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Effects of Quercetin Fraction from *Moringa oleifera* Leaf Extract on Oxidative Markers and Histological Profile of Carotid and Coronary Arteries: An Experimental Animal Study

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ABSTRACT

Stroke and coronary disease are mainly caused by atherosclerosis. Quercetin in *Moringa oleifera* leaf extract may protect against oxidative stress. This study aimed to determine the role of quercetin in improving dyslipidemia and inhibiting atherosclerosis onset using laboratory and histological examinations. This experimental laboratory study used a double-blind randomized sampling technique and a pre- and post-test control group design. A double-blind randomized technique referred to which neither the participants nor the experimenters know who is receiving a particular treatment. This procedure is utilized to prevent bias in research results. Experimental animals were divided into the control and treatment groups that received quercetin at a dose of 25 mg/kg body weight and a high-fat diet for 10 weeks from January to March 2023. The Friedman's test of the effect of quercetin administration on malondialdehyde (MDA), intercellular adhesion molecule (ICAM), C-reactive proteins (CRP), and low-density lipoprotein (LDL) levels revealed significant differences between the pre- and post-test. The Wilcoxon signed-rank test for the effect of quercetin administration on MDA levels revealed a significant difference in MDA levels after quercetin administration. Paired sample statistics revealed an average decrease in ICAM and CRP levels and an increase in LDL levels after quercetin administration. Cramer's V value demonstrated a strong relationship between quercetin administration and the intensity of the carotid and coronary arteries. A one-way analysis of variance indicated significant differences in the average sizes of the coronary and carotid arteries after quercetin administration. The quercetin fraction of *M. oleifera* leaves has a satisfactory therapeutic effect in an atherosclerotic rat model.

Keywords: atherosclerotic, moringa oleifera, oxidative stress, quercetin

INTRODUCTION

Atherosclerosis causes vascular diseases, such as stroke, coronary disease, and peripheral arterial disease, through arterial disruption, inflammation, and endothelial dysfunction. Stroke is associated with high rates of mortality and morbidity worldwide (Sujatha & Kavitha, 2017). Annually, 6–7 billion deaths are due to stroke, ranked after coronary disease (Benjamin *et al*., 2018). In Indonesia, the stroke prevalence is 713.783 annually (Riskesdas, 2018) and 12.3 per 1000 population in Central Java, concentrated

around Surakarta (Sekartuti, 2013). Corrective efforts to reduce atherosclerotic plaques are urgently required to prevent strokes. Atherosclerosis begins with inflammation, which leads to dysfunction of the endothelium, the innermost layer of blood vessels responsible for maintaining vessel walls and circulation. The endothelium possesses properties that counteracts atherosclerosis, inhibits cell growth, reduces inflammation, and regulates the vascular tone for optimal blood flow (Chimowitz *et al*., 2013). Several studies have demonstrated that various risk factors and oxidative stress can lead to endothelial dysfunction. Oxidative stress can be prevented by the administration of antioxidants. Therefore, the use of antioxidants has been studied extensively. *Antioxidants* inhibit the oxidation of other molecules. *Oxidation* is a chemical reaction in which electrons or hydrogen is transferred from a substance to an oxidizing agent. Oxidation reactions produce free radicals. Consequently, the radicals can initiate chain reactions, which can cause cell damage or cell death (Turgeon, 2020).

Hyperlipidemia is characterized by elevated total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels. This contributed to the development of atherosclerosis.(Helmy *et al*., 2017) Modified LDL, like ox-LDL, is absorbed by macrophages and forms foam cells in the arterial wall, leading to atherosclerotic plaques.(Ito *et al*., 2019) Diets high in saturated fat and cholesterol can increase cholesterol levels (especially LDL) and the risk of atherosclerosis (Pellizzon, 2014). *Moringa oleifera*, a plant belonging to the Moringaceae family, has gained attention for its potential therapeutic properties, including anti-cancer, anti-diabetic, anti-rheumatoid arthritis, anti-fungal, anti-microbial, and anti-atherosclerotic effects. Quercetin, an antioxidant present in *M. oleifera*, possesses remarkable antioxidant activity surpassing that of vitamins C and E (Sutrisno, 2011b). Recent research has suggested that *M. oleifera* leaf extract (Moringa leaves have been turned into powder and soaked in ethanol solution with a concentration of 70%) exhibits antioxidant properties and may offer protection against oxidative damage (Sathya *et al*., 2010). This study provides a method for determining the role of antioxidants in *M. oleifera* in improving atherosclerosis in an animal model.

