LenteraBio
Berkala Ilmiah Biologi

LenteraBio, 2025; Volume 14, Issue 1: 48-55

p-ISSN: 2252-3979 e-ISSN: 2685-7871
https://journal.unesa.ac.id/index.php/lenterabio/index

Effect of African Leaf Ethanol Extract in Total Cholesterol Levels and Aorta Histological Features of Mice with Diabetes Mellitus Type 2

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Article History:
Received:
15-August-2024
Revised:
11-December-2024
Available online:
07-January-2025
Published regularly:
31-January-2025

Abstract
This study aims to determine the effect of African leaf ethanol extract on reducing total cholesterol levels and aortic histology of mice (*Mus musculus*) with diabetes mellitus type 2. This study used a complete randomized design with a negative control group (standard purified feed), a positive control group (HFD + alloxan monohydrate), a simvastatin group and groups treated with ethanol extract of African leaves at doses of 75, 100 and 125 mg/kgBB with four replicates and treatment duration of 7 days. Total cholesterol levels were measured before the high-fat diet, on day 0 (after HFD) and day 8. The Histology of rat aorta was examined by light microscopes at 100x and 400x magnifications. Data of total cholesterol levels and changes were subjected to ANOVA and Duncan tests. Data of aortic lumen diameter were analyzed using Welch's ANOVA test. Atherosclerosis score was analyzed using Kruskal-Wallis and Mann-Whitney tests. The results of the analysis showed that the administration of African leaf ethanol extract at different doses reduced total cholesterol levels and atherosclerosis scores. In conclusion, the dose of ethanol extract of African leaf affects total cholesterol levels and aortic histology of mice with diabetes mellitus type 2 The dose 125 mg/kgBB was the optimal dose that can reduce total cholesterol levels and repair the aortic histology of mice with diabetes mellitus type 2.

Keywords: Atherosclerosis; High Fat diet; Hypercholesterolemic
How to Cite: Astri A, Qomariyah N, Fudhaili A, 2025. Effect of African Leaf Ethanol Extract in Total Cholesterol Levels and Aorta Histological Features of Mice with Diabetes Mellitus Type 2. LenteraBio; 14(1): 48-55
DOI: https://doi.org/10.26740/lenterabio.v14n1.p48-55

INTRODUCTION

Lifestyle changes and unhealthy diets can lead to various diseases, including increased blood cholesterol levels (hypercholesterolemia) (Nobertson *et al*., 2018). Cholesterol is a fat produced by metabolism. It contains sterol substances and widely distributed in cell membranes and blood circulation (Husen *et al*., 2022). Increased cholesterol levels caused by foods containing high cholesterol can reduce the transcription of the LDL receptor gene leading to decreased LDL receptor synthesis and increased LDL levels in the body (Hijriani *et al*., 2023). The required total cholesterol in human beings is normally <200 mg/dL. The incidence of hypercholesterolemia globally is around 45%, Southeast Asia 45%, and Indonesia 35% (Kemenkes RI, 2017; Balitbangkes, 2013; WHO, 2019). High levels of blood cholesterol may lead to atheroschlerosis (Meidiyanti, 2021).

Atherosclerosis is an acute inflammatory condition due to fat accumulation in the intima of blood vessels. Atherosclerosis begins with endothelial dysfunction. Monocytes that enter the endothelium will undergo differentiation into macrophages in the tunica intima. Lipoproteins, especially LDL, that accumulate in the intima will be oxidized by free radicals and macrophages. Macrophages phagocytize oxidized LDL through scavenger receptors. This leads to foam cell formation, the initial stage of atheroma formation (Prameswari, 2019; Santosa dan Baharuddin, 2020). Increased cholesterol leading to atherosclerosis can be found in patients with type 2 diabetes mellitus. Excessive free fatty acids can lead to insulin resistance in muscle and liver (Muhammad, 2018).

