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EFFECT OF GROUND AND NON-GROUND BAY LEAF (*Syzygium polyanthum*) ON AORTIC THICKNESS IN WISTAR RATS

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Abstract

Background: Hypercholesterolemia promotes aortic wall thickening and increases cardiovascular risk. Although *Syzygium polyanthum* (bay leaves) has antioxidant and hypocholesterolemic properties, evidence comparing crushed and non-crushed decoctions remains limited.

Objective: This study aimed to compare the effects of crushed and non-crushed bay leaf decoctions on aortic wall thickness in hypercholesterolemic Wistar rats (*Rattus norvegicus*).

Methods: A randomized post-test only control group study was conducted using 40 male Wistar rats assigned to eight groups. Bay leaf decoctions (crushed or non-crushed; 0.52%, 0.72%, and 0.92%) were administered orally for 14 days. Aortic wall thickness was assessed histologically and analyzed using the Kruskal–Wallis and Dunn's tests.

Results: A significant difference in aortic wall thickness was observed among the groups ($p = 0.040$). The lowest mean aortic wall thickness was observed in the 0.92% crushed bay leaf decoction group (92.00 ± 29.20 µm), which was lower than that of the hypercholesterolemic control group (188.00 ± 29.24 µm).

Conclusions: Crushed bay leaf decoction, especially at 0.92%, more effectively reduced aortic wall thickness than the non-crushed preparation. Further studies are needed to confirm the underlying mechanisms.

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INTRODUCTION

Coronary heart disease (CHD) is the leading cause of cardiovascular mortality worldwide. It occurs when coronary arteries become narrowed or blocked, reducing oxygen and nutrient supply to the myocardium. Blockage of blood vessels can trigger a sudden heart attack. In severe cases, blood flow to the heart muscle stops completely, and the heart's ability to pump blood throughout the body decreases drastically, which can be fatal (1). High blood cholesterol levels are an important factor in this blockage process because they can narrow the arterial lumen, reduce blood flow, and decrease oxygen supply to the heart, ultimately contributing to the development of CHD (2).

Hypercholesterolemia, a disorder of lipid metabolism characterized by elevated blood cholesterol levels, is closely associated with the onset of CHD. The World Health Organization (WHO) estimates that there are 16–33 million cases of hypercholesterolemia worldwide. In Indonesia, the number of people with high cholesterol is estimated to reach 76 million out of a total population of 273 million in 2023 (3). The incidence of hypercholesterolemia has consistently increased over the years, with cases rising from 23,636 in 2004 to 100,231 in 2016 (4)

Atherosclerosis is the main mechanism linking hypercholesterolemia to CHD. It is characterized by thickening of the aortic wall and the formation of foam cells in microscopic lesions (5). The arterial wall consists of three layers: the tunica intima (endothelial cell layer), the tunica media (smooth muscle cells and elastic tissue), and the tunica adventitia (connective tissue) (6). The normal aortic wall thickness is approximately 72.3 µm, whereas it increased to 86 µm in the positive control group reported in a previous study (7). Thickening of the aorta is caused by the accumulation of fat and cholesterol, proliferation of smooth muscle cells, and formation of a connective tissue matrix composed of collagen, elastin fibers, and fibrous tissue.

Measurement of aortic cross-sectional thickness typically focuses on the intima and media layers, as these regions are most susceptible to atherosclerotic changes. The accumulation of LDL cholesterol on the arterial walls triggers plaque formation, which obstructs blood flow and damages blood vessels. Risk factors for hypercholesterolemia include unhealthy lifestyle habits, excess weight, consumption of high-fat foods, lack of physical activity, smoking, alcohol consumption, and stress. Statins are the most commonly prescribed lipid-lowering drugs, but their use can cause side effects such as liver damage, myopathy, gastrointestinal disorders, insomnia, and central nervous system disturbances (8). These limitations encourage the search for safer, natural alternatives.

Previous studies have reported that *Syzygium polyanthum* (bay leaves) possesses hypocholesterolemic activity and may reduce blood cholesterol levels in experimental animals (9). These effects have been associated with the presence of bioactive compounds that may enhance cholesterol metabolism and excretion. However, the effects of bay leaf

preparations on vascular structural changes, particularly aortic wall thickness, remain insufficiently investigated (10).