MATERIALS AND METHOD

Subject Preparation

This study utilized a laboratory experimental research design in the Clinical Pathology Laboratory of Dr. Moewardi General Hospital, with a pre-test and post-test control group design. White rats were divided into two groups: a treatment group that received 25 mg/kg body weight of quercetin and a high-fat diet, and a randomly selected control group, this aims to analyze and prove the role of the quercetin fraction in hyperlipidemia conditions so that the research subjects are divided into a control group (giving distilled water with normal diet) and a treatment group (giving quercetin fraction with a high fat diet). This study focused on rats with body weights between 300 g and 350 g without physical defects. The sample size was determined using the formula n = 10/k +1, and a double-blind random sampling technique was employed. There were 5 treatment groups with 3 different drug doses. With this division and based on the formula, it was obtained that each selected group had 5 mice and met the requirements, namely a minimum of 3 and a maximum of 5 subjects per group. The total number of subjects followed was 25 mice. where the subject does not know the type of treatment or dose of drug given and the researcher randomizes the treatment given to the research subjects so that they do not know the type of treatment given to each group of subjects. The effects of quercetin on various parameters and histological profiles, including malondialdehyde (MDA), intercellular adhesion molecule-1 (ICAM), C-reactive protein (CRP), LDL as well as carotid and coronary arteries, in atherosclerosis were investigated.

Initial Procedure

Rats were selected as the experimental animals, and a high-fat diet was prepared using duck egg yolk 2cc/200gBB, oxidized oil (leftover frying oil) 1cc/200gBB, beef tallow 2cc/200gBB (2:1:2). *Moringa* leaf extract was obtained by drying, powdering, soaking in 70% ethanol solution, and filtering. The production of moringa leaf extract initiates by obtaining fresh, vibrant green moringa leaves sourced from the Lawu mountains. The process involves drying the fresh leaves for 48h at a temperature of 40°C. Next, a specialized grinding machine is utilized to transform the dried leaves into a fine powder with a sieve diameter of 1 mm. Subsequently, the powdered moringa leaves are immersed in a 70%

ethanol solution for a duration of 72 hours. After stirring the mixture for 30 min, it is allowed to rest for 24h before undergoing filtration to acquire the moringa leaf filtrate. Following this, the filtrate is evaporated using a rotary vacuum evaporator, with a water bath heated to 70°C, resulting in the formation of a moderately thick extract. Careful pouring of the obtained extract into porcelain cups is followed by gentle stirring at a temperature of 70°C, yielding the ethanol extract of M. oleifera ready for utilization in research. Finally, the ethanol extract of M. oleifera undergoes further processing to produce quercetin fractions for experimental treatment purposes. Quercetin fractions were obtained from ethanol extracts of *M. oleifera* leaves. The dose of quercetin from Moringa leaf extract given was based on findings in previous research. research by Jia, *et al*. (2019) used a quercetin solution at a dose of 12.5 mg/kg for 12 weeks. will significantly reduce the area of atherosclerotic plaque, lipid accumulation and increase collagen fibers in atherosclerotic plaque. Another study conducted by Jiang *et al*. (2020) using quercetin (20 mg/kg/day) intragastrical was able to alleviate atherosclerotic lesions both in vivo and in vitro through the mechanism of reducing cellular apoptosis and increasing mitochondrial membrane potential and reducing free radicals or Reactive Oxygen Species (ROS). Based on several considerations in the literature review, in this study 3 doses were chosen, namely; 1) Treatment group 1: 12.5 mg/kg body weight, 2) Treatment group 2: 25 mg/kg body weight, 3) Treatment group 3: 50 mg/kg body weight and those dissolved in 2 cc of distilled water for administration. To ensure the fraction is quercetin we use the ultrasonication method.

Experimental Studies

The experimental animals underwent a 3-day adaptation period and were randomly divided into two groups. The negative control group received distilled water and a high-fat diet, whereas the treatment group received 25 mg/kg quercetin and a high-fat diet for 10 weeks (in order to assess reduced atherosclerotic plaque formation, both in the aorta and in the carotid arteries) from January to March 2023. A hypercholesterolemia model was induced by feeding mice with a high-fat diet for 8 weeks. This is in accordance with research by Juzwiak, *et al*., (2005) The hypolipemic properties of quercetin were associated with reduced atherosclerotic plaque formation, both in the aorta (12-week study) and in the carotid arteries (4-week study) in animals fed a high-fat diet. Blood samples were collected after 8 weeks of treatment to assess LDL, MDA, ICAM, and CRP levels. Quercetin administration from *Moringa* leaf extract was continued for an additional 10 weeks, while the high-fat diet was maintained. Blood samples were collected again after 18 weeks of treatment to evaluate LDL, MDA, ICAM, and CRP levels. Subsequently, the animals were euthanized to obtain blood vessel specimens for histological examination. After the administration of the final extract (administration of quercetin from Moringa leaf extract), the rats were euthanized by ether aerosol inhalation. Blood samples were collected before euthanasia. Decapitation was performed to obtain the carotid and coronary artery specimens for histological examination. The carotid and cardiac arteries were fixed and trimmed, followed by dehydration, clearing, impregnation, and paraffin embedding. Slices with a thickness of 4–5 µm were obtained and stained with hematoxylin and eosin. Tissue sections were examined under a light microscope at 1000× magnification. A computer software was used to analyze the presence of atherosclerotic plaques. MDA and CRP levels were estimated by measuring thiobarbituric acid reactive substances (TBARS). ICAM levels were analyzed by serum analysis using high-performance liquid chromatography. LDL levels were measured before and after treatment using a spectrophotometer.