Simvastatin is one of the drugs to lower cholesterol levels because it has statin compounds which inhibit the synthesis of cholesterol in the body (Ariyanti, 2018). Prolonged consumption of simvastatin has an impact on the occurrence of myopathy from mild myalgia to fatal rhabdomyolysis leading to disruption of glucose homeostasis (Makinen, 2020). Alternative traditional medicine can be

applied by using African leaves (*Vernonia amygdalina* Del.), a plant that has been used in traditional medicine to cure various diseases, such as diabetes mellitus (Suryati *et al*., 2016).

African leaves can reduce cholesterol levels because of their secondary metabolite compounds such as flavonoid, saponin, and tannin. Flavonoids are considered capable of lowering cholesterol levels by inhibiting HMG-CoA reductase activity in order to reduce cholesterol synthesis (Ekananda, 2015). Saponins inhibit cholesterol absorption, bile salt reabsorption, and cholesterol synthesis because their interaction with bile salts forms an insoluble mixture, thus cholesterol unable to be absorbed by the intestine and excreted through feces (Benge *et al*., 2020). Tannins lower cholesterol by inhibiting adipocyte cell formation and cholesterol absorption in the intestine (Mutia *et al*., 2018).

Based on previous research, it has been found that African leaves can reduce total cholesterol levels in *Rattus novergicus*(Nobertson *et al*., 2018). Compared to rats, mice are smaller in size, reproduce quickly, and share 99% of their genes with humans (Stevani, 2016). Based on this information, this study was conducted by giving different doses of ethanol extract from African leaves to determine the optimal dose for total cholesterol levels and repairing aortic histology in mice with type 2 diabetes mellitus, combined with a High Fat Diet (HFD) and alloxan monohydrate.

MATERIALS AND METHODS

This was laboratory experimental research using a complete randomized design consisting of negative control treatment (standard feed), a positive control (High Fat Diet (HFD) + alloxan monohydrate), a simvastatin group (HFD + alloxan monohydrate + simvastatin), and three other treatment groups with different doses of African leaves (75 mg/kg BW, 100 mg/kg BW, 125 mg/kg BW) given HFD and alloxan monohydrate before treatment. Each treatment group was subjected to four replicates. This research was conducted from February to June 2024 in the laboratory of experimental animals, Basic Biology, and microtecnics Biology State University of Surabaya. The research required materials including African leaves, Deutschland Dunken Yoken (DDY) male mice aged 2 months and weighing 25–30 g, 96% ethanol, standard purified feed, goat fat oil, duck egg yolk, alloxan monohydrate, NaCMC 1%, distilled water, Neutral Buffer Phosphate solution, alcohol (70%, 80%, 96%, 100%), xylene, paraffin, mayer's albumin, hematoxylin-eosin, acid alcohol, and entellan. The tools used were 46×30×12 cm cages, oven, blender, sieve, rotary evaporator, measuring cup, digital balance, 1 ml syringe, sonde needle, lancet, glucometer (*EasyTouch*®*GCU*), strip test (*EasyTouch*®*GCU*), latex, dissecting set, urine pot, tissue cassettes, rotary microtome, glass slides, cover glasses, and microscope.

African leaves were extracted by maceration using 96% ethanol. The leaves, which were sorted, cleaned, and dried, were then oven-dried for 3 days at a temperature of 60℃. The dried leaves were pulverized into powdered simplisia. This simplisia was soaked with 96% ethanol solvent three times in stages, with a simplisia and ethanol ratio of 1:3 on the first day of soaking and 1:2 on the second and third days with 1×24 hours in each soaking process. After maceration, the filtrate was obtained. The filtrate was evaporated with a rotary evaporator at 55°C until a thick extract with a concentration of 100% was obtained (Tuldjanah *et al*., 2020). The thick extract was then diluted into doses of 75, 100, and 125 mg/kg body weight (BW) with 1% NaCMC.

