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Research Article

# The Therapeutic Effects of West Indian Elm (*Guazuma ulmifolia*) Leaf Extract on Coronary Artery Atherosclerosis in Hypercholesterolemic Wistar Rats

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## ABSTRACT

This study aimed to determine the therapeutic effect of West Indian elm leaf extract on coronary artery atherosclerosis in hypercholesterolemia Wistar rats. Cholesterol, a key factor in the pathophysiology of coronary heart disease, is present in fat-containing diets. Orlistat is one of the medications that can reduce blood cholesterol levels, but since it has side effects, herbs are more preferable to it. One such herbal plant, West Indian elm (*Guazuma ulmifolia*), contains tannins, saponins, alkaloids, and flavonoids, secondary metabolites that carry the properties necessary to reduce blood cholesterol levels, in its leaves. In this experimental study, which focused on histopathological parameters to see the scale of progression of coronary artery atherosclerosis, 30 Wistar rats were induced with a high-fat diet and divided into 6 sample groups, consisting of three control groups (normal, negative, and positive) and three treatment groups (which received a West Indian elm leaf extract at 0.2, 0.4, and 0.8 g/kgBW). Significant results were obtained from both Kruskal-Wallis and Mann-Whitney tests ($p<0.05$) between the negative control group and the positive control group, between the negative control group and treatment group 2 (0.4 g/kgBW), and between the negative control group and treatment group 3 (0.8 g/kgBW). In conclusion, this study proved that West Indian elm (*Guazuma ulmifolia*) leaf extract reduces the development of coronary atherosclerosis and shares a similarity in therapeutic effect with orlistat.

## INTRODUCTION

People who frequently consume fast foods, eat less fruits and vegetables, engage in less physical activity, drink alcohol and too much soda, and smoke cigarettes are pursuing an unhealthy lifestyle. Such a way of living affects their likelihood of developing heart disease, particularly Coronary Heart Disease (CHD), which is caused by the existence of obstructive atherosclerotic plaques that reduce the blood flow to the heart's myocardium (Severino *et al.* 2020). According to the findings of a 2017 research study, cardiovascular diseases were responsible for 17.8 million global deaths, and 35.6 million people survived with impairments (Roth *et al.* 2020). Coronary heart disease is seen as posing a serious danger to the 21st-century sustainable development (Khan *et al.* 2020).

The proportion of the national population consuming fatty foods once a day was reported to be fairly high at 40.7% (Zulkarnain 2018). Foods high in saturated fatty acids or trans-fatty acids (i.e., red meat, full-fat dairy products, butter, and coconut oil) cause an increase in lipid levels in the blood (Schoeneck & Iggman 2021). In fact, fatty foods contain cholesterol, which is an important factor in the pathogenesis of coronary heart disease (Kandaswamy & Zuo 2018). High intake of saturated fat can increase Low-Density Lipoprotein (LDL) (Nurdin *et al.* 2016) in the bloodstream and cause atherosclerotic plaques to form within the walls of the arteries (Jesch & Carr 2017).

Numerous clinical trials have demonstrated that lowering LDL-C (cholesterol-rich lipoprotein) levels can lower the risk of cardiovascular events (Lechner *et al.* 2020). One medication, orlistat, deactivates lipase by covalently attaching to the serine residue in the active site. Triglyceride breakdown is prevented by lipase inactivation, and as a result, free fatty acids are not absorbed (Bansal & Khalili 2022). However, because medications, especially orlistat, can have side effects like osteoporosis, stature problem, and acute kidney failure, it is crucial to learn more about natural food ingredients that can inhibit cholesterol absorption so that food ingredients and food supplements can be developed to treat people who wish to manage plasma cholesterol levels through non-pharmacological therapy (Jesch & Carr 2017).

