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Original Research

Anti-Atherosclerosis Effect of Soy Milk in Rats Fed an Atherogenic Diet

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ABSTRACT

Background. Atherosclerosis is a problem of cardiovascular disease. Consuming food containing excessive fat is a risk factor for atherosclerosis. Soy milk has been studied for its isoflavones which has antihyperlipidemic effect on the development of atherosclerosis. The aim of this study was to find out the effect of soy milk (*Glycine max* (L.) Merr.) administration on aortic's histopathology profile (the length of wall thickness and number of foam cells) in male white rats (*Rattus norvegicus*) which were given the atherogenic diet that consists of goat's fat and quail egg yolk. **Material and Methods.** A total of 30 rats were used in the true laboratory experiment which were distributed into 5 groups (n=6) using post-test only design, there were a normal diet group, an atherogenic diet group, an atherogenic and simvastatin 10 mg group, and another 2 groups that were given an atherogenic diet with soy milk doses variation of 12.5 g and 25 g respectively. Aortic's histopathology was prepared with the paraffin method and stained with Hematoxylin-Eosin. **Results.** ANOVA test showed that soy milk variation dose was significantly ($p<0.05$) reduce the aortic wall thickness and foam cell.

Introduction

Atherosclerosis is a progressive inflammatory response to blood vessel injury. The formation of atherosclerotic lesions is characterized by the accumulation of fat, foam cells, platelet aggregation, proliferation and migration of smooth muscle cells that cause the thickening and hardening of the artery walls. It results in the stiffness and brittleness of the arteries.[1,2]

The most influencing risk factor of atherosclerosis formation is hyperlipidemia [3,4]. Low-density lipoprotein (LDL) has validity as a predictor of cardiovascular disease. The presence of LDL in the bloodstream can penetrate the artery wall's endothelium and undergo oxidation, becoming oxidized LDL (Ox-LDL) [1,5]. Then the Ox-LDL will be endocytosed by macrophages through the scavenger receptors to become foam cells, and then it will form fatty streaks [6]. The continuous accumulation of fat will induce myocyte proliferation, extracellular matrix synthesis, and fibrosis tissue formation [1]. Atherosclerotic plaque blockage in the coronary arteries is the cause of coronary heart disease (CHD).[7]

Cardiovascular diseases are the top cause of death globally, with an estimated of 17.9 million people died in 2016, and 85% of these are due to heart attack and stroke. The 2016 Heart Disease and Stroke Statistics Update of the American Heart

Association (AHA) has recently reported that 15.5 million people ≥20 years of age in the USA have CHD [8]. The 2013 Basic Health Research (Riskesdas) has reported that 1.5% of Indonesia's population have CHD, and Aceh has a prevalence of 2.3%. [9]

There are various efforts to control atherosclerosis, one of which is by overcoming hyperlipidemia [2]. AHA recommends using statin drugs to lower the lipid profile to suppress atherogenic activity [10]. Anti-hyperlipidemic drugs must be consumed for a long time and require high costs and can cause unwanted side effects [2]. Based on this information, it is necessary to have alternative medicines to overcome it, one of those can be used is soybean (*Glycine max* (L.) Merr). [2,11]

Soybean contains essential amino acids, high protein and fiber, low saturated fat, cholesterol, and lactose-free, and as a source of omega-3 fatty acids. The content of vitamin E in soybeans is called tocopherol, which functions as an antioxidant. Also, soybeans contain high phytoestrogens, especially isoflavones [12]. The estrogenic activity of isoflavones serves as protection, affecting α and β estrogen receptors in the arteries. Isoflavones are also important for lowering cholesterol levels and repressing the development of atherosclerotic plaques in primates by inhibition of lipid peroxidation and LDL oxidation [13]. The provision of soy milk is expected to reduce and prevent thickening of the aortic wall and formation of foam cells, so that atherosclerosis does not occur.

Material and Methods

This is an experimental laboratory study using a completely randomized design and the post-test only method with a control group design. The study was conducted in the experimental animal laboratory and the anatomical pathology laboratory at Syiah Kuala, on November – December 2017.

