

## Effect of Mangrove (*Rhizophora* sp) Fruit Extract on Foam Cell Formation at the Initiation Stage of Atherosclerosis

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<http://dx.doi.org/10.13005/bpj/1902>

(Received: 23 November 2019; accepted: 19 March 2020)

To evaluate mangrove (*Rhizophora* sp) fruit extract effect on foam cell formation at the initiation stage of atherosclerosis. The study was conducted on 18 rats divided into 3 groups (n=6). Group 1 was given a standard diet, Group 2 was given a high cholesterol diet, and Group 3 was given a high cholesterol diet and mangrove fruit extract 500 mg/kg. The mangrove fruit extract effect on foam cell formation was assessed by histopathologic examination using hematoxylin eosin staining. The results showed that the number of foam cells was increased significantly in high cholesterol diet-fed rats compared to standard diet-fed rats (21.17 vs. 11.75). Mangrove fruit extract reduced this number remarkably (21.17 vs. 12.67). In conclusion, mangrove fruit extract inhibits the formation of foam cells at the initiation stage of atherosclerosis.

**Keywords:** Atherosclerosis; Foam Cell; Mangrove Fruit Extract.

Atherosclerosis is a major cause of cardiovascular disease and is a significant contributor to death in the world, including in Indonesia<sup>1</sup>. Atherosclerosis is a vascular inflammatory disease characterized by lipid accumulation, cell death and fibrosis in the arteries<sup>2</sup>. Inflammation has an important role and is the main mechanism underlying atherosclerosis pathogenesis. The formation of free radicals that trigger inflammatory reactions in the vascular lining is the basis of the course of this disease. Exogenous antioxidants are useful for eliminating these free radicals so they can prevent further vascular damage<sup>3</sup>.

The early stage of atherosclerosis begins with the entry of low-density lipoprotein (LDL) into the intima of artery. The LDL will undergo oxidation to oxidized LDL (ox-LDL) which will activate endothelial cells.

Activated endothelial cells express leukocyte adhesion molecules such as Vascular Cell Adhesion Molecule 1 (VCAM-1) on the surface of the arteries. VCAM-1 then interacts with monocytes in the circulation. Monocytes that have been attached to vascular endothelial cells then migrate into the arterial intima layer mediated by Monocyte Chemoattractant Protein-1 (MCP-1)<sup>4</sup>. Monocytes will then differentiate into macrophages in response to Macrophage-Colony Stimulating Factor (M-CSF)<sup>5,6</sup>. These macrophages then devour excessive cholesterol via scavenger receptors which will lead to foam cell formation<sup>4,5</sup>. Formation of foam cells that occurs in the initial stages of atherogenesis is a major hallmark of atherosclerotic disease<sup>6</sup>.

One of the antioxidants that can be developed is mangrove (*Rhizophora* sp), whose habitat is abundant in Riau Province, Indonesia, especially in coastal areas. Mangrove (*Rhizophora*

sp) contains flavonoid, tannin, saponin, polyphenols, sterols and alkaloid found in both stems, leaves and fruit so that various studies have been carried out to determine the pharmacological effects of this natural product. Based on previous studies *Rhizophora* sp has been shown to have pharmacological effects as anti-inflammatory, anti-oxidant, and anti-hypercholesterolemia<sup>7-10</sup>. The existence of these effects provides an opportunity to be used as an anti-atherosclerosis<sup>11</sup>.

The purpose of this study was to evaluate the effect of mangrove (*Rhizophora* sp) fruit extract on foam cell formation at the initiation stage of atherosclerosis.

## MATERIALS AND METHODS

### Experimental Animals

Eighteen healthy male Wistar rats (purchased from School of Pharmacy, Universitas Riau, Pekanbaru, Indonesia), 150-250 g body weight, 10 weeks of age were placed in cages individually in a room with proper ventilation, room temperature between 20–26 °C, and allowed for food and water ad libitum. Lighting of the room was regulated light and dark alternately for 12 hours. Animals underwent an acclimatization period at least 7 days before use in our study. During the acclimatization period the rats got a normal diet. Rats were treated in accordance with the Helsinki convention. Ethical approval was obtained from the Ethical Review Board for Medicine & Health Research, Faculty of Medicine, Universitas Riau (No:099/UN.19.5.1.1.8/UEPKK/2019).

