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ANTI-ATHEROSCLEROTIC EFFECTS OF *SONNERATIA ALBA* FRUIT EXTRACT IN ATHEROSCLEROTIC-INDUCED RATS

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ABSTRACT

Objective: The aim of this research was to investigate the potential of *Sonneratia alba* fruit extract to prevent atherosclerosis formation.

Methods: Eighteen male Wistar rats were divided into standard or negative control (S), positive control (P) and treatment (T) groups. Atherosclerosis was induced in groups P and T by orally administering a single dose of Vitamin D3 and a high-fat diet for three days. *Sonneratia alba* fruit extract was given to the T-group for three days. Lipids were enzymatically measured and foam cells were counted in 10 fields of a microscopic view of the abdominal aorta.

Results: This study showed higher cholesterol, LDL, HDL and triglyceride levels in the T-group, compared to the other groups. The average number of foam cells in the S-, P-and T-groups were 11.8±3.3 cells, 21.2±2.2 cells and 11.7±2.9 cells, respectively. Statistical analysis with One-Way Anova showed a significant difference in the average number of foam cells ($p = 0.042$). Further analysis showed a significant difference in the average number of foam cells in groups S with P ($p = 0.041$) and P with T ($p = 0.024$).

Conclusion: *Sonneratia alba* fruit extract showed a potential effect to inhibit atherosclerosis process but could not suppress lipid levels in the blood.

Keywords: atherosclerosis, foam cells, lipid, mangrove, *Sonneratia alba*

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INTRODUCTION

Atherosclerosis is inflammation in blood vessels, especially medium-sized arteries, and is a significant risk factor for cardiovascular disease. The process of atherosclerosis in the early stages is characterized by impaired vascular endothelial function due to inflammation and oxidative stress. In the next stage, cell progression is marked by a high number of foam cells in the vascular system and the proliferation of smooth muscle cells found in vascular media tunica. Further stage is marked by the formation of thrombus due to atheroma plaque rupture, which can cause partial or complete blockages in the vascular system [1]. Thrombus and atheroma plaque release can cause serious problems; blockages in the blood vessels of the heart and brain can even result in death.

Mangroves are found in coastal areas of Riau Province, Indonesia. In addition to functioning as a barrier to coastal abrasion and reducing air and water pollution along the coast, mangroves also function as an environment for marine biota. *Sonneratia alba* is a type of mangrove that is easily found in various mangrove forests in Riau [2].

Several studies have been conducted on the use of stems, leaves and fruit of different types of mangroves for various diseases. It is well-known that the mangrove has antibacterial, anti-inflammatory, anti-cholesterol, anti-tumor and anti-diabetic potentiality due to the presence of flavonoids, tannins, saponins, polyphenols, sterols and alkaloids contained in the components and fruits of mangroves. Furthermore, it has been found that mangroves have a potential effect as an exogenous antioxidant. Research conducted by Simlai *et al.* found that methanol extract of the *Sonneratia caseolaris* stem had an ability as a DPPH free-radical scavenger with more than 90% scavenging activity [3]. In addition, research conducted by Krishnamoorthy *et al.* showed that the phenolic and flavonoid content in *Bruguiera cylindrica* and *Ceriops decandra* mangroves demonstrated strong anti-free-radical activity against DPPH, ABTS and OH radicals [4].

Thus far, there is still limited research that evaluates the effect of *Sonneratia alba* fruit administration on atherosclerosis formation. The potential of the mangrove as an antioxidant provides an opportunity for it to become an alternative therapy for atherosclerosis. Therefore, we are interested to know the effects of *Sonneratia alba* fruit extract administration on atherosclerosis formation in atherosclerotic-induced rats.

MATERIALS AND METHODS

Samples

This research obtained ethical approval from the Medical and Health Research Ethics Committee of our university, with ethical approval number 099/UN.19.5.1.1.8/UEPKK/2019. Eighteen male *Rattus norvegicus Wistar* rats, age 7–8 w, weight 180–250 grams, obtained from Animal House of STIFAR Pekanbaru were used in this experiment. These rats were divided into three groups: standard or negative control (S), positive control (P) and treatment (T) groups. Group S was fed standard chow, group P was atherosclerosis-induced rats and group T was atherosclerosis-induced rats that were given methanol extract of *Sonneratia alba* fruit. Adaptation of the rats was performed for a week prior to treatment. During treatment, the rats were kept in surroundings that were room temperature with a light-dark cycle of 12:12 h and free access to standard chow (Fivo 5.12) and water.

Atherosclerosis animal model

Atherosclerosis induction in rats was done by administering a high-fat diet with a composition of 2% cholesterol taken from chicken egg yolks, 5% goat fat, 0.2% cholic acid that was added into the standard diet (Fivo 5.12). A single dose of 700,000 i. u/kg bodyweight of Vitamin D3 was administered orally to initiate the atherosclerosis process, followed by a 20 gram/day high-fat diet for three days [5].