Bay leaves contain various secondary metabolites with significant pharmacological activity(11). Bay leaves contain several bioactive compounds, including flavonoids, tannins, alkaloids, and saponins, which have been reported to exhibit antioxidant and hypolipidemic activities(11),(12). Previous studies have suggested that these compounds may reduce lipid accumulation, oxidative stress, and inflammation associated with hypercholesterolemia (13). Previous studies have suggested that alkaloids may inhibit pancreatic lipase activity, thereby reducing lipid absorption. Likewise, saponins may bind cholesterol and bile acids, promoting cholesterol excretion and contributing to lower serum cholesterol levels (14). However, the present study did not investigate these mechanisms directly; therefore, they serve only as the biological rationale for the study.

Decoction is one of the most commonly used traditional methods for preparing *Syzygium polyanthum* because it is simple, inexpensive, and capable of extracting water-soluble bioactive compounds. Crushing the leaves before boiling may increase the release of these compounds by enlarging the surface area exposed to the solvent. However, limited studies have evaluated whether crushed and non-crushed bay leaf decoctions produce different biological effects on vascular tissues (15).

Wistar rats (*Rattus norvegicus*) were selected because they are widely used as experimental models for hypercholesterolemia and cardiovascular research due to their physiological similarity to humans and ease of maintenance under laboratory conditions (16).

Although previous studies have demonstrated the hypocholesterolemic properties of *Syzygium polyanthum*, most have focused on serum lipid profiles or plant extracts, while limited information is available regarding the effects of different physical preparations of bay leaf decoction on aortic wall thickness. To the best of our knowledge, no previous study has directly compared crushed and non-crushed bay leaf decoctions in a hypercholesterolemic Wistar rat model. Therefore, the novelty of this study lies in evaluating whether the physical preparation of bay leaves influences their effect on aortic wall thickness. We hypothesized that crushed bay leaf decoction would produce a greater reduction in aortic wall thickness than the non-crushed preparation because crushing may enhance the extraction of water-soluble bioactive compounds during the decoction process.

MATERIALS AND METHODS

Research Design

This experimental study employed a randomized post-test only control group design involving eight experimental groups and was conducted at the laboratories of Padjadjaran University and Rajawali Health Institute, West Bandung, Indonesia. Tools and Materials, Experimental and Adapted Animals (17), Hypercholesterolemia feed, Treatment

Of Eight Groups, Making and Giving Bay Leaf Decoction, Cholesterol Level Measurement, Euthanasia and Tissue Isolation, Aortic Thickness Observation, Data Analysis.

Tools and Materials

The tools used in this study included: POCT (Lipid Pro), lancet pen (OneMed), cholesterol strip (Lipid Pro), stirring rod (Medica), 100 mL glass beaker (Iwaki), blender (Cosmos), funnel (Herma), coverslips (VWR), disposable syringe (OneMed), hot plate (Thermo), Büchi evaporator, needle (Kimble), watch glass (Corning), measuring cup (Duran), filter paper, measuring flask (Iwaki), microscope (Olympus), glass object (VWR), oven (Memmert), Paraplast paraffin, tweezers, strainer, Safe Gloves gloves, razor goal (Gillette), blue and yellow tips (Eppendorf), and wipes (Paseo).

The ingredients used included: 0.9% physiological NaCl (Merck), 10% neutral buffer formalin (Statlab), 30–98% graded alcohol (Merck), xylol (Merck), liquid paraffin (Leica Biosystems), Mayer's egg albumin (Merck), aquadest (Aqua RO), hematoxylin solution (Merck), eosin (Thermo Fisher), absolute alcohol (Merck), Canada balsam (Thermo Fisher), chloroform (Merck), simvastatin (Kimia Farma), bay leaf (*Syzygium polyanthum*), high-fat feed, and alcohol swabs (OneMed).

