;7(5):1-13. https://doi.org/10.3390/antiox7050069
19. Huang J, Wang Y, Ying C, Liu L, Lou Z. Effects of mulberry leaf on experimental hyperlipidemia rats induced by high-fat diet. Exp Ther Med. 2018; 16(2):547–556. https://doi.org/10.3892/etm.2018.6254
20. Peng CH, Lin HT, Chung DJ, Huang CN, Wang CJ. Mulberry Leaf Extracts prevent obesity-induced NAFLD with regulating adipocytokines, inflammation and oxidative stress. J Food Drug Anal. 2018; 26(2):778–787. https://doi.org/10.1016/j.jfda.2017.10.008
21. Lee YJ, Choi DH, Kim EJ, Kim HY, Kwon TO, Kang DG, Lee HS. Hypotensive, hypolipidemic, and vascular protective effects of *Morus alba L*. in Rats fed an atherogenic diet. Am J Chin Med. 2011; 39(1):39–52. https://doi.org/10.1142/S0192415X11008634
22. Himawan HC, Isa AF, Wiharja DS. Antioxidant Activity Of 70% Ethanol Extract Combination Of Kemangi Leaf (*Ocimum Americanum Linn*) and Binahong Leaf (*Anredera cordifolia (Ten.) Steenis*) Using DPPH. J Phys Conf Ser. 2021; 1764(1):1-7. https://doi.org/10.1088/1742-6596/1764/1/012009
23. Ihedioha JI, Noel-Uneke OA, Ihedioha TE. Reference values for the serum lipid profile of albino rats (*Rattus norvegicus*) of varied ages and sexes. Comp Clin Path. 2013; 22(1):93–99. https://doi.org/10.1007/s00580-011-1372-7
24. Isdadiyanto S, Fajar S. Histopathology of White Rat Aorta After Giving Kombucha Tea Concentration of 75% Based on Fermentation Time. Bul Anat dan Fisiol. 2023; 8(2):130–137. https://doi.org/10.14710/baf.8.2.2023.130-137
25. Sakamoto K, Nagamatsu S, Yamamoto E, Kaikita K, Tsujita K. Atherosclerotic coronary plaque development visualized by in vivo coronary imaging. Circ J. 2018; 82(7):1727–1734. https://doi.org/10.1253/circj.CJ-18-0516
26. Cui Y, Hou P, Li F, Liu Q, Qin S, Zhou G, Xu X, Si Y, Guo S. Quercetin improves macrophage reverse cholesterol transport in apolipoprotein E-deficient mice fed a high-fat diet. Lipids Health Dis. 2017; 16(9):1-7. https://doi.org/10.1186/s12944-016-0393-2
27. Kobayashi Y, Miyazawa M. Effects of *Morus alba L*. (Mulberry) Leaf Extract in Hypercholesterolemic Mice on Suppression of Cholesterol Synthesis. J Pharmacogn Nat Prod. 2016; 2(1)1-9 https://doi.org/10.4172/2472-0992.1000113
28. Huang H, Liao D, Dong Y, Pu R. Effect of quercetin supplementation on plasma lipid profiles, blood pressure, and glucose levels: a systematic review and meta-analysis. Nutr Rev. 2020; 78(8):615–626. https://doi.org/10.1093/nutrit/nuz071
29. He L, Xing Y, Ren X, Zheng M, Yu S, Wang Y, Xiu Z, Dong Y. Mulberry Leaf Extract Improves Metabolic Syndrome by Alleviating Lipid Accumulation *In Vitro* and *In Vivo*. Mols. 2022; 27(16):1-20 https://doi.org/10.3390/molecules27165111
30. Ahmed A. Flavonoids And Cardiovascular Risk Factors: A Review. Pharmadvances. 2021; 3(3):521-547. https://doi.org/10.36118/pharmadvances.2021.15
31. Aman AM, Soewondo P, Soelistijo SA, Arsana PM, Wismandari, Zufry H, Rosandi R. Guidelines for Dyslipidemia Management in Indonesia in 2021. Revise 202. Jakarta: PB PERKENI; 2021. 76 p.
32. Siriyong T, Boonya-arunnate T, Ninbodee K, Kaewmuean T, Mettriyasakul T, Jawang K, Thongmongkol P, Chanwanitsaku S, Kaewnoi K, Buachum B, Saeloh D, Noosak C, Voravuthikunchai SP. Effects of Traditional Thai Herbal Formulations in Patients with Obesity and Borderline Hyperlipidemia - A Preliminary Pilot Study. Trop J Nat Prod Res. 2022; 6(1):29–33. https://doi.org/10.26538/tjnpr/v6i1.6
33. Chan KC, Yang MY, Lin MC, Lee YJ, Chang WC, Wang CJ. Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in cultured aortic vascular smooth muscle cells. J Agric Food Chem. 2013; 61(11):2780–2788. https://doi.org/10.1021/jf305328d
34. Li J, Ji T, Su S, Zhu Y, Chen X, Shang E, Guo S, Qian DW, Duan JO. Mulberry leaves ameliorate diabetes via regulating metabolic profiling and AGEs/RAGE and p38 MAPK/NF-κB pathway. J Ethnopharmacol. 2022; 283:114713.1-14 https://doi.org/10.1016/j.jep.2021.114713
35. Batiha GE-S, Al-Snafi AE, Thuwaini MM, Teibo JO, Shaheen HM, Akomolafe AP, Teibo TKA, Al-kuraishy HM, Al-Garbeeb AI, Alexiou A, Papadaki M. *Morus alba*: a comprehensive phytochemical and pharmacological review. Naunyn Schmiedebergs Arch Pharmacol. 2023; 396(7):1399–1413. https://doi.org/10.1007/s00210-023-02434-4