



## EFFECTS OF *GARCINIA MANGOSTANA LINN PERICARP* EXTRACT AND PHYSICAL EXERCISE ON RENAL ATHEROEMBOLIC HISTOPATHOLOGY IN WISTAR RATS WITH METABOLIC SYNDROME

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### ABSTRACT

**Background:** Renal atheroembolism is an under-recognized cause of renal failure. Atheroembolism is caused by cholesterol crystals from ulcerated atherosclerotic plaques and is influenced by inflammation and endothelial dysfunction. The formation of complicated atherosclerotic lesions is a prerequisite for the development of cholesterol crystal emboli. The exact incidence of atheroembolism remains unclear due to frequent underdiagnosis. In a previous report, the rate of embolism in the kidney among patients dying after surgery for acute atherosclerosis of the aorta was 77%. Non-pharmacological therapeutic management (such as diet and physical exercise) is an important factor in preventing and reducing the risk of atherogenesis. Prior research suggests that controlling body weight, primarily through physical exercise, is a critical factor in preventing and reducing the risk of atherogenesis, in addition to medication adherence and other therapeutic management alternatives. Another study found that mice with metabolic syndrome given *Garcinia mangostana* pericarp can reduce inflammatory cell infiltration, thereby reducing physiological symptoms, metabolic syndrome, liver disorders, and cardiovascular symptoms. The combination of physical exercise and metabolic supplements in the treatment was even able to significantly reduce atheroma plaque area as well as the incidence of plaque rupture, and prolong survival time. **Objective:** To investigate the effect of *Garcinia Mangostana Linn Pericarp* administration along with Physical Exercise on the histopathologic features of renal atheroembolism in Wistar rats with metabolic syndrome. **Methods:** This study is a *true experimental* study with research subjects in the form of male *Rattus norvegicus* Wistar rats which were randomly divided into 3 groups. All groups were induced metabolic syndrome through the administration of a diet similar to the "Western" purified atherogenic diet patent Envigo® formulated as a high fat diet (20 - 23% BW; 40 - 45% kcal from fat), saturated fatty acids (SFA >60% of total fatty acids), and drinking tap water ad libitum. However, in group K (no additional treatment), P1 (*garcinia pericarp* extract 800 mg / kg / day with physical exercise), and P2 (*nanoemulsion garcinia pericarp* extract 50 mg / kg / time of administration with physical exercise). **Results:** In the group treated with *garcinia pericarp* extract 800 mg/kg bw/day with physical exercise and the group with *nanoemulsion* 50 mg/kg bw/day with physical exercise, there were no *fatty streaks*, inflammation, and impaired myocyte coherence. There was 0-5% fibrous connective tissue, one layer of foam cell layer and myocyte-lipid, no cholesterol crystal embolism was found. **Conclusion:** The administration of *Garcinia Mangostana Linn pericarp* extract and/or *nanoemulsion* with physical exercise can have an effect on the histopathological picture of the kidneys of Wistar rats with metabolic syndrome, although there was no difference in effect between the two treatments.

**Keywords:** *atheroembolic, atherosclerosis, Garcinia Mangostana Linn Pericarp extract, nanoemulsion, physical exercise*

### INTRODUCTION

Atheroembolic kidney disease is part of a multisystem disease caused by arterial blockage by cholesterol crystal emboli originating from ulcerated atherosclerotic plaques. Atheroembolic kidney disease is an important, but still underdiagnosed component of renal disease associated with atherosclerosis and an unexplored area of nephrology research.<sup>6</sup> The exact mechanisms underlying atheroembolic are not fully understood. Local tissue necrosis and inflammatory reactions caused by cholesterol

crystals (CCs) play an important role in atheroembolic pathogenesis.<sup>7</sup>

Histologically, cholesterol crystal emboli are identified in the lumen of arcuate and interlobular arteries as biconvex, *needle-shaped*, and empty gaps, referred to as *ghost cells* because they dissolve during specimen processing. Small crystals rarely lodge in afferent arterioles and glomerular capillaries. Tissue damage caused by these crystal emboli is usually patchy. Glomerular and interstitial changes are mainly ischemic with varying degrees



of glomerular damage and interstitial fibrosis. In the early phase, areas of acute tubular necrosis can be identified. Cholesterol crystal emboli are also identified in the skin, gastrointestinal tract and muscles.<sup>8,9</sup>