West Indian elm (*Guazuma ulmifolia*), belonging to the family Sterculiaceae, is a small genus of trees native to tropical America. Indonesian people have used the plant as traditional medicine, believing that the leaves can be used for slimming, the seeds can be used for treating diarrhea, constipation, and flatulence, the bark can be used as a diaphoretic, and the fruits or leaves can be used as treatment for diarrhea, cough, and abdominal pain and as tonic and astringent (Lumbantobing *et al.* 2019). According to research findings, West Indian elm has been utilized as hepatoprotective, anti-cancer, anti-obesity, antioxidant, and antibacterial medication. In animals with hyperlipidemia, West Indian elm can also lower blood cholesterol levels and the occurrence of atherosclerosis (Prahastuti *et al.* 2020) because the secondary metabolites found in the leaves and fruits, such as alkaloids, tannins, saponins, flavanoids, terpenoids, and steroids, are significant substances used in medicines (Kumar & Gurunani 2019). There has been a great deal of additional research on West Indian elm leaves in Indonesia. The majority of the research addresses West Indian elm leaves as an obesity therapy. However, not much of the research is associated with atherosclerosis, especially in the coronary arteries. In order to assess the therapeutic impact of West Indian elm leaf extract, this study concentrated on the histological characteristics of the coronary arteries of Wistar rats (*Rattus norvegicus*) living on a high-fat diet.

## METHODS

### Design, location, and time

The research is of a true experimental type with a randomized posttest-only control group design (the sample was randomly divided into groups, and the research data were taken after treatment). The research was conducted in the laboratory using experimental animals (in vivo). The purpose of this study is to analyze the existence of a causal relationship between the treatment (the administration of West Indian elm leaf extract) and the output (the histopathological images of the coronary arteries of the experimental animals). The preparation of the West Indian elm (*Guazuma ulmifolia*) leaf extract and the phytochemical analysis were carried out at BALITTRO, Bogor, and the experimental animal treatment was administered at the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, Padjadjaran University, Bandung. The study was carried out between April 2022 and December 2022. This research received an ethical clearance from the UPN "Veteran" Jakarta Ethics Committee (No: 393/IX/2022/KEPK).

### Materials and tools

***West Indian elm leaf extract.*** The extract was prepared through maceration with 96% ethanol and evaporation. In addition to 178.4 g of crude simplicia, 2,000 g of dried West Indian elm was used as raw material. With these amounts of raw material and simplicia, the yield was calculated using the following formula.

$$Yield = \frac{Extract\ weight}{Dry\ leaves\ weight} X100\%$$

***Phytochemical analysis.*** After simplicia was obtained from maceration, a phytochemical analysis was carried out to see the secondary metabolite contents of the extract. It was conducted following the test method explained in 1995 MMI Volume VI guidelines.

***Orlistat.*** In this study, orlistat was administered to experimental rats at 2.16 mg three times a day. This dose was determined in proportion to the dose given to humans (120 mg, three times a day) (Qi 2018).

***High-fat diet preparation.*** According to the Faculty of Medicine of Padjadjaran University (FK UNPAD) (2016), the high-fat diet given to the experimental rats was made of 8 kg of standard pellets, 15 duck egg yolks, 2.5 kg of flour, 0.75 l of coconut oil, 1 kg of goat fat, 3 g of cholesterol medication, 15 quail eggs, and enough hot water.

**Procedures**

***Determination of sample size and inclusion criteria.*** The sample size used was determined using Federer's formula (Widiyatno & Muniroh 2018). Thirty male Wistar rats (*Rattus norvegicus*) weighing 150–250 g and aged around 8 weeks (Thadeus *et al.* 2019) were utilized in this study. At 8–10 weeks, Wistar rats (*Rattus norvegicus*) reaches adulthood as they are ready for reproduction (Sengupta 2013).

***Acclimatization of experimental animals.*** Rats were kept in 150 $cm^2$ rectangular plastic cages to which sand was spread, with a covering of ram wire, to adapt for seven days. Every five rats were kept in one cage with a light intensity in the range from 1 to 25 lux. The lighting was set to be on for 12 hours and off for another 12 hours. The cage environment was kept from being damp, with its temperature maintained at around 25$^o$C. The rats were given standard feed and water ad libitum. During the acclimatization period, the rats were given 551 pellets and distilled water. After the acclimatization period was over, the rats were then treated according to their group.