Experimental and Adapted Animals(19)

Forty male Wistar rats (*Rattus norvegicus*), aged 2–4 months and weighing 200 ± 20 g, were used as experimental animals. Healthy rats were selected based on their active movement and good appetite. The animals were acclimatized for seven days in cages with wood shavings (changed twice per week), fed commercial-standard feed, and provided with ad libitum water under natural lighting and controlled room temperature. Initial cholesterol levels were measured from tail blood using a POCT Lipid Pro.

Hypercholesterolemia feed

The hypercholesterolemia feed was prepared from 5% egg yolks, 10% animal fat, 1% cooking oil, and 84% standard feed(12),(18),(19). The diet was prepared by thoroughly homogenizing all ingredients and forming them into pellets before administration. Rats were fed the high-fat diet for 14 consecutive days before treatment to induce hypercholesterolemia. Hypercholesterolemia was confirmed by measuring fasting total cholesterol levels using a Lipid Pro POCT device. Rats with total cholesterol levels exceeding 200 mg/dL were classified as hypercholesterolemic before receiving treatment..

Treatment of Eight Groups

The rats were randomly divided into eight groups, each consisting of five animals (20):

1. Negative Control: High-fat diet without treatment.
2. Positive Control: High-fat diet + simvastatin 0.018 g/kg BW/day for seven days.
3. Crushed 0.52%: High-fat diet + crushed bay leaf decoction at 0.52% of body weight (BW).
4. Crushed 0.72%: High-fat diet + crushed bay leaf decoction at 0.72% BW.
5. Crushed 0.92%: High-fat diet + crushed bay leaf decoction at 0.92% BW.
6. Non-crushed 0.52%: High-fat diet + non-crushed bay leaf decoction at 0.52% BW.
7. Non-crushed 0.72%: High-fat diet + non-crushed bay leaf decoction at 0.72% BW.
8. Non-crushed 0.92%: High-fat diet + non-crushed bay leaf decoction at 0.92% BW.

The concentrations (0.52%, 0.72%, and 0.92% BW) were selected based on previous studies reporting the hypocholesterolemic activity of *Syzygium polyanthum* in experimental animals (citation). These concentrations were used to evaluate the dose-dependent effects of crushed and non-crushed bay leaf decoctions on aortic wall thickness in hypercholesterolemic rats.

Making and Giving Bay Leaf Decoction(12),(20)

The preparation of bay leaf infusions was carried out using both ground and unground leaves at concentrations of 0.52%, 0.72%, and 0.92%, each boiled in 150 mL of distilled water for about 15 minutes until the volume was reduced to approximately 100 mL, then cooled to room temperature and filtered to separate the leaves. The decoctions were administered orally once daily using an oral gavage at a volume of 2 mL per rat, adjusted according to the average body weight (200 ± 20 g).

Cholesterol Level Measurement(21)

Before and after the intervention, blood was collected from the rat tail after an 8-hour fast, applied to a Lipid Pro strip, and measured using POCT(22),(19). Rats with cholesterol levels >200 mg/dL were categorized as hypercholesterolemic(6).

Surgery, Tissue Preparation, and Histological Procedures (17),(22),(19),(23)

Surgery and anesthesia were performed using 1.5 mL of chloroform per rat (23). Each rat was placed in a tightly closed container (22). Chloroform was poured onto cotton wool and inserted into the container containing the rats. The animal was left for approximately two minutes to ensure complete loss of consciousness. The rat was then positioned on a surgical board using needles, and an incision was made from the lower abdomen to the ribs using surgical scissors.

The preparation of tissue blocks was conducted to obtain aortic tissue samples for histological analysis (17). The procedures were carried out as follows:

1. Tissue Collection and Fixation (23): The mouse aorta, about 0.5 cm from the aortic arch, was collected and fixed in 10% formalin to preserve tissue integrity and prevent decomposition.
2. Trimming and Tissue Processing (17)
   - Trimming: The tissue was cut into smaller pieces (2–3 mm) to fit into the tissue processor.
   - Tissue Processing: The tissue was processed automatically through three main stages: Dehydration: Tissue fluids were gradually removed using alcohol solutions of increasing concentrations (70%, 90%, and 100%). Clearing: The tissue was immersed in xylene to remove lipids, facilitating paraffin infiltration. Infiltration: The tissue was immersed in liquid paraffin until all tissue cavities were completely filled.
3. Paraffin Block Preparation(17) : The paraffin-infiltrated tissue was embedded in a mold with liquid paraffin, solidified, and formed into a block for sectioning
4. Block Sectioning(17)

The paraffin block containing the tissue was sectioned using a microtome at a thickness of 3–5 μm. This thickness allowed adequate light penetration for microscopic observation.