RESULTS AND DISCUSSION

Oxidative Markers

Researchers examined oxidative markers in the rats involved in this study before administering quercetin treatment, with the average results of MDA, ICAM, and CRP pre-treatment in the control group (MDA=103.593, ICAM=0.415, and CRP=0.588) and in the *quercetin* 25 group (MDA=85.375, ICAM=0.377, and CRP=0.378).

To assess the effect of quercetin administration on MDA, ICAM, CRP, and LDL levels simultaneously (multivariate), the multivariate normality assumption was tested using the Shapiro–Wilk test ($p < 0.05$). As the data did not meet the multivariate normality assumption, a Friedman test was conducted. The Friedman test revealed a significant effect of quercetin administration on MDA, ICAM, CRP, and LDL levels ($p = 0.000$, < 0.05, respectively). Thus, quercetin administration significantly affected all four biomarkers simultaneously.

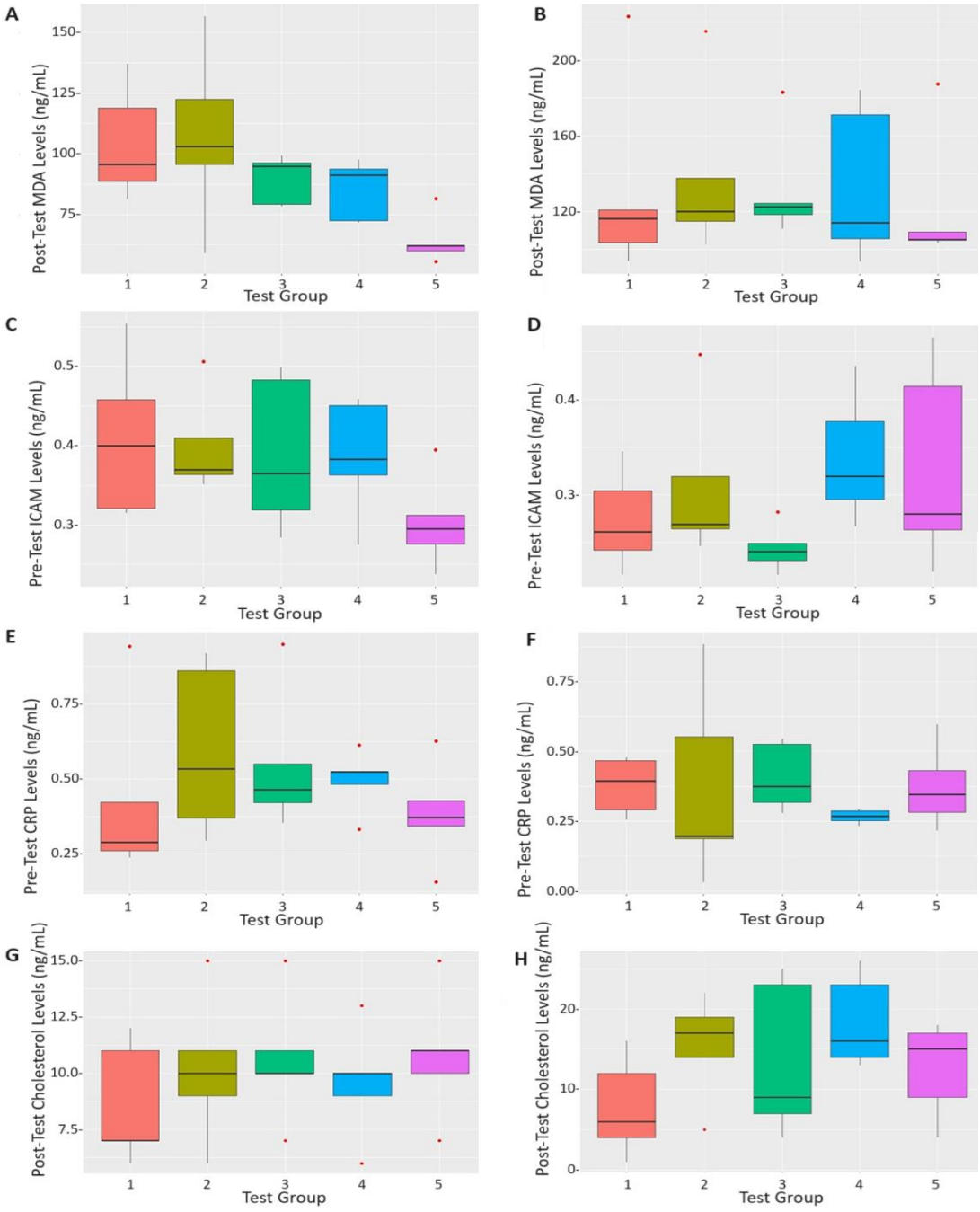

Figure 1. Boxplot diagram of pre-treatment MDA levels (a); Boxplot diagram of post-treatment MDA levels (b); Boxplot diagram of pre-treatment ICAM levels (c); Boxplot diagram of post-treatment ICAM levels (d); Boxplot diagram of pre-treatment CRP levels (e); Boxplot diagram of post-treatment ICAM levels (f); Boxplot diagram of pre-treatment LDL levels (g); Boxplot diagram of post-treatment LCL levels (h).