***Treatment groups.*** The sample was divided into three control groups (normal, negative, and positive) and three treatment groups (given West Indian elm leaf extract at different doses). Each group contained five rats. Group K0 (normal control) was given normal feed and water for 56 days. Group K1 (negative control) was given a high-fat diet for 56 days. Group K2 (positive control) was given a high-fat diet for 56 days and 2.1 mg of orlistat medication three times a day from day 29 to day 56. Groups K3, K4, and K5 (treatments 1, 2, and 3) were given a high-fat diet for 56 days and West Indian elm leaf extract at 0.2, 0.4, and 0.8 g/kgBW once a day, respectively, starting from day 29 to day 56.

***Termination and surgery.*** After the rats underwent their respective experimental treatments, they were given 0.3 mL of ketamine by intramuscular injection to put them to sleep. Ketamine was selected because it is simpler to administer, acts quickly, and has a potent analgesic impact (Yulianti & Astari 2020). The rats were terminated and dissected, and, after that, the coronary artery of each rat (in the rat's heart) was extracted.

***Preparation and staining of slides.*** The paraffin technique was used to prepare histological slides in several steps, namely, dehydration, fixation, clearing, infiltration, cutting, attachment, and deparaffinization (Harijati *et al.* 2017). Furthermore, Hematoxylin and Eosin (HE) staining was used to stain the samples (Cardiff *et al.* 2014). A 400x magnifying microscope was used to read the histopathological images.

***Determination of coronary artery atherosclerotic lesion indicators***. Atherosclerotic lesion indicators were used to determine the degree of coronary artery damage. Score 0 indicates that the histological image was in the normal condition. Score 1 indicates that there was an atherosclerotic plaque less than half the thickness of the tunica media with some form of endothelial dysfunction. Score 2 indicates that there were atherosclerotic plaques at least half the thickness of the tunica media with an accumulation of intracellular lipids, macrophages, and smooth muscle cells. Score 3 indicates that there were atherosclerotic plaques as thick as the tunica media with numerous macrophages, smooth muscle cells, and connective tissue. Score 4 indicates that atherosclerotic plaques were found to be thicker than the tunica media with a large intracellular lipid core and inflammatory cell infiltration (Kabiri *et al.* 2011).

**Data analysis**

The IBM® SPSS® Statistics computer program, version 24.0, was used to process all of the collected data. This study's data analysis applied the Kruskal-Wallis test to determine whether there was an average difference between at least two group pairs. The next test was the Mann-Whitney post-hoc test, aimed to get the average difference between each pair of groups. The confidence interval of both tests was 95%.

## RESULTS AND DISCUSSION

**West Indian elm leaf extract**

The yield of West Indian elm (*Guazuma ulmifolia*) leaf extract in this study was 8.92%. Yield is essential in making extracts because the yield value is related to the number of bioactive compounds in the extract (Whika *et al.* 2017). The higher yield value, the greater the resulting extract. This means that more bioactive compounds are contained in plant leaves (Nahor *et al.* 2020). The results of the phytochemical analysis of West Indian elm (*Guazuma ulmifolia*) leaf extract in this study are presented in Table 1.

**Table 1. Phytochemical analysis test**

| Phytochemical analysis | Results |
|---|---|
| Alkaloid | + |
| Saponin | + |
| Tannin | + |
| Phenolic | + |
| Flavonoid | + |
| Triterpenoid | + |
| Steroid | + |
| Glycoside | + |

(+): Positive; (-): Negative

Table 1 shows that the West Indian elm (*Guazuma ulmifolia*) leaf contains secondary metabolites such as saponins, tannins, alkaloids, phenolics, triterpenoids, steroids, glycosides, and flavonoids. Since this study used qualitative phytochemical analysis, the results were either positive or negative to indicate the presence of secondary metabolites. Secondary metabolism describes metabolic pathways and the tiny molecules they produce that are unnecessary for the organism's growth and reproduction. In plants, secondary metabolic pathways produce a diversity of compounds called Plant Secondary Metabolites (PSMs). Many PSMs have positive, beneficial effects on human health and agriculture production, contributing significantly to the economy (Pang *et al.* 2021).