The most important risk factor for atheroembolism is atherosclerosis and most other risk factors, such as diabetes mellitus, hypertension, hyperlipidemia, and smoking are actually also risk factors for atherosclerosis. The severity of atherosclerosis is a determinant of atheroembolic risk.<sup>10</sup>

The formation of complicated atherosclerotic lesions is a prerequisite for the development of cholesterol crystal emboli. The process of atherogenesis begins with inflammation of the endothelial lining of the blood vessels. The endothelial layer that functions to produce vasodilator and vasoconstrictor molecules, such as nitric oxide (NO) and endothelin, will experience an imbalance in the production of these vasoactive substances resulting in impaired function referred to as endothelial dysfunction.<sup>11</sup>

If it is related to the current condition, the lower and middle classes tend to believe in the benefits of herbal medicine as an alternative therapy management, both as a complement and alternative.<sup>12</sup>

The potential effects of medicinal plants in improving and maintaining human health, low cost, and low side effects, have taken center stage. *Mangosteen (Garcinia mangostana)* is a tropical fruit used for centuries in Southeast Asia for medicinal purposes. Mangosteen rind contains *xanthones*, which are bioactive compounds that have potential benefits, such as anti-inflammatory, antioxidant, neuroprotective, cytotoxic, and antiproliferative responses. Xanthones from *Garcinia mangostana* peel exhibited anti-inflammatory effects in inflammatory model mice through the reduction of TNF, IL-6, iNOS, and COX-2.<sup>13</sup> Atheroembolic animal models have been developed, whereby atherosclerotic plaque suspensions are injected into the left carotid artery or aorta of animals.<sup>14</sup>

Non-pharmacological therapeutic management that is easy to do is an effort to control body weight, mainly by exercise (physical exercise). The form of physical exercise in mice includes

running on a *treadmill* following an exercise protocol. From previous research, exercise on a *treadmill* at a speed of 12 m/min with a duration of 60 minutes per day, 5 times a week for 6 weeks, prevents the development of atherosclerosis and has a favorable effect on the composition of atherosclerotic plaques.<sup>13</sup>

Renal atheroembolism is an underdiagnosed and under-researched clinical disease in the field of nephrology. To date, no studies have been conducted that prove the potential of *Pericarp Garcinia Mangostana Linn* as an anti-inflammatory herbal medicine in renal atheroembolism. So it is necessary to conduct this research. Incidence of atheroembolic is not well known. Clinical experience is confined to isolated case reports and clinicopathologic discussions of cases. Thurlbeck and Castlemann reported an incidence of 4% in elderly subjects (aged 65 years and older) presenting minimally atherosclerosis. However, incidence increased to 77% in older patients with severe atherosclerosis.<sup>1</sup>

## METHODS

This study is a *true experimental* with *The Randomized*. Maintenance and treatment were carried out at the UNISSULA Biomolecular Laboratory from December 2022 to February 2023. Paraffin block making and preparation processing, including HE painting were carried out at the Anatomical Pathology Laboratory Universitas Diponegoro-RSND. This study has obtained *ethical clearance* granted by the Ethics Commission for Health Research Universitas Diponegoro No.44/EC-H/KEPK/FK-UNDIP/V/2023.

The research subjects were male *Rattus norvegicus* Wistar rats that met the inclusion criteria (healthy male rats, white fur and active appearance, age  $\pm$  2 months (6-8 weeks), healthy rats on physical examination characterized by clear eyes, shiny fur, white, clean and thick, agile movements, and feces that are not mushy or watery) and exclusion (defects, appearing sick (standing fur, mushy feces), rats whose conditions decreased and / or died during the study). The sampling method used was *simple random sampling* and 16 rat samples were obtained.