Note : Red boxplot= Kel. K (Control Group); Yellow boxplot= Kel. KN (Negative Control Group); Green boxplot= Kel. P1 (Treatment Group 1); Blue boxplot= Kel. P2 (Treatment Group 2); Purple boxplot= Kel. P3 (Treatment Group 3)

Table I. ICAM, CRP, LDL Paired T-Test Results, Paired Samples Test Results, Paired Samples Effect Sizes Results

		Paired Samples Statistics				Paired Samples Test	Paired Samples Effect test
		Mean	N	Std. Deviation	Std. Error Mean		
ICAM	Pre-Test	.37784	25	.083145	.016629	Sig (2-tailed) .005	Cohen's d = .616
	Post Test	.29860	25	.074610	.014922		
CRP	Pre-Test	.49044	25	.222862	.044572	Sig (2-tailed) .019	Cohen's d=.505
	Post Test	.35940	25	.173524	.034705		
LDL	Pre-Test	9.96000	25	2.730690	.546138	Sig (2-tailed) .027	Cohen's d = -.470
	Post Test	13.56000	25	7.280568	1.456114		

Boxplot diagrams demonstrating MDA levels before and after the quercetin administration in each group (Figures 1a and 1b). The normality test results using the Shapiro–Wilk method obtained a *p*-value of 0.018 (<0.05); therefore, the Wilcoxon signed-rank test method was used. The Wilcoxon signed rank test revealed a significant difference in MDA levels ($p < 0.05$) after quercetin administration.

Boxplot diagrams illustrating the ICAM levels before and after quercetin administration in each group (Figures 1c and 1d). The normality test conducted using the Shapiro–Wilk method resulted in a p-value of 0.746, indicating that the paired t-test can be employed. Upon examining the paired sample statistics, an average reduction in ICAM parameters was evident following quercetin administration. Additionally, the paired sample test yielded a p-value of 0.005, signifying a significant difference in the ICAM parameters after quercetin administration. The impact of quercetin administration on ICAM parameters was observed in the paired sample effect sizes (Cohen's d point estimate = 0.616) (Table I), suggesting a significant and moderate difference in ICAM parameters after quercetin administration.

Boxplot diagrams demonstrate the CRP levels before and after quercetin administration of in each group (Figures 1e and 1f). The results of the normality test using the Shapiro–Wilk method was $p = 0.284$ (>0.05); therefore, the paired t-test was used (Table I). The results of the paired sample statistics demonstrated an average decrease in the CRP parameters after quercetin administration. The results of the paired sample test ($p = 0.019$) revealed a significant difference in the CRP parameters after quercetin administration. The paired sample effect sizes (Cohen's d point estimate = 0.505) indicated a significant and moderate difference in the CRP parameters after quercetin administration.

The boxplot diagrams (Figures 1g and 1h) illustrate the LDL levels before and after quercetin administration in each group. The normality test conducted using the Shapiro–Wilk method yielded a *p*-value of 0.778, indicating that the paired t-test can be applied. Analysis of paired sample statistics revealed an average increase in the LDL parameters following quercetin administration. The paired-samples test ($p = 0.027$) demonstrated a significant difference between the pre- and post-test scores. The effect of quercetin on LDL parameters was observed in the results of paired sample effect sizes, with a Cohen's d point estimate of 0.47. Consequently, a significant but weak difference in LDL parameters was identified after quercetin administration.

Histological Profile

To assess the impact of quercetin administration on the intensity of the carotid vessels, the chi-square test was employed. Using Fisher's exact test ($p = 0.011$), we determined that a relationship existed between quercetin administration and the intensity of the carotid vessels. Cramer's V value (>0.3) indicated a strong association between quercetin administration and the intensity of carotid vessels.

To examine the effect of quercetin administration on the histopathological size of the carotid vessels, a one-way analysis of variance (ANOVA) was performed. The normality tests, conducted using the Shapiro–Wilk test, indicated p-values >0.05, satisfying the assumption of normality. The homogeneity of variance was assessed using Levene's test, resulting in a p-value of 0.27 (<0.05), indicating that the variances were not homogeneous across groups.

Considering the research design and the fulfilment of the assumption of independence between groups (as each group used five different rats), a one-way ANOVA test was conducted using Welch's ANOVA. The results of the one-way ANOVA test indicated a Welch significance value of <0.001, indicating a significant difference in the average carotid vessel size after quercetin administration among the different test groups.