Mice were acclimated for 7 days in cages and given purified feed and drinking water ad libitum. After acclimation, the mice were induced with a high fat diet (HFD) at 5 g daily per mice. The high fat diet was made from 80% purified feed, 15% goat fat oil, and 5% duck egg yolk (Tatto *et al*., 2017). Mice were fed a HFD for 7 weeks until they developed hypercholesterolemia. The high fat diet was given to all mice except negative control group, which was given standard purified feed. After developing hypercholesterolemia, the mice were injected with alloxan monohydrate at a dose of 110 mg/kg BW via intraperitoneal. Three days after the alloxan monohydrate injection, fasting blood sugar was measured to ensure that the mice were already hyperglycemic, with blood glucose levels above 126 mg/dL (Firdaus *et al*., 2017).

Cholesterol levels were measured before HFD induction, on day 0 (after HFD induction and on day 8. Blood was drawn from the caudal vein using a blood lancet and measured with an Easy Touch GCU and cholesterol test. Cholesterol levels in mice are considered high or indicative of hypercholesterolemia when their blood cholesterol are >130 mg/dL (Erni *et al*., 2014).

Preparation of aortic histology is the final stage after 7 days of extract administration. Before dissection, mice were anesthetized using chloroform. The aortic organs, which had been washed with physiological NaCl solution, were then immersed in 70%, 80%, and 96% alcohol, followed by absolute alcohol. The clearing process was carried out with xylene twice: first with xylene 1 for 15 minutes, and

then with xylene 2 overnight. The infiltration process began by immersing the organs in a paraffin (1:1) solution for 30 minutes, followed by immersion in pure paraffin three times for 1 hour each. The organs were then embedded in paraffin in a standing position to facilitate cross-sectioning. Sectioning was performed using a microtome to obtain slices with a thickness of 4-5 µm. the paraffin-embedded organ slices were then immersed in a water bath at 40°C and transferred onto glass slides that had been smeared with mayer's albumin. The slides were dried in an oven at 50°C for at least 2 hours. The preparations were then stained with hematoxylin-eosin dye (Khaleyla *et al*., 2021)

Aortic preparations were observed using a light microscope at 400x and 100x magnification. The diameter of the aortic lumen was measured using Image J. The measurement was obtained from the average lenghth of the lumen in the horizontal (x) and vertical (y) directions. Meanwhile, atherosclerosis lesions were determined using a scoring method based on Ismawati *et al.* (2016), as presented in Table 1.

Table 1. Atherosclerosis score

Score	Definition
0	No Atherosclerosis
1	Elastic fiber alignment with few foam cells
2	Medial lipid infiltration, smooth muscle growth, and fibrosis or calcification in the elastic lamella fragmentation
3	Thrombus or ulcerative plaque

Total cholesterol levels and aortic wall thickness were tested for normality with Saphiro-Wilk and continued with ANOVA test. Data on total cholesterol levels were tested with Duncan's test. Data on aortic wall thickness was not homogenous so it was tested using ANOVA-Welch test. Atherosclerosis score data were analyzed using Kruskal-Wallis test and post hoc Mann-Whitey Test.

RESULTS

Based on the research conducted, there were differences in the mean total cholesterol levels between the control and treatment groups. This include total cholesterol levels before HFD induction, after HFD (day 0), day 8, and changes in total cholesterol levels after application of African leaf ethanol extract (Table 2).

Table 2. Effect of ethanol extract of African leaf on total blood cholesterol of type 2 diabetes mellitus mice.

Treatment	Total Cholesterol Levels (mg/dL) ±SD			Retrieved (mg/dL) ± SD
	Before HFD	D-0	D-8	
KN (A)	102,75 ± 2,68^{a}	115.75 ± 3,27^{a}	121,5 ± 2,06^{a}	↑5,75 ± 1,48^{e}
KP (B)	110,25 ± 5,80^{ab}	159,25 ± 4,71^{cd}	157,75 ± 6,83^{c}	↓1,5 ± 2,18^{d}
KS (C)	115 ± 3,81^{b}	160,75 ±2,38^{d}	127 ± 4,18^{a}	↓33,75 ± 2,86^{a}
K I (D)	104 ± 2,74^{a}	150,75 ± 5,07^{b}	138 ± 3,54^{b}	↓12,75 ± 2,38^{c}
K II (E)	109,25 ± 6,87^{ab}	152 ± 4,85^{bc}	122,5 ± 5,59^{a}	↓29,25 ± 2,18^{b}
K III (F)	114 ± 5.79^{b}	158,5 ± 4,33^{cd}	123,25 ± 4,32^{a}	↓35,25 ± 1,92^{a}