All rats were induced with a diet similar to the "*Western*" *purified atherogenic diet* patented by Envigo<sup>®</sup> with *high fat diet* formulation (20 - 23%



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BW; 40 - 45% kcal from fat), saturated fatty acids (SFA >60% of total fatty acids), and drinking from tap water *ad libitum* for 8 weeks. Control group rats (K) were not given additional treatment. Rats in treatment group-1 (P1) were supplemented with *Garciana Mangostana Linn Pericarp* Extract at a dose of 800 mg/kgBB/day, divided into 3 doses, per round, for 8 weeks. This dose is supported by previous studies, 2520 mg/day of *Garcinia mangostana Linn* (GMLE) extract divided into three doses<sup>15</sup> *mangosteen pericarp extract, 200, 400, 800 mg/BB over a period of 90 days.*<sup>16</sup>

The average weight of rats is in the range of 300 grams, so the dose used is 240 mg / day divided into 3 doses, which is 80 mg per administration / rat. Accompanied by physical exercise with a *treadmill* speed of 12 m/min, duration of 60 minutes per day, 5 days a week, for 8 weeks. Rats of treatment group-2 (P2) plus the administration of *Nanoemulsion of Garciana Mangostana Linn Pericarp* Extract 50 mg/kgBB once a day, per round, for 8 weeks. Grounded upon the previous study, administering  $\alpha$ -mangostin-loaded self-microemulsion, with oral administration of 20 mg/kg and increased the oral dose up to 50 mg/kg.<sup>17</sup> Physical exercise with a *treadmill* speed of 12 m/min, duration of 60 minutes per day, 5 days a week, for 8 weeks. Treatment was done individually.

At the end of the 8th week, rats that were still alive until the end were terminated, then the kidney organs were taken. Preparations were taken from the interstitial arteries and histopathologic tissue processing, paraffin blocking, HE staining, and interpretation of the histopathologic images by an anatomic pathologist.

## RESULTS

All study subjects were given an atherogenic diet to trigger the onset of metabolic syndrome. The diagnostic criteria for metabolic syndrome were the presence of at least three of the following five risk factors; a) obesity with *elevated* waist circumference ( $\geq 102$  cm or  $\geq 40$  inches for men;  $\geq 88$ cm or  $\geq 35$  inches for women); b) elevated triglycerides ( $>150$  mg/dL or 1.7 mmol/L) or being on therapy to lower triglyceride levels; c) decreased HDL ( $<40$  mg/dL or 40 mg/dL or 1.03 mmol/L in men;  $<50$  mg/dL or 1.3 mmol/L in women) or on therapy to lower HDL levels; d) elevated blood pressure ( $\geq 130$  mmHg

systolic or  $\geq 85$  mmHg diastolic or on antihypertensive drug therapy in patients with a history of hypertension); and e) elevated fasting glucose levels ( $\geq 100$  mg/dL or on drug therapy to lower elevated sugar levels.<sup>18</sup>

**Table 1.** Study Groups and Number of Mice

		Freque ncy	Perce ntage	Presentas Valid	Percentage Cumulative
Valid	K	5	31.3	31.1	31.3
	P1	6	37.5	37.5	68.8
	P2	5	31.3	31.3	100.0
	Total	16	100.0	100.0	

During the research, the following facts were obtained:

1. K was found that all rats died before the end of the study period, consisting of rat K.3 died on the second day of the fifth week of the study (Tuesday, 3/1/2023), rat K.5 died on the fifth day of the fifth week of the study (Friday, 6/1/2023), rats K.1 and K.4 died on the third day of the sixth week (Wednesday, 11/1/2023). Rat K.2 died on the fourth day of the sixth week (Thursday, 12/1/2023).
2. P1 obtained three rats died before the end of the study period, consisting of rat P1.4 died on the first day of the first week of the study (Tuesday, 6/12/2022). Rats P1.3 and P1.6 died on the first day of the fifth week of the study (Monday, 2/1/2023).
3. P2 obtained one rat died before the end of the study period, which consisted of rat P2.3 died on the first day of the fifth week (Monday, 2/1/2023).