The chi-square test was used to assess the impact of quercetin administration on the intensity of coronary blood vessels. The Fisher's exact test ($p < 0.001$) revealed a relationship between quercetin administration and the intensity of coronary blood vessels. Cramer's V value > 0.3 indicates a strong relationship between quercetin administration and the intensity of coronary blood vessels.

To evaluate the effect of quercetin administration on the histopathological size of the coronary arteries, a one-way ANOVA was applied. The Shapiro–Wilk normality test yielded p-values > 0.05, indicating a normal distribution. The homogeneity of variance was assessed using the Levene test, which resulted in a significance value of 0.75, suggesting that the variances in each group were homogeneous. Therefore, a one-way ANOVA test can was conducted.

The results of the one-way ANOVA indicated a p-value < 0.001 (< 0.05), indicating a significant difference in the average size of the coronary arteries after quercetin administration among the different test groups.

Discussion and Comparison of Previous Research

This study provides evidence that quercetin administration offers a promising therapeutic effect on MDA, ICAM, CRP, and LDL levels, as well as on the histological profiles of carotid and coronary arteries in atherosclerosis. Quercetin, which is present in Moringa leaves, is an antioxidant known for its potency, surpassing the strength of vitamins C and E by 4–5 times (Sutrisno, 2011a; TN *et al*., 2010).

The inflammation of vascular lesions is one of the main factors in the development of atherosclerosis. The additional product, MDA protein, has been detected in ox-LDL in the Apo B fraction in atherosclerotic lesions; therefore, it can be used as a biomarker of disease risk related to oxidative stress and its progression (Ito *et al*., 2019b). MDA will result in damages the endothelial glycocalyx (Lankin *et al*., 2022) which suppresses the ability of the endothelium to control arterial tone according to changes in wall shear stress. MDA can be used as a biomarker for oxidative stress because MDA will increase in formation in response to oxidative stress, can be measured accurately using various methods, is stable in isolated samples, is not affected by fat content and diurnal variations, and is a specific product of lipid peroxidation (Mulianto N, 2020). The Wilcoxon test revealed a significant difference in the MDA levels after quercetin administration. Lower MDA levels were observed in the K2 group (25 mg/kg quercetin fraction for 10 weeks). The results obtained are in accordance with those of Senyigit *et al*., who stated that the decrease in MDA levels in the quercetin group is an indicator of the protective role of quercetin against toxicity in the rat brain (Senyigit *et al*., 2019). In a study conducted by Mehany *et al*., quercetin also succeeded in reducing MDA levels in brain tissue. These results demonstrate a positive effect of quercetin on brain damage (Mehany *et al*., 2022).

Increased levels of dissolved ICAM-1, VCAM-1, and PAI-1, together with decreased levels of omentin-1, cause a proinflammatory imbalance that triggers the onset of subclinical atherosclerosis and increases the risk of both cardiovascular and cerebrovascular diseases (Nakashima *et al*., 1998; Varona *et al*., 2019). Based on the paired t-test and Cohen's d coefficient values, significant and moderate differences in ICAM levels after administration of quercetin fraction. Other factors that may have an effect are the three other molecules involved in strengthening leukocytes during oxidative processes that were not measured in this study, such as VCAM-1, P-selectin, and E-selectin, which play important roles in the early steps of the tethering and rolling of monocytes and lymphocytes. The results of the statistical analysis in this study are in accordance with the research conducted by Cheng *et al*. who stated that quercetin can suppress the expression of matrix metalloprotease-9 and cell-to-cell adhesion molecule-1 (ICAM-1) to achieve anti-inflammatory effects on stimulated tumour necrosis factor-α by human retinal pigment epithelial cells (ARPE-19) (Cheng *et al*., 2019). Bian *et al*. (2018) have also reported that quercetin can inhibit the production of inflammatory cytokines and chemokines induced by interleukin-1β in ARPE-19 cells (Bian *et al*., 2018).

CRP binds to damaged cell membranes and triggers an inflammatory response to sustain damage, especially in atherosclerotic plaques (Pathak *et al*., 2020). In this plaque, monocyte adhesion and withdrawal of molecules such as e-selectin and monocyte chemoattractant protein-1 (MCP-1) occur. CRP mediates LDL uptake by macrophages and activates the complement system, leading to atherosclerosis. Plaques that undergo apoptosis via caspase-3 will increase the number of apoptotic cells and the plaque will become wider (Sproston & Ashworth, 2018). Based on the paired t-test and Cohen's d coefficient values, significant and moderate differences in CRP levels after the administration of the quercetin fraction were observed. CRP is an essential protein expressed under various inflammatory conditions, and CRP levels increase rapidly in conditions such as tissue injury, infection, cancer, and kidney and cardiovascular diseases (Khan & Mujahid, 2020). Mirsafaei *et al*. (2020) have reported that quercetin administration reduced CRP levels in rats with metabolic syndrome (Mirsafaei *et al*., 2020). Several mechanisms have been proven to be related to the role of quercetin in reducing CRP, inhibiting the nuclear factor-kB (NF-kB) signalling pathway, decreasing the formation of leukotriene B4 in leukocytes, and suppressing nitric oxide production (Tabrizi *et al*., 2020). Quercetin and its metabolites physiologically suppress the expression of key molecules involved in monocyte recruitment such as VCAM 1, ICAM 1, and MCP-1 gene expression. Quercetin has been demonstrated to suppress the formation of IjB kinase and C-Jun kinase, which in turn can result in the suppression of NF-kB activation, and significantly influence the suppression of the inflammatory process mediated by oxidized LDL, which appears through the regulation of the NF-kB Toll-like receptor signalling pathway (Bhaskar *et al*., 2016).