5. Staining(23) : The tissue sections were deparaffinized with xylene, rehydrated with ethanol, rinsed, and stained with H&E—hematoxylin for blue-purple nuclei and eosin for pink cytoplasm.
6. Observation and Analysis(23),(17)
The aortic tissue slides were observed under a light microscope at 40× magnification. Images were captured using a digital camera positioned at the eyepiece.

Data Analysis (24),(25)

Data normality was assessed using the Shapiro–Wilk test. Because the data were not normally distributed, differences among groups were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparison test. Statistical analyses were performed using IBM SPSS Statistics (specify version), and a p-value <0.05 was considered statistically significant.

Ethical Considerations

This study did not obtain formal approval from an institutional animal ethics committee. However, all experimental procedures were conducted in accordance with standard laboratory practices to minimize animal suffering.

RESULTS AND DISCUSSIONS

This research was carried out at the Cytotechnology Laboratory of the Rajawali Health Institute for the surgical procedures of Wistar rats (*Rattus norvegicus*), as well as at the Cytotechnology Laboratory, Ministry of Health, Bandung, for tissue staining using hematoxylin-eosin. The sample consisted of 40 male Wistar rats aged 2–3 months, which were randomly divided into eight treatment groups, namely Group I (negative control) rats fed only a high-fat diet, Group II (positive control) rats fed a high-fat diet and simvastatin, Groups III, IV, and V rats fed a high-fat diet and crushed bay leaf decoction at doses of 0.52%, 0.72%, and 0.92% of body weight (BW), respectively, and Groups VI, VII, and VIII rats fed a high-fat diet and non-crushed bay leaf decoction at the same doses of 0.52%, 0.72%, and 0.92% BW(12),(18).

Bay leaves (*Syzygium polyanthum*) used in this study were known to contain active compounds such as flavonoids, saponins, and tannins, which have the potential to lower blood cholesterol levels (10),(12). Lowering cholesterol is expected to prevent the formation of atherosclerotic plaques in the walls of blood vessels, which is one of the main causes of cardiovascular disease. Therefore, this study aimed to evaluate the effect of administering bay leaf decoction on the aortic thickness of rats that had previously been induced with a high-fat diet. Prior to use, bay leaves were qualitatively tested to ensure the presence of active compounds.

Data analysis in this study began with a normality test using the Shapiro–Wilk test and a variance homogeneity test using Levene's test. If the data met the assumptions of normality and homogeneity, a One-Way ANOVA was performed to determine whether significant differences existed between groups. The data did not meet the assumptions of normality or homogeneity, the non-parametric Kruskal–Wallis test was used as an alternative method of analysis(25).

Histology of Aortic Thickness in Wistar Rats

In this study, the initial stage involved tissue processing and Hematoxylin–Eosin (HE) staining at the Histology Laboratory of the Bandung Health Polytechnic. HE staining is a standard method in histology for observing tissue morphology. Hematoxylin stains the cell nucleus, which is basophilic, while eosin stains the cytoplasm, which is acidophilic. The combination of these two dyes produces a clear contrast between the nucleus and cytoplasm, allowing the structure of cells and tissues to be observed in greater detail(26). The use of HE staining in this study aimed to evaluate the histological structure of the aorta in Wistar rats (*Rattus norvegicus*) that had hypercholesterolemia and were treated with bay leaf decoction at various doses. Microscopic image of the aorta with 400x magnification are shown in Fig.1.