There are various possible causes underlying the death of rats in this study, including the use of solvents in drugs, such as ethanol and aquabides. In this study, aquabides was used to dissolve the mangosteen peel extract and ethanol to dissolve the *nanoemulsion*. The use of ethanol as a solvent is better than aquabides because aquabides only dissolves water-soluble compounds. Meanwhile, compounds that are not water soluble will be wasted which results in compounds that are needed and not water soluble cannot be used. Other studies also show that the use of aquabides gives results that are not better than ethanol.

In addition, the use of drug sachets administered to rats several times a day is also suspected to be the cause of rat deaths. The



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frequency and method of using the drug sonde that is not appropriate or exceeds the prescribed time can lead to errors.

Therefore, the rats in the control group could not be statistically analyzed and compared with the treatment group because the control rats had all died before the end of the study period.

**Table 2.** Pre and Post Foam Cell Test Results

Group	Pre Foam Cell	Post Foam Cells		P
		1 layer	>1 layer	
P1	1 layer	6 (100)	0 (0)	1,000 <sup>†</sup>
	>1 layer	0 (0)	0 (0)	
P2	1 layer	4 (100)	0 (0)	1,000 <sup>†</sup>
	>1 layer	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the paired t-test between pre-foam cells and post-foam cells using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 3.** Differences in Foam Cells of Groups P1 and P2

Group	Group	Foam cell		P
		1 layer	>1 layer	
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre foam cells and post foam cells, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 4.** Test Results of Pre Lipid Myocytes and Post Lipid Myocytes

Group	Myocyte lipid pre	Myocyte lipid post		P
		1 layer	>1 layer	
P1	1 layer	6 (100)	0 (0)	1,000 <sup>†</sup>
	>1 layer	0 (0)	0 (0)	
P2	1 layer	4 (100)	0 (0)	1,000 <sup>†</sup>
	>1 layer	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the paired t test between pre lipid myocytes and post lipid myocytes using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 5.** Differences in Myocyte Lipids of Groups P1 and P2

Group	Group	Lipid myocytes		P
		1 layer	>1 layer	
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre lipid myocytes and post lipid myocytes, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 6.** Test Results of Pre External Lipid Deposition and Post External Lipid Deposition

Group	Pre external lipid deposition	Post external lipid deposition		P
		None	Available	
P1	None	6 (100)	0 (0)	1,000 <sup>†</sup>
	Available	0 (0)	0 (0)	
P2	None	4 (100)	0 (0)	1,000 <sup>†</sup>
	Available	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the paired t-test between pre and post external lipid deposition using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 7.** Differences in External Lipid Deposition of Groups P1 and P2

Group	Group	External lipid deposition		P
		None	Available	
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre external lipid deposition and post external lipid deposition, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

From the results of the paired t-test between pre cholesterol crystal embolism and post cholesterol crystal embolism using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there





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is no significant difference.

**Table 8.** Test Results of Pre Cholesterol Crystal Embolism and Post Cholesterol Crystal Embolism

Group	Pre cholesterol crystal embolism	Post cholesterol crystal embolism		p
		None	Available	
P1	None	6 (100)	0 (0)	1,000 <sup>†</sup>
	There is	0 (0)	0 (0)	
P2	None	4 (100)	0 (0)	1,000 <sup>†</sup>
	There is	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the unpaired difference test between groups P1 and P2 using the Mann-Whitney alternative test on pre cholesterol crystal embolism and post cholesterol crystal embolism, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 9.** Differences in Cholesterol Crystal Embolism in Groups P1 and P2

Group	Cholesterol crystal embolism	P		
			None	Available
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the paired t test between pre atherosclerosis and post atherosclerosis using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 10.** Test Results of Pre Atherosclerosis and Post Atherosclerosis

Group	Pre atherosclerosis	Post atherosclerosis		p
		Normal	Inflammation	
P1	Normal	6 (100)	0 (0)	1,000 <sup>†</sup>
	Inflammation	0 (0)	0 (0)	
P2	Normal	4 (100)	0 (0)	1,000 <sup>†</sup>
	Inflammation	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre atherosclerosis and post atherosclerosis, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 11.** Differences in Atherosclerosis in Groups P1 and P2