High LDL levels are considered a risk factor for ischemic and haemorrhagic cerebral events. This process is caused by damage to the endothelial layer of arterial walls. Damage to this layer is most likely triggered by hyperlipidaemic conditions because if the blood LDL concentration is high, cholesterol can precipitate on the arterial endothelium and narrow the arteries (Rahmayanti *et al*., 2021).The antihyperlipidemic oleifera leaf extract were investigated through the inhibition of pancreatic lipase, formation of cholesterol micelles, pancreatic cholesterol esterase, and binding of bile acids. Pancreatic lipase and cholesterol esterase play important roles in the hydrolysis of dietary triglycerides and cholesterol esters.

Based on the Fisher's exact test and Cramer's V value of >0.3, the administration of the quercetin fraction of M. oleifera leaves reduced the intensity level of intima thickness ($p < 0.001$), which was classified by Subbotin in a previous study (Subbotin, 2016). The appearance of blood vessels with various fibrous tissues or myointimal cells between the endothelium and internal elastic lamina is defined as the thickness of the tunica intima (Ruengsakulrach *et al*., 1999).

Atherosclerosis of the intracranial arteries causes changes in vessel walls, ranging from minor vessel wall thickening to luminal stenosis, which significantly affect hemodynamic (Chimowitz *et al*., 2011). Existing comparisons demonstrated that the greatest decrease in the intensity of tunica intima thickness was observed in the carotid vessels ($p = 0.003$). This is in accordance with previous research conducted by Chimowitz *et al*.,(Chimowitz *et al*., 2011) which is also reinforced by the explanation of Peace *et al*.,(Peace *et al*., 2018) who stated that both the carotid and coronary arteries respond to specific vasoactive stimuli during atherosclerotic pathogenesis. However, the carotid arteries can provide information regarding vascular function more quickly and accurately, which has prognostic value for vascular events in the future. Research conducted by Hoehmann *et al*. also supports the evidence that the peripheral arteries have a statistically significant moderate correlation ($r=0.372$) with the coronary and carotid arteries in determining the risk of vascular events (HOEHMANN *et al*., 2017; Poredos *et al*., 2003). The Bonferroni and Games–Howell post-hoc test results demonstrated a significant difference in the average size of the carotid and coronary blood vessels after the administration of the quercetin fraction between each test group, with the smallest size in the control group. This is in accordance with the findings of Juzwiak *et al*.(Juźwiak *et al*., 2005) who stated that the administration of quercetin significantly reduced the area of atherosclerotic plaques, lipid accumulation, and TC levels and increased collagen fibres in atherosclerotic plaques. Quercetin can alleviate atherosclerotic lesions both in vivo and in vitro by simultaneously decreasing cellular apoptosis, increasing the mitochondrial membrane potential, and decreasing free radicals (Li *et al*., 2023).