Notes: KN or Negative Control, KP or Control Positive (HFD + alloxan), KS or Simvastatin Group (HFD + alloxan + simvastatin), KI (HFD + alloxan + African leaf ethanol extract 75 mg/kg BW), KII (HFD + alloxan + African leaf ethanol extract 100 mg/kg BW), KIII (HFD + alloxan + African leaf ethanol extract 125 mg/kg BW). ↑ indicating increase, ↓ indicating decrease of total cholesterol levels. Different subset notations indicate significant differences based on Duncan's test ($P<0.05$).

The induction of a high fat diet (HFD) for 7 weeks, consisting of a mixture of purified feed, goat fat oil, and duck egg yolk leads to an increase in total cholesterol levels (>130 mg/dL), except in mice in the negative control group that don't consume the HFD. Based on ANOVA analysis, the ethanol extract of Africa leaves significantly reduced total cholesterol levels (P= 0,000). The decrease of total cholesterol levels in the positive control group was significantly different from the simvastatin and African leaf ethanol extract treatment groups. The KIII treatment (125 mg/kg BW) and 10mg doses of simvastatin showed no significant difference (P= 0,416). However KIII decreased significantly compared to KI and KIII.

Atherosclerotic lesions due to high fat feeding were observed under a microscope with 400x magnification and analyzed using a scoring method. The scoring method was applied to assess the

severity of atherosclerosis in the aorta of mice induced by diabetes mellitus and HFD. Based on the results of the study, the mean score of atherosclerosis is presented in Table 3.

Table 3. Atherosclerotic lesion score

Treatment	Average Atherosclerotic Lesion Score ±SD
KN (A)	0 ± 0[a]
KP (B)	0,83 ± 0,17[b]
KS (C)	0,17 ± 0,29[a]
KI (D)	0,33 ± 0,33[b]
KII (E)	0,17 ± 0,29[a]
KIII (F)	0,17 ± 0,17[a]

Notes: KN or Negative Control, KP or Control Positive (HFD + alloxan), KS or Simvastatin Group (HFD + alloxan + simvastatin), KI (HFD + alloxan + African leaf ethanol extract 75 mg/kg BW), KII (HFD + alloxan + African leaf ethanol extract 100 mg/kg BW), KIII (HFD + alloxan + African leaf ethanol extract 125 mg/kg BW). ↑ indicating increase, ↓ indicating decrease of total cholesterol levels. Different subset notations indicate significant differences based on Duncan's test (P<0.05).

Based on Table 3, the aorta of mice with a score of 0 (no atherosclerosis) in each replicate represents the negative control group, as indicated by the tight arrangement of the aortic wall and the absence of fat accumulation. In the positive control group, macrophages, foam cells, and irregular endothelial cells were observed. In the dose I control group, foam cells were present, though in fewer numbers, along with irregular endothelial cells. In the simvastatin, KII, and KIII groups, foam cells were still present but in smaller numbers compared to the positive control and KI groups (Figure 1).