The study subjects were 30 male Wistar rats (*Rattus norvegicus*) aged 2-3 months, which were divided into five groups used 6 repetitions, consisting of 2 control groups (C-, and C+) and 3 treatment groups (T1, T2, and T3). Group C- without treatment, group C+ was given atherogenic diet, group T1 was given atherogenic diet and simvastatin 10 mg (1.8 mg/200 g BW animal dose), group T2 was given atherogenic diet and 12.5 g (2.25 g/200 g BW) soy milk, and group T3 was given atherogenic diet and 25 g (4.5 g/200 g BW) soy milk. The atherogenic diet was made from a mixture of 150 g of goat fat and 100 g of quail egg yolk. Soy milk was made by first washing and draining the soybeans. Then it was roughly mashed and boiled in water that has reached 60˚C for 30 minutes. The soybeans were left to soak in the water for 4 hours. Then added more water to that soaked water and finely ground. The ground product was filtered to obtain a solution of soy milk. Then, it was reheated at a temperature of 60˚C.

Rats were treated simultaneously for 60 days. At the end of the study, the rats were dispatched using chloroform. Then, tissue samples were surgically removed from the rat's aorta. Aortic's histopathology was prepared with the paraffin method and stained with Hematoxylin-Eosin, and utilized to observe the wall thickness and sub-endothelial foam cell expression under microscope with 40 x 10 magnification in eight fields of view. The results will be analyzed using ANOVA and Tukey post hoc analysis.

Results

Table 1. Size of Aortic Wall Thickness and the Number of Foam Cell

Parameter	Kelompok				
	C-	C+	T1	T2	T3
Foam cells	0.00±0.00	4.67±1.75	0.67±0.82	0.17±0.41	0.00±0.00
Aortic wall thickness (µm)	203.3±57.30	419.6±28.47	319.7±48.06	252.3±37.73	214.8±29.69

C-: Without treatment

C+: Treatment of atherogenic feed

T1: Treatment of atherogenic feed and simvastatin 10 mg

T2: Treatment of atherogenic feed and soy milk 25 g

T3: Treatment of atherogenic feed and soy milk 12.5 g

Based on the ANOVA results, a significant value was obtained, which indicated a significant difference of $p = 0.00$ ($p < 0.05$), so it could be concluded that soy milk affected the aortic wall thickness and the number of foam cells.