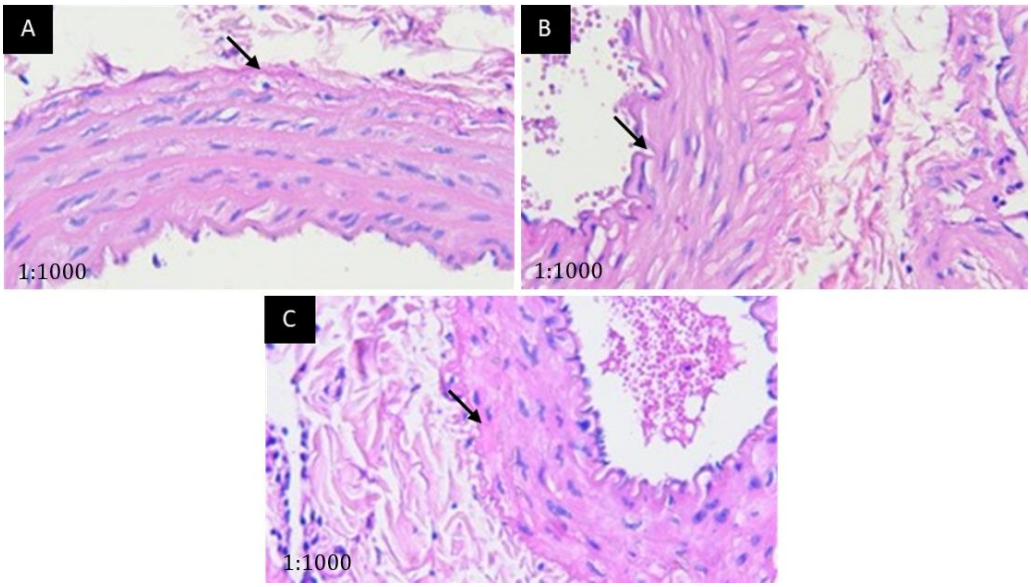

Figure 2. illustrates the results of histological analysis using HE staining on the carotid artery. The thickness of the blood vessel walls was observed under a light microscope at a magnification of 1000x and measured in pixel units using computer-assisted analysis. (a) In the Negative control group (normal rats), the measurement of blood vessel wall thickness was 440 pixels. (b) In the Control group (atherosclerosis rats without quercetin), the measurement of blood vessel wall thickness was 520 pixels. (c) In the Quercetin Group (rats with atherosclerosis treated with quercetin at a dose of 12.5mg/kg), the measurement of blood vessel wall thickness was 460 pixels.

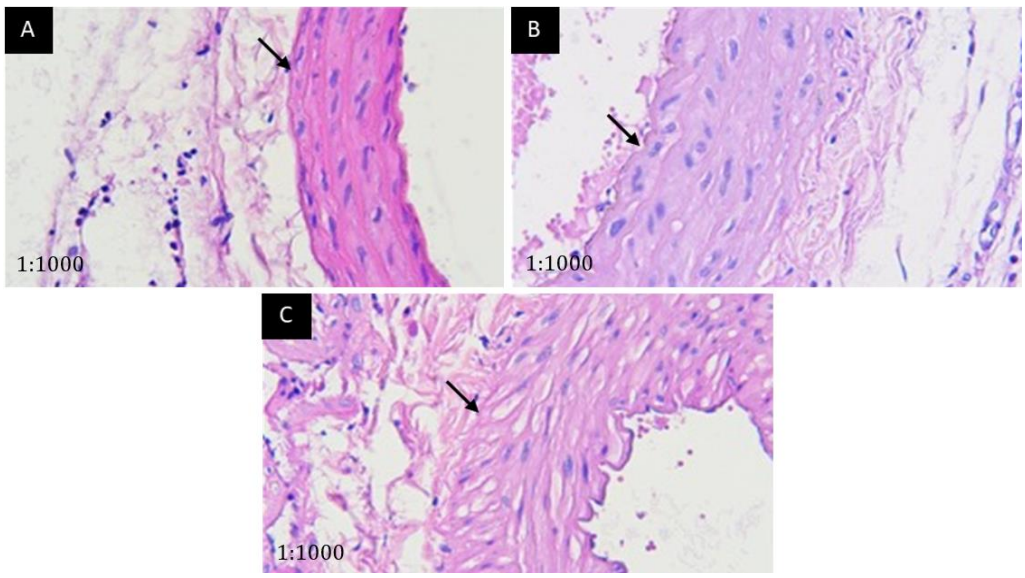

Figure 3. Depicts the results of a histological analysis using HE staining on the coronary artery. The examination was conducted with a light microscope at 1000x magnification, and the thickness of the blood vessel walls was measured using computer-assisted analysis in pixel units. (a) The blood vessel wall thickness measurement in the Negative control group was 380 pixels. (b) The blood vessel wall thickness measurement in the Atherosclerosis rats without quercetin was 480 pixels. (c) The blood vessel wall thickness measurement in the Quercetin Group (quercetin 25mg/kg) was 450 pixels. Additionally, the margin area of the blood vessels exhibited a homogeneous and continuous arrangement of cells.