Mangrove *Sonneratia alba* fruit extraction

Mangrove *Sonneratia alba* fruits were obtained from the coastal area of Meranti District, Riau Province, Indonesia. Plant identification was carried out in the Faculty of Mathematics and Natural Sciences of our university to ensure the *Sonneratia alba* species was used. Mangrove

fruits were washed clean, thinly cut and dried for 7–10 d in an open space. Pieces of mangrove fruit that had been dried were mashed to flour. The extraction process was carried out by dissolving the flour in methanol in a ratio of 1:7 (w/v) and stored for 24 h using the Soxhlet Apparatus. The extracts formed were then evaporated to dryness using a rotary evaporator at 50 °C, and the evaporated flour was stored at 4 °C until it was tested**.** The extract was given orally to T-group at a dose 400 mg/kg body weight per day for three days.

Blood lipid examination and histopathological analysis of abdominal aorta

After three days of treatment with *Sonneratia alba* fruit extract, a necropsy was performed. Anesthetizing with ether was used to euthanized the rats. A cardiac aspiration was performed to obtain blood samples. Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels were enzymatically measured using a direct examination method.

Histological examination of the abdominal aorta was performed to view the atherosclerosis process that occurred by administering a high-fat diet and the effects caused by the administration of *Sonneratia alba* fruit extracts to the atherosclerosis process. Tissues were fixed in 10% phosphate-buffer formalin and embedded in paraffin blocks. The abdominal aortic paraffin blocks were cut into thin slices and stained with hematoxylin-eosin (HE). The initial phase of the atherosclerosis process was assessed by counting the number of foam cells found in 10 fields of a microscopic view at 40-times magnification.

Statistical analysis

The data obtained were delivered in means and standard error of means. The statistical analysis used was the One-Way ANOVA test for total cholesterol, HDL and foam cells number, followed by Post Hoc; the Kruskal-Wallis test was used for LDL and triglyceride levels, followed by Mann-Whitney U. Values were considered statistically significant when $p<0.05$.

RESULTS

The effect of *Sonneratia alba* mangrove extract on the lipid profile at the initiation stage of atherosclerosis is shown in table 1. Administration of a high-fat diet mainly affected total cholesterol and LDL levels in the rats. The total cholesterol level of rats in the P-group and T-group was higher than in S-group: 82.8±13.1 mg/dl, 113.8±12.6 mg/dl, and 62.5±6.2 mg/dl, respectively. Statistical analysis using One-Way Anova showed a significant difference with p = 0.039. After further analysis using the Post Hoc test, this significant difference was seen between the S-and T-groups (p = 0.046).

Table 1: Effects of giving sonneratia alba fruit extracts on lipid profile

Group	Total cholesterol mean+SEM (mg/dl)	p	HDL mean+SEM (mg/dl)	p
Negative control	62.5+6.2		36.7+4.3	
Positive control	82.8+13.1	0.039*	34.8+5.1	0.645
Treatment	113.8+12.6		40.0+2.4	
Group	LDL mean+SEM (mg/dl)	p	Triglyceride mean+SEM (mg/dl)	p
Negative control	15.5+2.3		140.3+26.5	
Positive control	33.5+14.9	0.029*	95.8+21.4	0.086
Treatment	66.5+11.3		170.7+25.4	

*indicate a significantly different among negative control, positive control, and treatment group according to One-Way Anova test (cholesterol) and Kruskal Wallis test (LDL).

LDL levels in P-group (33.5±14.9 mg/dl) and T-group (66.5±11.3 mg/dl) were also found to be higher than in S-group (15.5±2.3 mg/dl). Statistical analysis using the Kruskal-Wallis test showed a significant difference in LDL levels (p = 0.029). Further statistical analysis using the Mann-Whitney U test showed that the significant difference was seen between S-and T-groups (p = 0.010). Furthermore, HDL levels in T-group (40.0±2.4 mg/dl) were higher than either S-group (36.7±4.3 mg/dl) or P-group (34.8±5.1 mg/dl), but it was not statistically different (p = 0.645). Meanwhile, triglyceride levels in T-group (170.7±25.4 mg/dl) was found to be higher than the other groups, but the triglyceride levels of P-group (95.8±21.4 mg/dl) that was administered a high-fat diet were lower than S-group (140.3±26.5 mg/dl), which received standard chow. There were no statistically differences between the three groups (p = 0.086).