### Experimental Design

Rats were randomly divided into 3 groups (n=6), Group 1 was given a standard diet. Group 2 was given a high cholesterol diet of vitamin D3 (cholecalciferol) 700.000 IU/kg (Sigma, St. Louis, MO, USA) on the first day followed by 5% goat fat, 2% cholesterol, 0.2% cholic acid (Sigma, St. Louis, MO, USA) and 92.8% standard diet for 3 days to induce atherosclerosis initiation stage<sup>11,12</sup>. Group 3 was given a high cholesterol diet and treated with mangrove fruit extract 500 mg/kg. Mangrove fruit extract was given orally once a day using a gastric tube.

### Evaluation of Foam Cell Formation

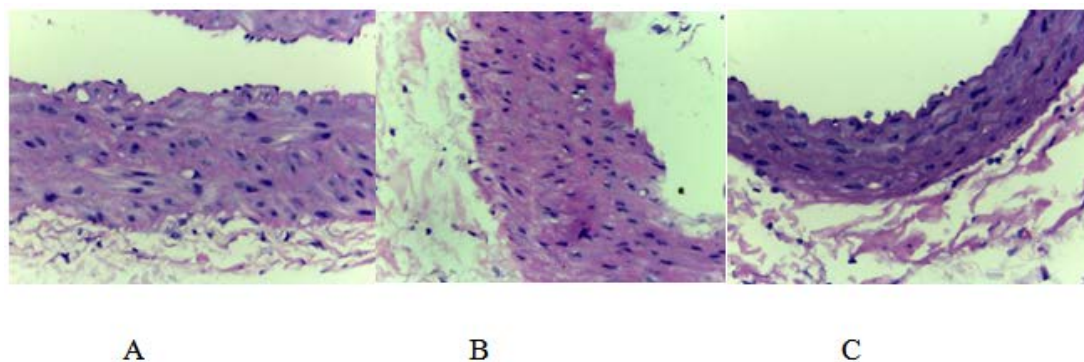
At the end of the experiment, the rats were sacrificed with ether anesthesia. Subsequently, abdominal aortic tissues were rapidly excised. We fixed the aortic sample in 10% neutralized formaldehyde in 0.1 M phosphate buffer and embedded in paraffin. A hematoxylin and eosin (H&E) staining was performed to count the number of foam cells in the abdominal aortic in rats. Foam cells were calculated at 10 fields of view with  $\times 400$  magnification. Images were obtained using a light microscope equipped with a digital camera (Leica, Wetzlar, Germany) connected to a PC monitor.

### Statistical Analysis

Data are presented as mean  $\pm$  SEM.

## RESULTS

The H&E staining showed that in Group 1 receiving standard diet and Group 3 receiving a high cholesterol diet and treated with mangrove



**Fig. 1.** Photomicrographs showing histological sections of aorta abdominal of rats. (A) Group 1 was given a standard diet, (B) Group 2 was given a high cholesterol diet, (C) Group 3 was given a high cholesterol diet and treated with mangrove fruit extract 500 mg/kg, at magnification of  $\times 400$  stained with H&E. The red arrow indicated foam cell.

fruit extract demonstrated few foam cells (Figures 1A and 1C), while in Group 2 receiving a high cholesterol diet showed abundance of foam cells in the aortic lesion (Figure 1B). These results demonstrated that the administration of high cholesterol diet induced foam cell formation and mangrove fruit extract treatment inhibited the accumulation of these cells in aortic lesion.

Figure 2 shows the number of foam cells among different experimental groups. High cholesterol diet-fed animals exhibit increased number of foam cells in the aortic lesions compared to control rats ( $21.17 \pm 2.21$  vs.  $11.75 \pm 3.33$ ). Mangrove fruit extract treatment significantly reduced the accumulation of foam cells in the aortic lesions ( $21.17 \pm 2.21$  vs.  $12.67 \pm 4.62$ ).

## DISCUSSION

During the recent years, many researchers have paid attention to phytochemicals, which are regarded as important sources for drug development<sup>13</sup>. Mangroves are plants that live on the coastline and live in tropical and subtropical regions. This plant is known as halophyte which is very tolerant of high salt content and easily adapts to environments with low oxygenation and muddy. In Indonesia, approximately 47 species of

mangrove plants were found, both of which belong to the family Rhizophoraceae, Sonneratiaceae, Avicenniaceae and Meliaceae<sup>14</sup>. The results of this study showed that mangrove fruit extract may reduce foam cell formation in the early stages of atherosclerosis.