Group	Atherosclerosis	p		
			Normal	Inflammation
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the paired t test between pre fatty streaks and post fatty streaks using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 12.** Fatty Streaks Pre and Fatty Streaks Post Test Results

Group	Fatty streaks pre	Fatty streaks post		p
		None	Available	
P1	None	6 (100)	0 (0)	1,000 <sup>†</sup>
	Available	0 (0)	0 (0)	
P2	None	4 (100)	0 (0)	1,000 <sup>†</sup>
	There is	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the unpaired difference test between groups P1 and P2 using the Mann-Whitney alternative test on pre fatty streaks and post fatty streaks, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 13.** Differences in Fatty Streaks of Groups P1 and P2

Group	Fatty streaks	p		
			None	There is
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney

From the results of the paired t test between pre myocyte coherence and post myocyte coherence using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre myocyte coherence and post myocyte coherence, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.



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**Table 14.** Test Results of Pre Myocyte Coherence and Post Myocyte Coherence

Group	Pre myocyte coherence	Post myocyte coherence		P
		Normal	Distracted	
P1	Normal	6 (100)	0 (0)	1,000 <sup>†</sup>
	Distracted	0 (0)	0 (0)	
P2	Normal	4 (100)	0 (0)	1,000 <sup>†</sup>
	Distracted	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon

From the results of the paired t-test between pre fibrous connective tissue and post fibrous connective tissue using the Wilcoxon alternative test in groups P1 and P2, the p value > 0.05 was obtained, so it can be concluded that there is no significant difference.

**Table 15.** Differences in Myocyte Coherence of Groups P1 and P2

Group	Group	Myocyte coherence		P
		Normal	Distracted	
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

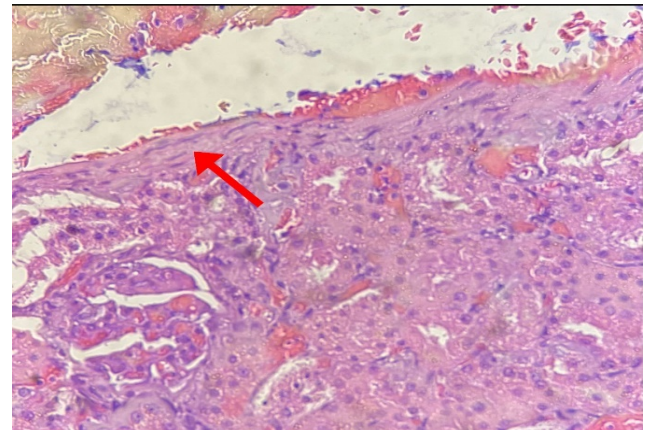
Description: <sup>‡</sup> Mann-Whitney

Atheroembolic lesions consist of fat covered by fibrous connective tissue (fibrous cap), which may lead to narrowing of the arterial lumen. Plaque grows along with the proliferation of fibrous connective tissue. Consumption of a high-fat diet may trigger fat deposition which causes fibrous cap become atheroma. In the treatment group, 0-5% fibrous connective tissue was found. Physical exercise and administration of *garcinia mangostana linn pericarp* extract can reduce the accumulation of fibrous connective tissue

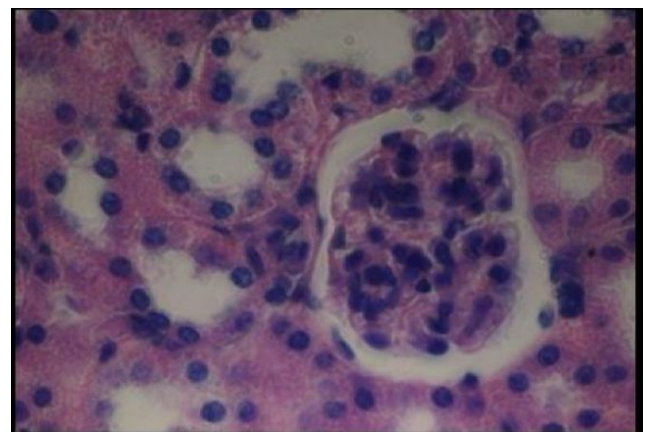
**Table 16.** Test Results of Fibrous Tissue Pre and Fibrous Tissue Post