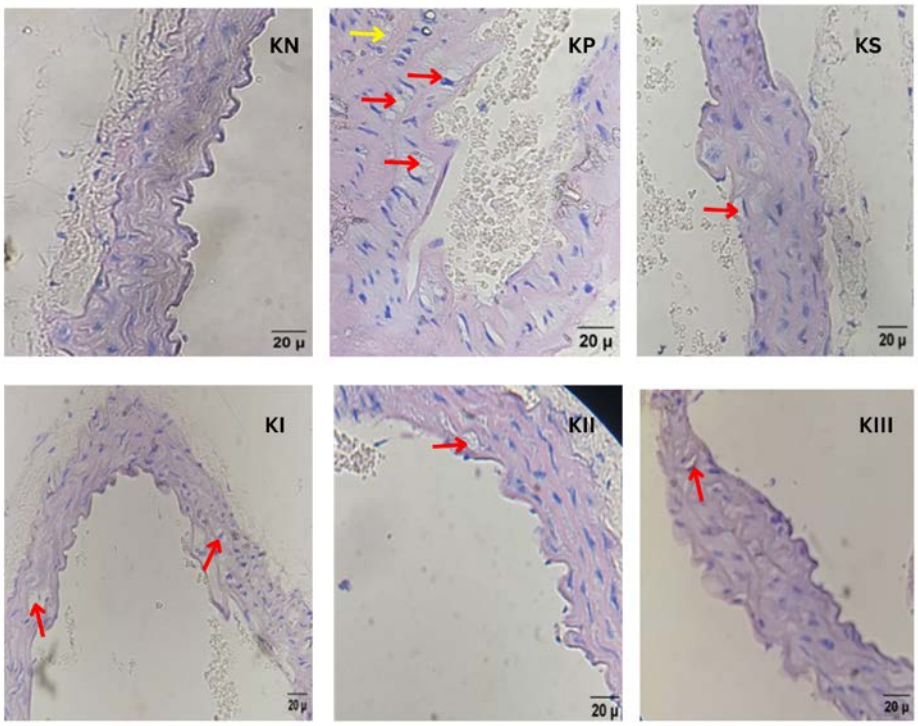

Figure 1. KN or Negative Control, KP or Control Positive (HFD + alloxan), KS atau Simvastatin Group (HFD + alloxan + simvastatin), KI (HFD + alloxan + African leaf ethanol extract 75 mg/kg BW), KII (HFD + alloxan + African leaf ethanol extract 100 mg/kg BW), KIII (HFD + alloxan + African leaf ethanol extract 125 mg/kg BW). ↑: Foam Cell. ↑: Macrofag.

Atherosclerotic lesions were analyzed using the Kruskal-Wallis Test, with a result of P=0.032, indicating that the ethanol extract of African leaves has an effect on atherosclerotic lesions. The data were then tested with the Mann-Whitney test, which showed that the positive control was significantly different from the negative control, KS, KII, and KIII. However, the positive control did not indicate a significant difference compared to KI.

The effect of African leaf ethanol extract on aortic wall thickness is presented in Table 4 and Figure 2.

Table 4. Aortic wall thickness of type 2 diabetes mellitus mice

Treatment	Average Wall Thickness (µm) ±SD
KN	15,65 ± 1,16[a]
KP	19,89 ±2,20[a]
KS	16,11 ±0.26[a]
KI	17,73 ±1.16[a]
KII	16,26 ±1.39[a]
KIII	16.04 ±1,09[a]

Notes: KN or Negative Control, KP or Control Positive (HFD + alloxan), KS atau Simvastatin Group (HFD + alloxan + simvastatin), KI (HFD + alloxan + African leaf ethanol extract 75 mg/kg BW), KII (HFD + alloxan + African leaf ethanol extract 100 mg/kg BW), KIII (HFD + alloxan + African leaf ethanol extract 125 mg/kg BW). Different subset notations indicate significant differences based on Anova-Welch Test (P<0,05).

Based on Table 4, the largest aortic wall thickness was observed in the positive control group, with an average thickness of 19,89µm. Meanwhile, the smallest aortic wall thickness was found in the negative control group, with a thickness of 15,65 µm**.** The following are the results of aortic histology preparations observed under a 100x magnification microscope and measured using Image J.