***Histopathological image results***

Histopathological observations of the coronary arteries of rats were interpreted with indicators of atherosclerotic lesions in the coronary arteries. The atherosclerotic indicators referred to are presented in Table 2.

Based on Table 2, there was no progression of coronary artery atherosclerosis in the normal control group because all the rats in the group had a progression score of 0. The normal control group was fed with sodium carboxymethyl cellulose at all times. This group represented a disease-free state against which the intervention groups were to be compared.

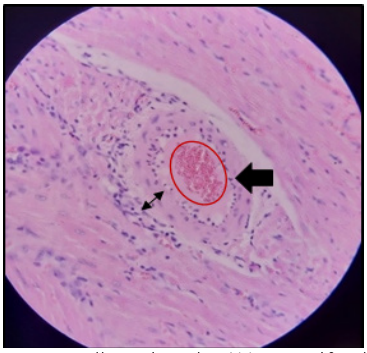
The progression results show no changes in the architecture of the coronary arteries of the rats because there were no risk factors to induce it (Figure 1). Hyperlipidemia and hyperglycemia are significant risk factors for the pathogenesis of atherosclerosis because they increase Reactive Oxygen Species (ROS) levels, thereby damaging the vascular endothelium (Rafieian-Kopaei *et al.* 2014).

The negative control group was a group of rats that were given a high-fat diet with the addition of neither orlistat nor West Indian elm leaf extract. Based on Table 2, the highest atherosclerosis progression was scored by the negative control group. This is because the high-fat feed given contained many lipid-derived macromolecules, such as triacylglycerols, cholesterol, and fatty acids, which would then be absorbed by the small intestine and enter the blood circulation. In this study, the risk factor for atherosclerosis in the coronary arteries of rats was hypercholesterolemia. High levels of cholesterol, especially LDL, followed by chronic inflammation of the endothelium, will cause endothelial dysfunction. The injured endothelium

**Table 2. Results of atherosclerotic lesion assessment indicators in coronary arteries**

| Nomenclature | Groups | Score | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| K0 | Normal control | 5 | - | - | - | - |
| K1 | Negative control | - | - | 5 | | - |
| K2 | Positive control | 4 | - | 1 | - | - |
| K3 | Treatment 1 | 1 | - | 4 | - | - |
| K4 | Treatment 2 | 2 | - | 3 | - | - |
| K5 | Treatment 3 | 3 | 1 | 1 | - | - |

K0: Normal feed and water; K1: High-fat diet; K2: High-fat diet and 2.1 mg of orlistat; K3: High-fat diet and West Indian elm leaf extract 0.2 g/kgBW; K4: High-fat diet and West Indian elm leaf extract 0.4 g/kgBW; K5: High-fat diet and West Indian elm leaf extract 0.8 g/kgBW



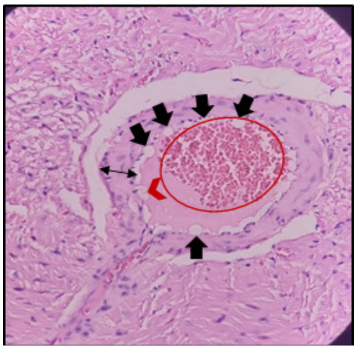
Hematoxylin eosin stain; 400x magnification

○ : Lumen filled with erythrocytes
⬅ : Tunica intima endothelium
↔ : Tunica media

**Figure 1. Picture of atherosclerosis progressive score 0**

can be penetrated by circulating LDL in the blood vessels, which will enter the space in the subintima. This will give rise to atheroma plaques (Aziz & Yadav 2016).