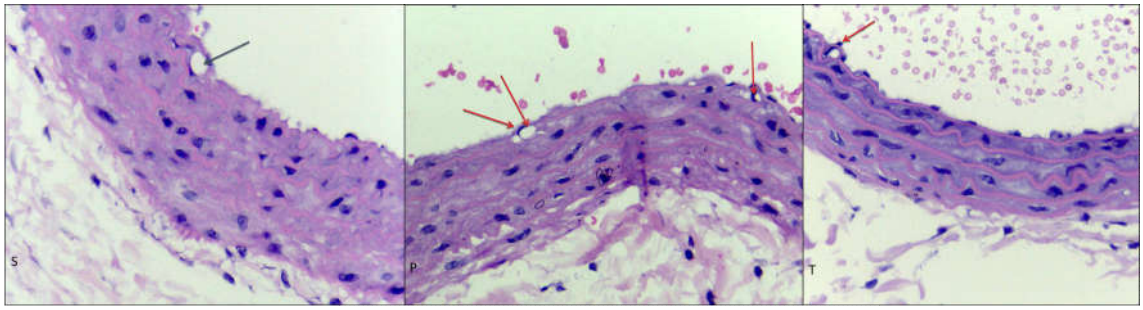

Fig. 1: Histological appearance of abdominal aorta. The left picture was negative control, the middle picture was positive control and the right picture was treatment group. The arrows showed foam cells in abdominal aorta of the three groups

The atherosclerosis initiation phase is characterized by the presence of foam cells. In this study, the number of foam cells was calculated from 10 fields of a microscopic view. The results showed foam cells were found in varying amounts in all three groups (fig. 1). As shown in table 2, the average number of foam cells in the positive control (P) group was higher than the negative control (S) and treatment (T) groups, which were 21.2±2.2 cells, 11.8±3.3 cells and 11.7±2.9 cells, respectively. Analysis using the One-Way Anova test showed a statistical difference in the number of foam cells in the three groups (p = 0.042). Further analysis using the Post Hoc test showed a significant difference between P-and S-group (p = 0.041) and P-and T-group (p = 0.024), but not a statistical difference between S-and T-group (p = 0.984).

Table 2: Average number of foam cells in the negative control, positive control and treatment groups

Group	Foam cell mean+SEM	p
Negative control	11.8+3.3	
Positive control	21.2+2.2	0.042*
Treatment	11.7+2.9	

*indicates a significantly different among groups according to the One-Way Anova test.

DISCUSSION

Several factors are known to trigger atherosclerosis, one of which is an increase in plasma lipid levels, especially LDL. In situations of high plasma LDL levels, these molecules are able to enter the vascular subendothelium through the damaged endothelium and are oxidized (OxLDL). Large amounts of OxLDL are potent oxidants that can trigger the formation of reactive oxygen species (ROS) through nitric oxide (NO) activation [6]. NO is a potent oxidant that, when produced by vascular endothelial cells, will have a protective effect; but if produced by macrophages, it will be pro-atherogenic. Excessively formed ROS will cause activation and translocation of NF-kB p65 subunits. Activation of the NF-kB subunits will trigger an increasing expression of monocyte chemoattractant protein (MCP-1), thereby increasing monocyte extravasation to vascular subendothelium and triggering the release of various pro-inflammatory mediators, such as IL-1, IL-8 and NO by macrophages. The release of pro-inflammatory mediators by these macrophages will continue to activate NF-kB and trigger vascular smooth muscle proliferation [7].

In addition, observing NF-kB will provide signals to vascular endothelium to express vascular cell adhesion molecule-1, intracellular adhesion molecule-1, E-selectin and P-selectin. Similar to MCP-1, the adhesion molecule causes monocytes and T lymphocytes to extravasate to the sub-endothelium. In intact tunica, monocytes turn into macrophages and phagocytosis of the OxLDL through the SR-A and CD36 scavenger receptors and form foam cells. The formation of these foam cells is one of the markers in both the early and advanced stages of atherosclerosis [8].

In this study, the administration of *Sonneratia alba* mangrove extract did not seem to affect the lipid profile. Rats given an atherogenic diet showed an increase in total cholesterol, LDL and triglycerides, except in the positive control group. In contrast, the administration of *Sonneratia alba* fruit extracts showed an inhibitory effect on the initiation of the atherosclerosis process, which was characterized by a low average number of foam cells in the group given the atherogenic diet and mangrove extract, compared with the group that was only given an atherogenic diet.

Mangroves stems, fruits and leaves are known to be rich in antioxidants. Some active ingredients of mangrove are known to have antioxidant effects by inhibiting the stress signaling pathway, either through the NF-kB, MAPK or JAK-STAT pathways, thereby suppressing free-radical activity as a free-radical scavenger that can inhibit superoxide formation. Thus, reactive oxygen species and cellular oxidative stress can be prevented [9]. This might explain the protective effect shown by *Sonneratia alba* in inhibiting the initiation of atherosclerosis in this study, even though plasma LDL, total cholesterol and triglyceride levels in the rats given *Sonneratia alba* extract were higher than in the rats that were not given *Sonneratia alba* fruit extract. The inhibition mechanism of *Sonneratia alba* fruit extracts against atherosclerosis needs further research to determine which pathway is most involved in the inhibition process.

CONCLUSION

The *Sonneratia alba* mangrove extract has the potential to inhibit atherosclerosis at the initiation stage, but it does not affect the lipid profile in the blood. It is necessary to further investigate which pathway is affected by the mangrove extract in inhibiting the atherosclerosis process.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no competing interest.

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