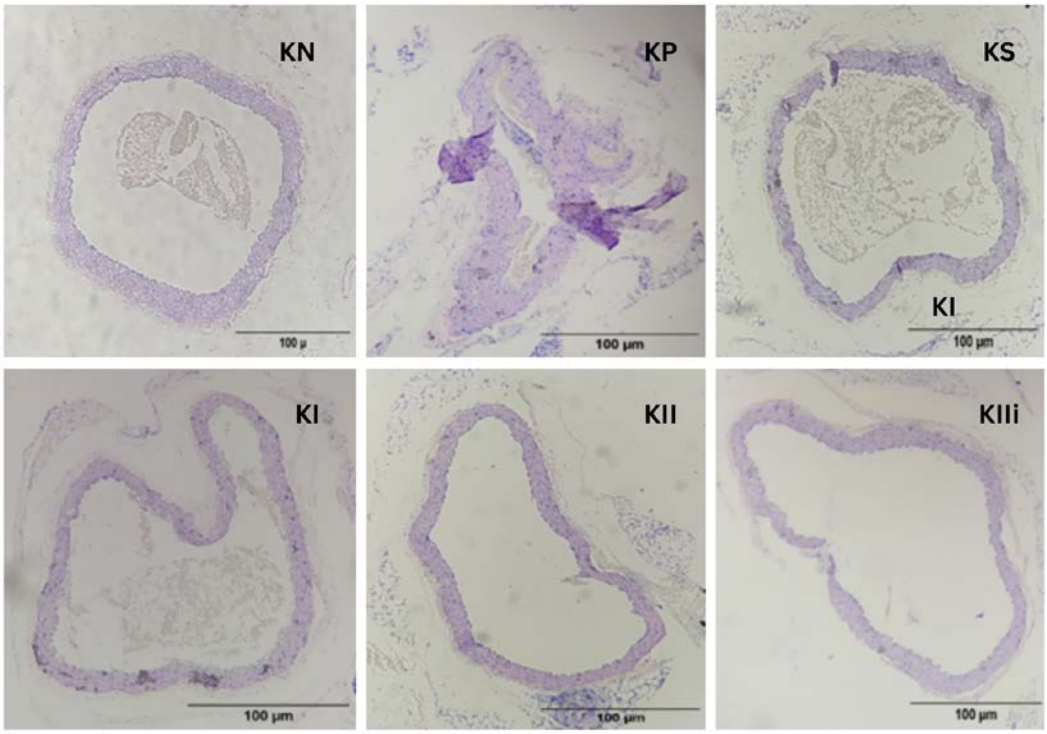

Figure 2. KN or Negative Control, KP or Control Positive (HFD + alloxan), KS or Simvastatin Group (HFD + alloxan + simvastatin), KI (HFD + alloxan + African leaf ethanol extract 75 mg/kg BW), KII (HFD + alloxan + African leaf ethanol extract 100 mg/kg BW), KIII (HFD + alloxan + African leaf ethanol extract 125 mg/kg BW).

Aortic wall thickness data is not homogeneous so it is continued with Welch's ANOVA test and obtained p=0.170, indicating that there is no difference in aortic wall thickness in each treatment.

DISCUSSION

Continuous consumption of fatty foods can lead to uncontrollable increases in cholesterol levels, resulting in hypercholesterolemia. In this study, total cholesterol levels in the positive control treatment, simvastatin group, and doses of 75, 100, 125 mg/kg BW increased due to the induction of High Fat Diet (HFD) for 49 days. The HFD consumed by the mice was a mixture of standard purified feed, goat fat oil, and duck egg yolk in a ratio of 80:15:5. Saturated fatty contained in goat fat oil and duck egg yolk can increase cholesterol levels. Goat fat oil contains 102 mg of cholesterol and 47.3 g of saturated fat in every 100 ml while duck egg yolk contains 102 mg of cholesterol.

The saturated fats in the HFD used are resistant to oxidation and free radical formation, resulting in increased cholesterol and LDL due to the absence of double bonds in saturated fatty acids

(Untari *et al*., 2023). Most lipids, in the form of triglycerides, are hydrolyzed into free fatty acids. To produce energy, free fatty acids. To produce energy, free fatty acids are then oxidized to acetyl-CoA. Excessive fat consumption increases the synthesis of acetyl-CoA, leading to an increase in cholesterol levels (Mulyani *et al*., 2019).