The formation of foam cells involves various processes such as taking ox-LDL, cholesterol esterification and cholesterol efflux. Ox-LDL uptake by macrophages is carried out through phagocytosis and pinocytosis processes mediated by CD36 and SR-A. About 75-90% of ox-LDL uptake by macrophages was carried out through both receptors<sup>15</sup>. Manning-Tobin *et al.* reported the results of his study which showed that SR-A suppression can prevent the formation of atherosclerotic lesions<sup>16</sup>, while research conducted by Xie *et al.* showed that down-regulation of CD36 can inhibit foam cell formation<sup>17</sup>.

The process of cholesterol esterification involves Acyl coenzyme A: cholesterol acyltransferase-1 (ACAT1) and neutral cholesteryl ester hydrolase (nCEH)<sup>18</sup>. The level of cholesterol esterification determines the number of macrophages that will turn into foam cells<sup>19</sup>. Xu *et al.* showed ACAT1 inhibition can suppress foam cell formation and inhibit the development of atherosclerosis<sup>20</sup>.

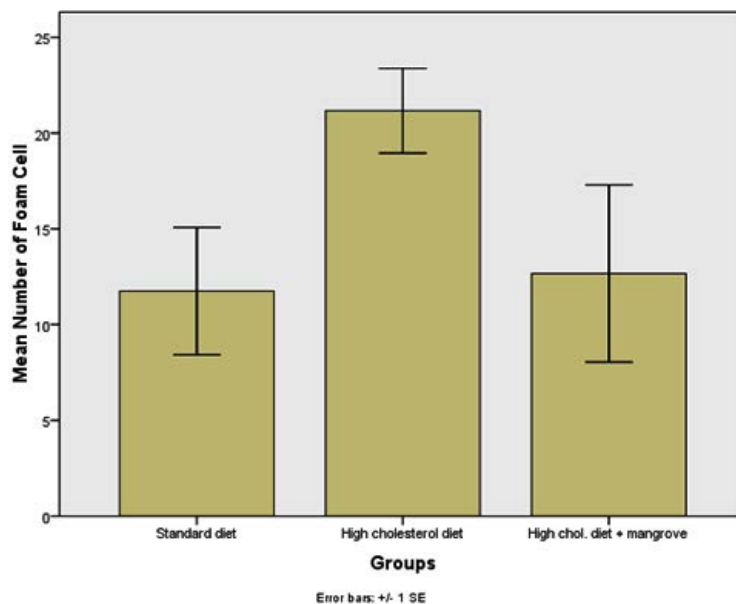


Fig. 2. Number of foam cells among different experimental groups. Data are presented as mean  $\pm$  SEM (N=6)

The process of cholesterol efflux involves the ATP-binding cassette (ABC) transporter A1 (ABCA1), ABCG1 and BI-receptor scavenger (SR-BI)<sup>21,22</sup>. Disruption in the process of cholesterol efflux results in cholesterol accumulation in macrophages<sup>23</sup>, whereas ABCA1 stimulation can inhibit foam cell formation<sup>24</sup>.

The balance between cholesterol uptake, cholesterol esterification and cholesterol efflux plays a role in preventing lipid accumulation in macrophages<sup>25</sup>. At the stage of atherosclerosis initiation there is an increase in CD36 and SR-A expression, an increase in cholesterol esterification due to an increase in ACAT1 levels or a decrease in nCEH levels and a decrease in cholesterol efflux due to a decrease in the expression of ABCA1, ABCG1 and SR-BI. This condition causes the accumulation of cholesterol esters in the macrophages which will result in the formation of foam cells<sup>26</sup>. The progression of atherosclerosis depends on the accumulation of monocytes on the walls of the arteries, the more monocytes/macrophages accumulate, the more foam cells are formed so that the development of atherosclerotic lesions will also accelerate<sup>27</sup>.

The initiation stage of atherosclerosis is characterized by the formation of foam cells. Foam cells found at the initiation stage of atherosclerosis are derived from monocytes found in the circulation. Monocytes that have been attached to vascular endothelial cells then migrate into the intima layer of arteries<sup>28</sup>. The mechanism of mangrove fruit extract in inhibiting foam cell formation is thought to be through the inhibition of monocyte migration into the arterial wall.

### CONCLUSION

In conclusion, mangrove fruit extract could inhibit the formation of foam cells at the initiation stage of atherosclerosis. Thus mangrove fruit extract is very potential to be developed as anti-atherosclerosis.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge that the present research is supported by Research Development Centre University of Riau.

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