Group	Fibrous pre	Fibrous post		P
		0-5%	5-25%	
P1	0-5%	6 (100)	0 (0)	1,000 <sup>†</sup>
	5-25%	0 (0)	0 (0)	
P2	0-5%	4 (100)	0 (0)	1,000 <sup>†</sup>
	5-25%	0 (0)	0 (0)	

Description: <sup>†</sup> Wilcoxon



**Figures 1.** Histopathology of Fibrous Tissue of the Treatment Group (HE staining, 100x magnification)



**Figures 2.** Normal Histopathology of the Treatment Group (HE staining, Magnification 400x)

Renal corpuscles and tubules appeared normal in the treatment group. No evidence of inflammation occurred after physical exercise. Providing *garcinia mangostana linn pericarp* extract is beneficial to the kidney as it prevents damage caused by free radicals, one of which is due to consumption of a high-fat diet..

**Table 17:** Differences in Fibrous Tissue of Groups P1 and P2

Group	Group	Fibrosa		P
		0-5%	5-25%	
Pre	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	5 (100)	0 (0)	
The post	P1	6 (100)	0 (0)	1,000 <sup>‡</sup>
	P2	4 (100)	0 (0)	

Description: <sup>‡</sup> Mann-Whitney



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From the results of the unpaired t test between groups P1 and P2 using the Mann-Whitney alternative test on pre fibrous connective tissue and post fibrous connective tissue, the  $p$  value  $> 0.05$  was obtained, so it can be concluded that there is no significant difference.

## DISCUSSION

The use of herbal medicines in recent times has begun to increase in the world, especially in various countries, such as Indonesia, China, and India. The use of herbal medicine has increased because it has pharmacological effects on almost all diseases with mild side effects. Common problems in herbal medicines are low bioavailability, solubility, absorption of active substances, and stability. To overcome these problems, the technology used for herbal medicine formulation is being developed. One example is nano technology. Nano technology is a technology in which drug particles are made on a nano scale (10 nm - 1000 nm). The use of nano technology is expected to overcome the problems in herbal medicine as well as improve therapeutic effects and reduce toxicity.<sup>19</sup>

The *nanoemulsion* form was chosen because it can increase the physical stability of a bioactive component, protect chemical damage and interactions with food ingredients, and can be well dispersed in aqueous systems. The use of *nanoemulsion* form in this extract can improve and maximize the work of the extract. The increase is due to the large surface area of nanoemulsion which can allow penetration of the active ingredients quickly so that it can be a drug carrier with a small size that will not damage normal human or animal cells.<sup>20</sup>

Based on the data obtained, for the number of foam cell layers, 100% of the number of rats in group P1 had 1 layer of foam cell layer. Similarly, 100% of the P2 group also obtained 1 layer of foam cell layer.

The main abnormalities in the lipid fraction are increased LDL, total cholesterol, and decreased HDL. Blood that contains too much cholesterol will easily stick to the inner wall of the blood vessels. After entering the intima, LDL will undergo oxidation and produce oxidized LDL which eventually leads to the synthesis of materials that can attach and attract monocytes through the

endothelial layer, then into the intima. Furthermore, oxidized LDL produces chemicals that can convert monocytes into macrophages that can then "eat" LDL through *scavenger receptors*. This phagocytosis will produce foam cells as a by-product.<sup>16</sup>

From the results of blood laboratory examinations of Wistar rats in this study, an increase in GDP, a decrease in HDL, a decrease in LDL, a decrease in total cholesterol was obtained in the group given the extract. Meanwhile, the group given *nanoemulsion* experienced a decrease in GDP, an increase in HDL, a decrease in LDL more, an increase in total cholesterol, a decrease in triglycerides more.

The main compound found in *mangosteen* rind is  $\alpha$ -*mangosteen*. In ripe, mangosteen fruits have about twice the  $\alpha$ -*mangosteen* than young mangosteen fruits. Recent studies have noted that mangosteen peels have antilipemic properties, lowering total cholesterol, triglycerides, and LDL, possibly increasing HDL cholesterol levels by increasing *Peroxisome Proliferators-Activated Receptors* (PPAR) activity.<sup>21</sup>

Based on the interpretation results for the number of myocyte-lipid layers, 100% of the number of rats in group P1 had 1 layer of myocyte-lipid layer. Similarly, 100% of the P2 group also obtained 1 layer of myocyte-lipid layer.