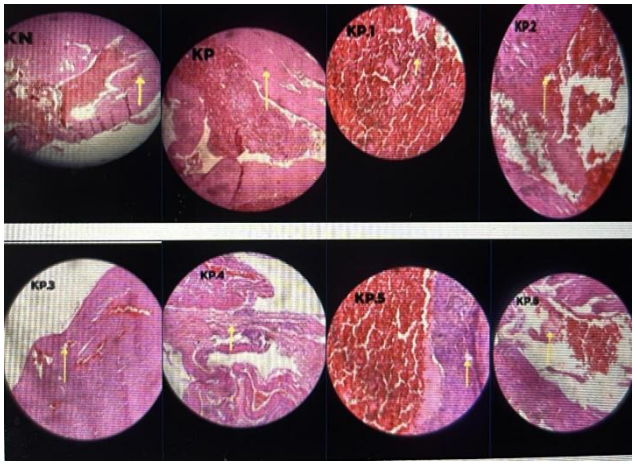


Figure 1 Microscopic image of the aorta with 400x magnification

Description : ⟶ Inflammatory cell infiltration

KN : the group given fat feed, KP : the group given fat feed + simvastatin, KP1 : the group given bay leaves was crushed at a dose of 0.52%, KP2 : the group given bay leaves was crushed at a dose of 0.72%, KP3 : the group given bay leaves was crushed at a dose of 0.92% KP4: the group given bay leaves was non-crushed at a dose of 0.52% KP5: the group given bay leaves was non-crushed at a dose of 0.72% KP5: The group given bay leaves was non-crushed with a dose of 0.92%(27).

Observations were made on aortic tissue preparations that had undergone hematoxylin-eosin (HE) staining. The evaluation focused on changes in microscopic structures, including the presence of inflammatory cells, lymphocytes, polymorphonuclear cells (PMNs), and plaque formation. Each tissue sample was then assessed and assigned a damage score based on the severity of the observed changes. The results of the assessment are presented as scores in Table 1, which served as the basis for further data analysis(27).

Test Results of Differences in Aortic Thickness of Wistar Rats in Each Group

Histological observations of the aorta of Wistar rats were performed to assess the damage score and aortic wall thickness in each treatment group. The data obtained were expressed as mean ± standard deviation (SD) to describe the differences between groups. The results of the aortic thickness measurements and histological damage scores for each group are presented in Table 1.

Table 1 Histological Damage Score

Group	Number of white rats (*Rattus norvegicus*)	Score	Mean±SD	Aortic thickness (µm)
Group (-)	1	3	2.40 ± 0.55	188.2 ± 26.0
	2	2		
	3	3		
	4	2		
	5	2		
Group (+)	1	2	1.60 ± 0.55	145.4 ± 26.0
	2	1		
	3	2		
	4	1		
	5	2		
Digestion dose 0.52%	1	1	1.20 ± 0.45	124.0 ± 42.5
	2	2		
	3	1		
	4	2		
	5	0		
Crushed dose 0.72%	1	1	1.00 ± 0.71	113.2 ± 34.5
	2	1		
	3	2		
	4	0		
	5	1		
Crushed dose 0.92%	1	1	0.60 ± 0.55	91.8 ± 28.0
	2	0		
	3	1		
	4	0		
	5	1		
non-crushed dose 0.52%	1	2	1.60 ± 0.89	145.2 ± 44.1
	2	3		
	3	1		
	4	1		
	5	1		
non-crushed dose 0.72%	1	2	1.20 ± 0.84	124.0 ± 42.5
	2	1		
	3	0		
	4	2		

Group	Number of white rats (*Rattus norvegicus*)	Score	Mean±SD	Aortic thickness (µm)
	5	1		
non-crushed dose 0.92%	1	1	1.00 ± 0.71	113.2 ± 34.5
	2	0		
	3	1		
	4	1		
	5	2		

Discussion

The Effect of High-Fat Feeding

Based on the results of serum LDL cholesterol level measurements in all groups of rats fed a high-fat diet for 7 days, no significant increase in LDL cholesterol levels was observed. Djajanti(10) reported that feeding a hypercholesterolemic diet consisting of 1% cholesterol, 5% egg yolks, 10% animal fat, 1% cooking oil, and standard feed up to 100% was not sufficiently effective in increasing blood cholesterol levels in Wistar rats.

One possible explanation may be that the feeding duration was too short, and the feeding was carried out ad libitum, resulting in variability in the amount of feed consumed by each individual. In addition, the composition of the feed may not have been high enough to significantly affect the lipid profile, particularly LDL cholesterol levels. The ad libitum method was chosen because oral gavage (sonde) feeding carries the risk of side effects such as vomiting and diarrhea, which can cause the animals to become sick and inactive, thus falling under the exclusion criteria(28).