Lipid accumulation in the subintima space is clearly visible in the histopathological image of the coronary arteries of the negative control group rats (Figure 2). Progressive lesions in the negative control group did not reach score 3 (marked by atherosclerotic plaques as thick as the tunica media and the presence of inflammatory cells and smooth muscle cell proliferation) or score 4 (marked by atherosclerotic plaques thicker than the tunica media and lots of inflammatory cell infiltration). This was because the level of fat in the diet consumed by the experimental rats during the 56 days' duration was not significant enough to cause atherosclerotic lesions.



Hematoxylin eosin stain; 400x magnification

❮ : Endothelial dysfunction
⬅ : Vacuoles contain intracellular lipids
↔ : Tunica media
○ : Lumen filled with erythrocytes

**Figure 2. Picture of atherosclerosis progressive score 2**

The positive control group, which was given a high-fat diet and an orlistat therapy at a dose of 2.16 mg three times a day, had low atherosclerosis progression, almost equivalent to the normal control group. Based on Table 2, only 1 rat in the group demonstrated atherosclerosis progression, while 4 others were normal. The positive control group proved that orlistat can reduce the progression of atherosclerosis. This is because orlistat covalently binds to the serine residue of the lipase active site and deactivates it. Lipase inactivation prevents triglyceride hydrolysis, and thus free fatty acids are not absorbed (Bansal & Khalili 2022). By decreasing the absorption of fat in the intestine, total cholesterol levels in the blood will also decrease, so that the risk factors for atherosclerosis in the coronary arteries can be inhibited. One rat that experienced atherosclerosis progression, K2-4, which scored 2 in the analysis, already had a greater body weight than the other rats from the beginning of the study. At the end of the study, it weighed 381 g, making it the rat with the greatest body weight in this study. Based on what happened to these rats, it can be concluded that the risk factors for atherosclerosis can also come from sources that cannot be modified, namely genetics, age, and sex (Johansson *et al.* 2021).

The results of progressive atherosclerotic lesion analysis of treatment groups 1, 2, and 3 show that the administration of West Indian elm leaf extract at doses of 0.2, 0.4, and 0.8 g/kgBW, respectively, had a therapeutic effect. Based on Table 2, treatment group 3 had the following results: one rat had a lesion progression score of 2, one had a lesion progression score of 1, and three had a lesion progression score of 0. These results were better than those of treatment groups 1 and 2. Although treatments 1 and 2 were considered ineffective in inhibiting the progression of atherosclerotic lesions, they had lower scores than the score of the negative control group. This means that at doses of 0.2 and 0.4 g/kgBW, West Indian elm leaf extract already showed a therapeutic effect.

The West Indian elm leaf extract contains secondary metabolites such as tannins, alkaloids, and saponins (Kumar & Gurunani 2019). These compounds can bind to cholesterol and fat molecules in food, reducing the amount of fat and cholesterol absorbed in the intestine. This will cause a decrease in cholesterol in the blood (Rozqie *et al.* 2012). In addition, the West Indian elm leaf extract also contains flavonoids, which act as antioxidants so as to prevent endothelial damage due to oxidative stress and prevent LDL oxidation. Flavonoids also act as anti-inflammatory, cholesterol-lowering, antihypertensive, and antiplatelet agents. They can also inhibit the proliferation of smooth muscle cells that occur in the pathogenesis of atherosclerosis (Ciumărnean *et al.* 2020). The secondary metabolites contained in the West Indian elm (*Guazuma ulmifolia*) leaf extract have different mechanisms of action in inhibiting the progression of atherosclerosis, so they are effective for use as therapy.

Atherosclerotic lesion assessment indicator data were analyzed using the Kruskal-Wallis test, yielding asymp. sig. results with $p<0.05$. This analysis was followed by the Mann-Whitney test to see the average difference of each experimental group (Table 3).