Based on the 7-day treatment presented in Table 2, it is known that total cholesterol levels decreased in all groups except in the negative control. The smallest decrease was found in the negative control group, with a total decrease of 1.5 mg/dL, as this group did not receive treatment or ethanol extract of Africa leaves. Meanwhile, the largest decrease was found in the KIII group (125 mg/kg BW dose), with a total decrease was 35.25 mg/dL. The average decrease in total cholesterol levels for the KIII and simvastatin groups showed no significant difference, with the simvastatin group experiencing a decrease of 33.75 mg/dL. Simvastatin is an anti-hypercholesterolemia drug that inhibits the HMG-CoA reductase enzyme. As a result, cholesterol synthesis decreases, and the number of receptors increases, leading to a reduction in LDL and total cholesterol levels (Wulandari and Rahmanisa, 2016; Faadilah and Ardiaria, 2016). The dose of African leaf ethanol extract 125 mg/kg BW (KIII) for 7 days decreased most significantly in mice with total cholesterol levels close to normal levels. Therefore, this dose can be considered the optimal dose for reducing total cholesterol levels.

According to Ardiani's research (2017), ethanol extract of African leaves is able to reduce total cholesterol levels in *Rattus novergicus* at doses of 100; 150; and 200 mg/kg BW significantly compared to negative control. Nobertson *et al.* (2018) in their research also mentioned that ethanol extract of African leaves at doses of 50; 100; and 150 mg/kg BW was also able to reduce total cholesterol levels in *Rattus novergicus* with the optimal dose of 100 mg/kg BW. African leaves act as anti-inflammatory, antioxidant, and anti-diabetic because of their bioactive compounds (Bestari, 2021).

Flavonoids are able to inhibit cholesterol synthesis. This reduction in cholesterol synthesis occurs due to the inhibition of HMG-CoA reductase formation, which leads to an increase in LDL receptors and a decrease in total cholesterol levels (Mutia *et al*., 2018). In addition, flavonoids prevent the activation of acyl-CoA cholesterol acyltransferase (ACAT) in HepG2 cells, which reduces cholesterol esterification in the liver and intestines (Benge *et al*., 2020). Another compound in African leaves that can reduce cholesterol levels is tannin. Tannins react with mucosal proteins and intestinal epithelial cells to inhibit fat absorption in the in the intestine (Ekananda, 2015). Saponins in African leaves are believed to have hypercholesterolemia effects. Saponins inhibit cholesterol absorption, the reabsorption of bile salts, and cholesterol synthesis due to their interaction with bile salts which form an insoluble mixture so that cholesterol cannot be absorbed by the intestines and excreted through the feces (Benge *et al*., 2020).

Lipid accumulation leads to lipid modification, which triggers inflammation in the endothelium. Inflammatory mediators then migrate to the tunica intima, where they adhere to the endothelium due to adhesion molecules such as interceluller adhesion molecule-1 (ICAM-1), selectin, and vascular cell adhesion molecule-1 (VCAM-). *Low Density Lipoprotein* (LDL) is phagocytosed by macrophage, which are derived from monocytes and act as inflammatory mediators. Foam cells, resulting from phagocytosis, accumulate to form an atheroma in the blood vessel wall (Ramadhian and Rahmatia, 2017).

The scoring method was used to measure the severity of the atherosclerosis lesions. The results presented in Table 3 indicate that the positive control group had the highest lesions score due to HFD induction and the absence of drug or extract treatment. Meanwhile, the negative control group was not detected atherosclerosis due to the absence of HFD containing saturated fat. The saturated fat is resistant to oxidation and free radical formation, which leads to increase cholesterol and LDL levels due to the absence of double bonds in saturated fatty acids (Untari *et al*., 2023). The simvastatin treatment group, 100 mg/kg BW dose of African leaf ethanol extract, and 125 mg/kg BW dose if African leaf ethanol extract had the same atherosclerotic lesion score. This indicates that African leaf ethanol extract at doses of 100 and 126 mg/kg BW has the ability to improve atherosclerosis lesions in the aorta of mice as an alternative to simvastatin for 7 days. Therefore, the optimal dose of ethanol extract that can repair atherosclerotic lesions to approach normal aortic histology score is at a minimum of 100 mg/kg BW.