To overcome this, the researchers extended the duration of high-fat feeding to 21 days and added propylthiouracil (PTU) as a cholesterol-increasing agent. This treatment was shown to be effective in increasing cholesterol levels, including serum LDL cholesterol.

Phytochemical Test of Bay Leaf (*Syzygium polyanthum*)

Qualitative phytochemical analysis confirmed the presence of flavonoids, tannins, and saponins, consistent with previous reports to determine the presence of secondary metabolite compounds in bay leaf powder, including tannins, flavonoids, and saponins. The tannin test was performed by adding 95% ethanol and a few drops of $FeCl_3$ solution to the bay leaf powder placed in a test tube. A color change to blackish-blue indicated a positive result for the presence of tannins. Tannins are polyphenolic compounds capable of binding and precipitating proteins(27).

The flavonoid test involved adding hot distilled water, HCl, and magnesium powder to bay leaf powder, with a brownish-yellow color and white deposits indicating a positive result. The yellow color is related to the properties of flavonoids as phenolic compounds, while the addition of magnesium powder and acid serves to reduce the benzopyran core in the chemical structure of flavonoids(29). The saponin test was carried out by mixing the bay leaf powder with distilled water, stirring, and then adding a few drops of HCl. The formation of foam or persistent froth indicated a positive result for the presence of saponins. The foam is due to the amphipathic nature of saponins, i.e., they contain both a hydrophilic

(polar) and a hydrophobic (non-polar) region(27). The results of the phytochemical tests are shown in Figure 2.

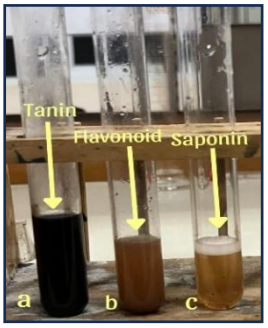
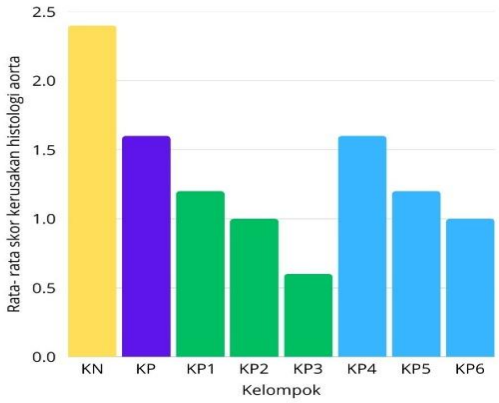


Figure 2 Phytochemical test of bay leaf *(Syzygium polyanthum)*

Effect of Bay Leaf Administration (*Syzygium polyanthum*) on Aortic Thickness of White Rats (*Rattus norvegicus*) Fed an atherogenic diet.

The results of the observations showed that aortic thickness varied depending on the atherogenic diet and the administration of bay leaf decoction at different doses and processing methods. For clarity, the data on aortic thickness measurements were then presented as a bar diagram to facilitate comparisons between the control group and the treatment groups with doses of 0.52%,0.72%, and 0.92%, as well as the differences between crushed and non-crushed preparations.



Based on the results presented in the bar chart, the highest histological damage scores of the aorta were observed in the negative control group, while the treatment groups receiving bay leaf decoction exhibited a gradual decrease in damage scores. In the groups treated with crushed bay leaf decoction at doses of 0.52%, 0.72%, and 0.92% BW, the reduction in histological damage was more pronounced compared to the corresponding non-crushed groups at the same doses, indicating that the crushing process enhanced the effectiveness of bioactive compounds in bay leaves(27). This finding is consistent with previous studies reporting that hypercholesterolemia increases foam cell formation, oxidative stress, and aortic wall thickness, whereas the administration of antioxidant agents or natural compounds can reduce intima–media thickness and improve the histological structure of the aorta in Wistar rats induced with a high-cholesterol diet(30).