A comparison of the Mann-Whitney post-hoc test results is presented in Table 3. The negative control group, which was the group with coronary artery atherosclerosis, was used as a benchmark to for the therapy groups. There were significant average differences between the negative control group and the positive control group, between the negative control group and treatment group 2, and between the negative control group and treatment group 3, statistically proving that orlistat and West Indian elm (*Guazuma ulmifolia*) leaf doses of 0.4 and 0.8 g/kgBW were effective in inhibiting the progression of atherosclerosis. In addition, the average difference was not significant between the negative control group and treatment group 1. This means that the administration of the West Indian elm (*Guazuma ulmifolia*) leaf extract at a dose of 0.2 g/kgBW was effective as therapy. Based on descriptive data and analytic tests, the most effective dose to provide a therapeutic effect on the progression of coronary artery atherosclerosis, and thus prevent and treat coronary heart disease, was 0.8 g/kgBW.

Based on Table 3, there were no significant average differences ($p>0.05$) between the normal control group and treatment group 3 ($p=0.136$) and between the positive control group and treatment group 3 ($p=0.606$). This statistically proves that the administration of West Indian elm (*Guazuma ulmifolia*) leaf extract at a dose of 0.8 g/kgBW had an effect equivalent to that of orlistat in inhibiting the progression of atherosclerosis. In addition, the West Indian elm (*Guazuma ulmifolia*) leaf extract dose of 0.8 g/kgBW did provide a therapeutic effect, so that the histopathological appearance of the coronary arteries in rats induced with a high-fat diet did not differ significantly from the histopathological appearance of the coronary arteries in normal rats. In other words, the West Indian elm (*Guazuma ulmifolia*) leaf extract dose of 0.8 g/kgBW was the most effective in providing a therapeutic effect on the progression of coronary artery atherosclerosis because the results were not significantly different from the results of rats given orlistat at 2.16 mg three times a day or

**Table 3. Mann-Whitney test of atherosclerotic lesion assessment indicators in coronary arteries**

| Rats (n=5) | Mean±SD | Normal control | Negative control | Positive control | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|---|---|---|---|
| Normal control | 0±0.000 | - | p=0.003 | p=0.317 | p=0.014 | p=0.050 | p=0.136 |
| Negative control | 2±0.000 | - | - | p=0.014 | p=0.317 | p=0.134 | p=0.017 |
| Positive control | 0.4±0.894 | - | - | - | p=0.072 | p=0.221 | p=0.606 |
| Treatment 1 | 1.6±0.894 | - | - | - | - | p=0.513 | p=0.106 |
| Treatment 2 | 1.2±1.095 | - | - | - | - | - | p=0.356 |
| Treatment 3 | 0.6±0.894 | - | - | - | - | - | - |

K0: Normal feed and water; K1: High-fat diet; K2: High-fat diet and 2.1 mg of orlistat; K3: High-fat diet and West Indian elm leaf extract 0.2 g/kgBW; K4: High-fat diet and West Indian elm leaf extract 0.4 g/kgBW; K5: High-fat diet and West Indian elm leaf extract 0.8 g/kgBW; SD: Standard Deviation the results of healthy rats. From this study, at a dose of 0.8 g/kgBW, West Indian elm leaf extract could slow the development of coronary artery atherosclerosis in Wistar rats (*Rattus norvegicus*) fed with a high-fat diet.

## CONCLUSION

Secondary metabolites like saponins, tannins, alkaloids, phenolics, triterpenoids, steroids, glycosides, and flavonoids can be found in West Indian elm (*Guazuma ulmifolia*) leaf extract. At a dose of 0.8 g/kgBW, West Indian elm leaf extract can slow the development of coronary artery atherosclerosis in Wistar rats (*Rattus norvegicus*) fed with a high-fat diet of a mix of duck egg yolks, flour, coconut oil, goat fat, and quail eggs. West Indian elm leaf extract is recommendable for individuals who frequently consume fatty foods to slow the development of coronary heart disease.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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