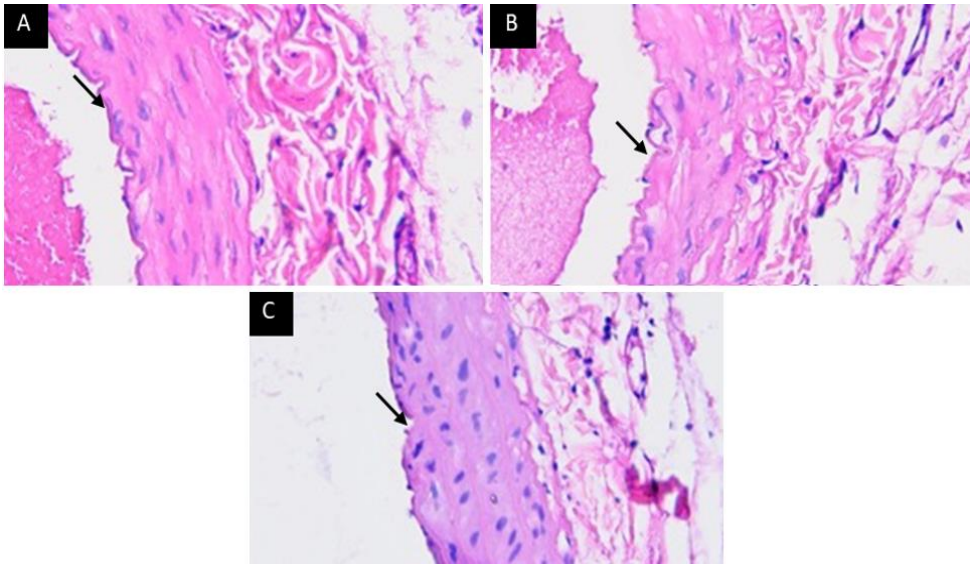

Figure 3. Presents the results of a histological examination using HE staining on the peripheral limb artery. The examination was conducted using a light microscope at 1000x magnification, and the thickness of the blood vessel walls was measured in pixel units through computer-assisted analysis. (a) In the Negative control group, the measurement of blood vessel wall thickness was 400 pixels. (b) In the Atherosclerosis rats without quercetin, the measurement of blood vessel wall thickness was 470 pixels. (c) In the Quercetin Group (quercetin 50mg/kg), the measurement of blood vessel wall thickness was 460 pixels.

The findings of this examination indicate that the carotid blood vessels of rats in the Negative control group had thinner walls compared to the atherosclerosis model rats. Furthermore, atherosclerosis model rats treated with quercetin at a dose of 12.5mg/kgBW (Quercetin Group) exhibited thinner blood vessel walls compared to atherosclerosis model rats without quercetin treatment (Control group) (Figure 2).

The findings of this examination revealed that the coronary blood vessels in rats from the Negative control group, who received only aquades, had thinner walls compared to the atherosclerosis model rats. Furthermore, the atherosclerosis model rats treated with quercetin at a dosage of 25mg/kgBW (Quercetin Group) displayed thinner blood vessel walls compared to the atherosclerosis model rats who received a placebo (Atherosclerosis rats without quercetin). It is worth noting that the margin area of the blood vessels demonstrated a consistent and uniform cell arrangement (Figure 3).

The examination revealed that the peripheral limb arteries of rats in the Negative control group, who received only aquades, exhibited thinner blood vessel walls compared to the atherosclerosis model rats. Moreover, atherosclerosis model rats treated with quercetin at a dosage of 50mg/kgBW (Quercetin Group) demonstrated thinner blood vessel walls compared to the atherosclerosis model rats receiving a placebo (atherosclerosis rats without quercetin) (Figure 4). The administration of quercetin at different doses (12.5 mg/kgBW, 25 mg/kgBW, and 50 mg/kgBW) in atherosclerosis model rats resulted in a reduction in blood vessel thickening in the carotid, coronary, and peripheral arteries. This finding aligns with a study conducted by Chis *et al*. (2019), which investigated the effects of quercetin administration on carotid blood vessels in diabetic sedentary rats (DSQ). In the Chis *et al*. study, quercetin was administered at a dose of 30 mg/kgBW for 5 weeks, and the reduction in thickening was assessed using ultrasound (USG) (Chis *et al.*, 2019).

Similarly, the study by Monori-Kiss *et al*. (2017) also reported a similar finding regarding the effects of quercetin on coronary blood vessels in atherosclerosis model rats. In this study, quercetin was administered at a dose of 30 mg/kgBW for 5 weeks, and the reduction in thickening was evaluated using arteriography (Monori-Kiss *et al.*, 2017).

Luo *et al*. revealed that quercetin effectively suppressed the levels and activity of myeloperoxidase (MPO) in the blood vessels of ApoE−/− animals and simultaneously decreased p47phox expression and NADPH oxidase activity. NADPH oxidase is the major source of H2O2 in vascular endothelial cells. Quercetin effectively decreases MPO/H2O2-mediated HOCl production and is toxic to human vascular endothelial cells (Luo *et al*., 2022). The inhibitory effect on MPO activity may occur because quercetin significantly inhibits NADPH oxidase-derived H2O2 formation in human endothelial cells and may act as an effective mediator of MPO intermediates, further preventing the production of HOCl by the MPO/H2O2 system, which would lead to an increase in the thickness of the tunica intima (Batiha *et al*., 2020).

CONCLUSION

Quercetin treatment significantly affected biomarkers associated with atherosclerosis, including ICAM, CRP, LDL, and MDA levels (where MDA marker is related to the mechanism of antioxidant action). This led to a decrease in MDA, ICAM, and CRP levels, indicating reduced oxidative stress and inflammation, whereas LDL levels increased slightly. Quercetin also improves the histopathological profile of the carotid and coronary arteries by reducing intima thickness. These findings suggest the potential therapeutic benefits of quercetin in atherosclerosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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