These results align with the theory of atherosclerosis, in which a high-fat diet promotes the accumulation and oxidation of LDL cholesterol in blood vessel walls,

triggering inflammatory cell infiltration and plaque formation, thereby causing aortic wall thickening(27). In contrast, the groups treated with bay leaf decoction exhibited a marked reduction in aortic thickness, indicating tissue repair.

The repair mechanism is supported by the bioactive compounds in bay leaves, including flavonoids, tannins, and saponins. Flavonoids (quercetin) act as antioxidants that prevent LDL oxidation, thereby reducing foam cell formation in the intima layer. Tannins inhibit fat absorption in the intestines, while saponins bind cholesterol to bile acids, facilitating its excretion in feces(36). This combination of pharmacological effects reduces lipid deposition in the aortic wall and prevents further thickening(31).

Dose comparison showed that the 0.92% BW dose resulted in the most significant reduction in aortic thickness compared to the 0.52% and 0.72% doses, confirming a dose–response relationship in which higher doses of bay leaf decoction exert greater protective and reparative effects(32). When comparing the crushed and non-crushed preparations, the bar diagram shows that the crushed bay leaf groups exhibited thinner aortic walls than the corresponding non-crushed groups at the same dose. This indicates that crushing enhances efficacy, likely because it increases the surface area of the leaf tissue, allowing more bioactive compounds to dissolve during boiling and resulting in a higher concentration of active compounds in the decoction(12).

Overall, the study results and visualization indicate that administration of bay leaf decoction can repair aortic damage induced by an atherogenic diet. The greatest effect was observed with the 0.92% BW crushed preparation, which resulted in aortic thickness closest to normal conditions. These findings support the potential of bay leaves as a natural therapy to reduce the risk of atherosclerosis through cholesterol-lowering effects and vascular protection. For future studies, hypercholesterolemia induction should be carried out over a longer period using a more optimized high-fat diet and should include a larger sample size and longer observation period to obtain more representative results. Practically, individuals are encouraged to maintain a healthy lifestyle, limit consumption of high-fat foods, exercise regularly, and consider the use of bay leaves as a natural support for cardiovascular health. In addition, governmental programs are expected to strengthen education on coronary heart disease prevention, conduct periodic cholesterol screenings, and support the development of bay leaves as a safe, affordable, and accessible complementary therapy.

Table 2 Normality Test Results

Tests of Normality							
		Kolmogorov-Smirnova			Shapiro-Wilk		
	group	Statistic			Statistic		
		s	Df	Sig.	s	Df	Sig.
Score	negative group	.367	5	.026	.684	5	.006
	Positive group	.367	5	.026	.684	5	.006
	0.52%	.231	5	.200*	.881	5	.314
	0.72%	.300	5	.161	.883	5	.325
	0.92%	.367	5	.026	.684	5	.006
	Non-crushed 0.52%	.349	5	.046	.771	5	.046

	Non-crushed 0.72%	.231	5	.200*	.881	5	.314
	Non-crushed 0.92%	.300	5	.161	.883	5	.325

Based on Table 2, the results of the normality test showed that $p < 0.05$, indicating that the data were not normally distributed. Thus, the assumption for using parametric tests was not met. Therefore, the analysis was continued using a non-parametric test, namely the Kruskal-Wallis test, to determine whether there were significant differences in aortic thickness between the treatment groups(25).

Table 3 Results of the Crosscal-Wallis H test

Test Statistics[a,b]	
	Score
Kruskal-Wallis H	14.693
Df	7
Asymp. Sig.	.040

Based on Table 3, the results of the Kruskal–Wallis test in this study showed $p < 0.05$, indicating that there were significant differences in aortic thickness between the treatment groups. Thus, administration of bay leaf decoction in Wistar rats fed an atherogenic diet had a significant effect on aortic thickness compared to the control group(33).

These findings may be associated with the biological activities previously reported in bay leaves (flavonoids, tannins, and saponins) exert a protective effect against vascular damage caused by a high-fat diet, thereby inhibiting the thickening of the aortic wall(34). The Kruskal–Wallis test shows overall differences, so a Duncan post-hoc test was used to identify which treatment groups significantly differed in reducing aortic thickness compared to the control and other doses. This analysis is essential to ensure that the effects of the treatment are not only general but also statistically significant in specific groups(12). The results of the Duncan test are presented in Table 4.