Flavonoids, as antioxidants, act as inhibitors of various oxidation reactions. The hydroxyl group in the flavonoids structure functions as a reactive oxygen species (ROS) scavenger, capable of halting the oxidation of LDL (Agustina *et al*., 2022). Flavonoids protect the body from oxidative stress by triggering nitric oxide synthesis, which helps inhibit inflammation in blood vessels (Panche *et al*., 2016). Antioxidant in tannins found in African leaves can inhibit fat oxidation and the formation of

foam cells (Permana *et al*., 2016). These active compounds serve as antioxidants. An important mechanism by which antioxidants prevent lipid peroxidation is by neutralizing free radicals. The reduction of ROS by bioactive compounds prevents LDL oxidation in the endothelium, making the effective in reducing the risk of atherosclerosis (Agustina *et al*, 2023).

Endothelial dysfunction due to oxidative stress can increase macrophage production. The accumulation of fat, macrophages, and platelets in the tunica intima and tunica media causes the blood vessel wall to thicken and the lumen diameter to narrow (Zhou *et al*., 2016). Aortic wall thickness in this study showed no significant differences across treatments. Based on Table 4, the smallest average aortic wall thickness was found in the positive control group, with a value of 15,65 µm, while the largest average aortic wall thickness was found in the negative control group, with a value of 19,89 µm. total cholesterol and blood glucose levels that did not exceed normal limits did not lead to atherosclerosis in the negative control, while the positive control group developed atherosclerosis and aorta wall thickening due to elevated total cholesterol and blood glucose levels. However, the increase in total cholesterol and type 2 diabetes mellitus in mice this study did not result in significant thickening of the aortic wall compared to the negative control. This is related to the severity of atherosclerosis in the mice. Regarding lesion scores, the highest score in each replicate was 1, where only a few foam cells were found in the tunica intima and tunica media. Thrombus that leads to thickening the aortic wall was not found in all treatment groups.

Atherosclerosis of the coronary vessels is a disease that begins at a young age and develops asymptomatically for many years, until the blood vessels become blocked and ischemic symptoms appear (Aini *et al*., 2021). In mice, most cholesterol is transported in HDL, rather than LDL as in humans. This results in a higher proportion of plasma cholesterol being carried by HDL particles, rather than LDL as in humans. Therefore, most of the plasma cholesterol is in HDL particles and as a whole, they have much lower cholesterol levels that can provide protection against atherosclerosis. Although mice are a model for HDL, there are some strain differences that need to be considered in lipoprotein metabolism and inflammatory susceptibility. The C57BI/6 strain is commonly used as a model for atherosclerosis because it has lower HDL levels (Oppi *et al*., 2019).

CONCLUSION

The ethanol extract of African leaves can reduce total cholesterol levels and affect the histology of the aorta in mice with type 2 diabetes mellitus. The optimal dose for reducing cholesterol levels and improving aortic histology due to HFD induction is 125 mg/kg BW, with a decrease of 35.25 mg/dL. Suggestion for further research are extending the duration of High Fat Diet (HFD) induction and selecting a feed with higher LDL to increase total cholesterol levels in mice until aortic wall thickening occurred.

ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to all those who have provided support and contributions to this research, especially to the Biology lecturers from Universitas Negeri Surabaya, who offered valuable assistance in terms of advice and facilities. We would also like to express our gratitude to the editors and reviewers who assisted in the publishing process of this article. Without their help and support, this research would not have been carried out successfully.

CONFLICT OF INTEREST

There is no conflict of interest.

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