Based on the results obtained from the research that has been carried out regarding the presence or absence of extracellular lipid core deposition, in group P1 no extracellular lipid core deposition was found in 100% of the total number of treated rats. Similarly, group P2 also found 100% no extracellular lipid core deposition.

Based on the examination of the presence or absence of cholesterol crystal embolism, the P1 group found 100% no cholesterol crystal embolism. Likewise, in group P2, 100% were found to have no cholesterol crystal embolism.

Based on the results obtained, all rats in groups P1 and P2 were normal, no inflammation or atherosclerosis was found. There was no significant difference between groups P1 and P2.

The  $\alpha$ -*mangosteen* compound found in this peel has also been shown to have better anti-inflammatory effects than anti-inflammatory drugs available in the market. *Mangosteen* is known to





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inhibit the release of cytokines involved in inflammatory responses, such as *interleukin-1 beta* (IL-1 $\beta$ ), *interleukin-6* (IL-6), and *tumor necrosis factor-alpha* (TNF- $\alpha$ ). By inhibiting the release of these cytokines, *mangosteen* can help reduce inflammation that occurs in the body. In addition, *mangosteen* may also have antioxidant activity that can keep cells protected from damage or defects caused by free radicals and oxidative stress associated with inflammation.<sup>22</sup>

Based on the results obtained, it can be seen that all rats in groups P1 and P2 did not have *fatty streaks*. There was no significant difference between groups P1 and P2.

The main pathological lesion associated with atherosclerosis is *fatty streaks* which are yellow-colored areas on arterial blood vessels, forming patches <1 mm or lines 1-2 mm wide, and up to one mm long.<sup>23</sup>

No disturbed myocyte coherence was found in group P1. Likewise in group P2. After statistical analysis, the difference has a p value > 0.05 which can indicate there is no significant difference.

Based on the examination of the presence or absence of fibrous connective tissue, it was found that 100% of the number of P1 treatment rats were included in score 0, which is based on the classification of 0-5% fibrous connective tissue. The same thing was found in group P2.

This is consistent with previous studies stating that exercise therapy significantly inhibits atherosclerosis (the main cause of atheroembolic) by inhibiting the recruitment and activation of bone marrow-derived macrophages and lower levels of oxidative stress markers. In addition, administration of mangosteen peel extract can reduce cytokine gene expression and proinflammatory cell infiltration, significantly decreasing the average size of adipocytes in retroperitoneal adipose tissue.

Inflammation has been shown to be key in the process of atherogenesis, which can result in atheroma plaque rupture. Therefore, therapeutic modalities for atheroembolic kidney disease can also be directed to address the inflammatory response.

## CONCLUSIONS

Both treatment combinations produced evidence of renal histopathology in Wistar rats with metabolic syndrome, namely in the group with the

administration of *Garcinia Mangostana Linn Pericarp Extract and Nanoemulsion* together with physical exercise, no disturbed myocyte coherence was found. All rats were found to have 0-5% fibrous connective tissue, no *fatty streaks*, no inflammation, a single layer of foam cell layer and lipid myocytes, no external lipid deposition, and no cholesterol crystal emboli.

When associated with the use of nanoparticles in this study, but there is no difference in effect between the administration of extract preparations and *nanoemulsion* which should be nanoemulsion better effect when compared with extracts. This may be influenced by several things, including particle size, dosage regimen, exposure route, surface chemistry, degree of aggregation, transmembrane diffusivity, excretion pathways, and immunogenicity. By controlling these factors, the interaction of nanomaterials with biological tissues, penetration, diffusivity, absorption, distribution, immune recognition, duration of deposition into various body tissues, and clearance from the body can be controlled to prevent unwanted nanotoxicity.

However, these results could not be compared with the untreated group as all the rats died before the end of the study.

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