Table 4 Duncan test results

Treatment Groups	**Number of white rats (*Rattus norvegicus*)**	**Mean ± SD (score)**	**Aortic thickness (µm) ± SD**
Negative Control	5	2.40 ± 0.55	188.0 ± 29.2a
Positive Control	5	1.60 ± 0.55	145.3 ± 29.2ab
Crushed 0.52%	5	1.20 ± 0.84	124.0 ± 44.6abc
Crushed 0.72%	5	1.00 ± 0.71	113.3 ± 37.7bc
Crushed 0.92%	5	0.60 ± 0.55	92.0 ± 29.2c
Non-crushed 0.52%	5	1.60 ± 0.89	145.3 ± 47.7ab
Non-crushed 0.72%	5	1.20 ± 0.84	124.0 ± 44.6abc
Non-crushed 0.92%	5	1.00 ± 0.71	113.3 ± 37.7bc

Based on Table 4, the results of the Duncan test showed significant differences between treatment groups in aortic thickness of Wistar rats with hypercholesterolemia. The

negative control group had the highest damage score (2.40 ± 0.55; 188.0 ± 29.2 µm) and differed significantly from the lowest score observed in the crushed 0.92% group (0.60 ± 0.55; 92.0 ± 29.2 µm). The positive control group and the 0.52% non-crushed group showed a decrease compared to the negative control, but this difference was not statistically significant. Increasing doses of bay leaf decoction, particularly in crushed form, resulted in lower damage scores, with the strongest protective effect observed at the 0.92% dose. These findings support the role of bioactive compounds in bay leaves, such as flavonoids and tannins, which possess antioxidant and antihypercholesterolemic properties, in improving aortic histology (35).

Dose comparisons showed that the 0.92% dose produced the most significant improvement in aortic thickness, approaching that of the negative control group. This indicates a dose-dependent effect, where higher doses confer greater protective potential(8). When comparing crushed and non-crushed preparations, the Duncan test results indicated that the crushed bay leaf groups exhibited better protective effects than the corresponding non-crushed groups at the same dose. This can be explained by the crushing process, which breaks down the cell walls of the leaves, allowing more optimal release of bioactive compounds into the decoction and thereby enhancing the pharmacological effect on aortic thickness (29).

CLINICAL IMPLICATION

This study suggests that *Syzygium polyanthum* (bay leaf) decoction, especially in crushed form at a 0.92% dose, significantly reduces aortic wall thickness in rats fed a high-fat diet. Clinically, the bioactive compounds in bay leaves may help protect blood vessels and lower cholesterol, indicating their potential as a natural, affordable complementary therapy to prevent atherosclerosis and support cardiovascular health.

LIMITATIONS

This study has several limitations. The small sample size (n = 40) may have reduced statistical power, and the use of male Wistar rats under controlled laboratory conditions limits the generalizability of the results to humans. The short duration of high-fat diet induction and treatment may not capture long-term effects, while aortic assessment was confined to histology without supporting biochemical data such as lipid profiles or oxidative stress markers.

CONCLUSIONS

The results of this study showed that bay leaf (*Syzygium polyanthum*) decoction, articularly at the 0.92% dose, was associated with a greater reduction in aortic wall thickness than the non-crushed preparation in hypercholesterolemic Wistar rats. A statistically significant difference was observed among the treatment groups (p = 0.040). Further studies are needed to confirm the underlying mechanisms and potential cardiovascular benefits of this finding.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest that could have influenced the results or interpretation of this study.

AUTOR CONTRIBUTIONS

SRNA designed and conducted the research, analyzed and interpreted the data, wrote, reviewed and revised the manuscript draft. FN designed and conducted the research and wrote the draft of the manuscript. HIS assisted with and supervised the experiments and provided the necessary resources. SB designed the research, supervised the process, analyzed and interpreted the data.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

ChatGPT (OpenAI, GPT-5) was employed to assist in improving the manuscript's language and readability. The authors carefully reviewed all revisions to ensure the accuracy and reliability of the final version.

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