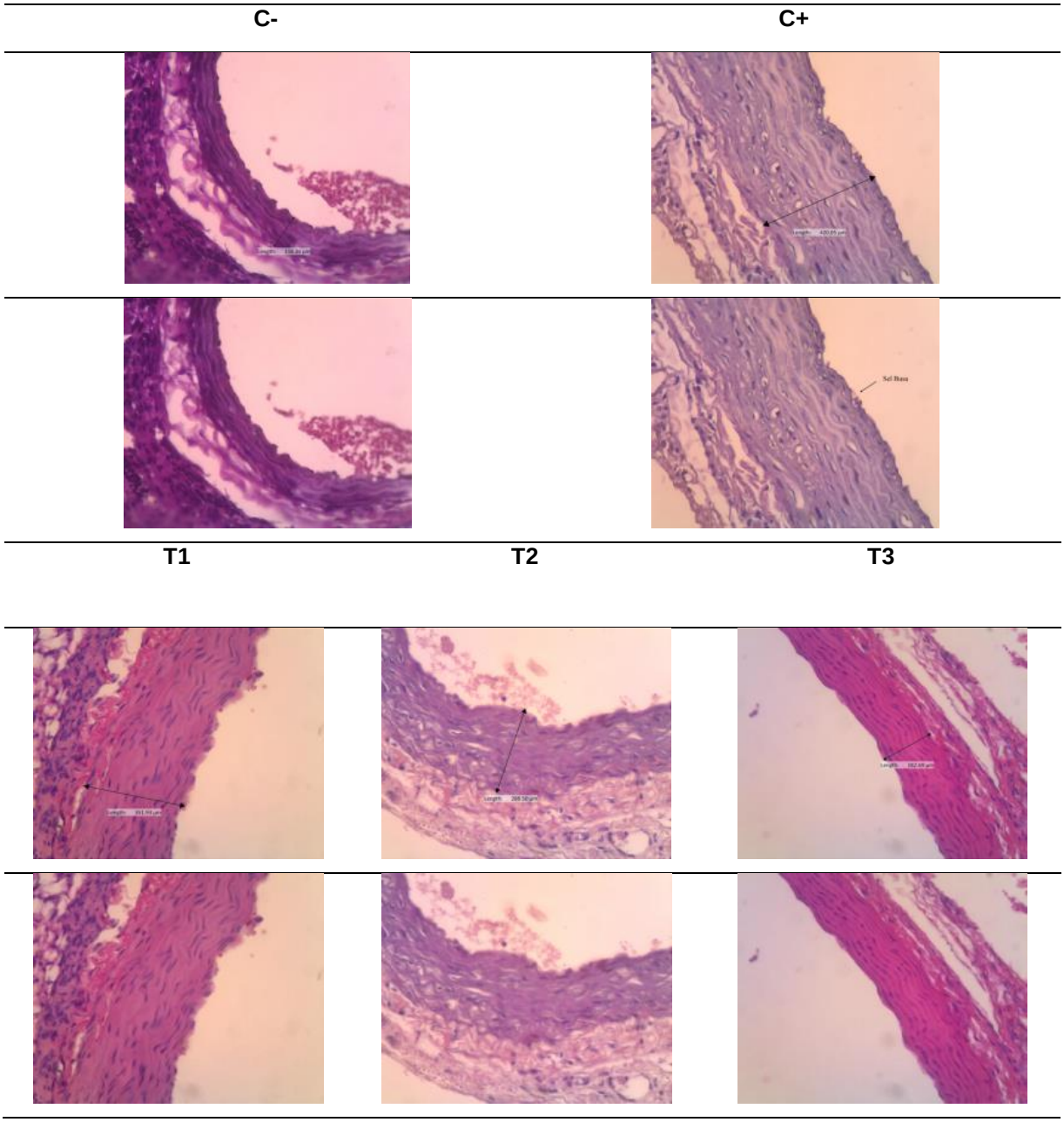

Figure 1. Aortic Cross-Section Preparation with HE Staining

The characteristics of foam cells on HE staining were white cells (colorless) because they contained fat, with a dark core located on the edge (fig. 1)

Table 2. Tukey test result: Aortic foam cells

Group	C-	C+	T1	T2	T3
C-	-	0,000*	0,689	0,997	1,000
C+	0,000*	-	0,000*	0,000*	0,000*
T1	0,689	0,000*	-	0,861	0,689
T2	0,997	0,000*	0,861	-	0,997
T3	1,000	0,000*	0,689	0,997	-

*significant result

Table 3. Tukey test result: Aortic wall thickness

Group	C-	C+	T1	T2	T3
C-	-	0,000*	0,001*	0,280	0,989
C+	0,000*	-	0,003*	0,000*	0,000*
T1	0,001*	0,003*	-	0,067	0,002*
T2	0,280	0,000*	0,067	-	0,538
T3	0,989	0,000*	0,002*	0,538	-

*significant result

Discussion

Based on Tukey's post hoc analysis results, it could be seen that the C- group had a significant difference to the C+ group with p value = 0.000. It was suggested that atherogenic feeding significantly increased the thickness of the aortic wall. It was related to the amount of LDL cholesterol [14]. An atherogenic diet containing high cholesterol and saturated fat caused the collection of LDL particles in the tunica intima. [15]

Hyperlipidemia could lead to the formation of free radicals and oxidative stress. Moreover, endothelial damage in the C+ group increased endothelial permeability, thereby facilitated LDL's entry into the intima and undergone oxidation, becoming Ox-LDL. This process stimulated the release of growth factors, namely platelet-derived growth factor (PDGF), so that smooth muscle cells in the tunica media migrated to the tunica intima and experienced proliferation therein, resulting in the accumulation of smooth muscle cells.[16] Injured endothelium would also release Reactive Oxygen Species (ROS), which increased the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin and increased the exposure to chemokine interleukins (IL) such as IL -1, IL-6 and IL-18 caused monocyte adhesion to the endothelium. Then, endothelial cells released the macrophage colony-stimulating factor (MCS-F), which converted monocytes into macrophages. Finally, the macrophages endocytosed Ox-LDL through the scavenger receptors to formed foam cells. [14,15,17]

Based on the analysis results, soy milk and simvastatin could reduce the aortic wall thickness and the number of aortic foam cells. The simvastatin (T1) treatment group was used as a comparison control because it was an effective hypolipidemic agent to lower cholesterol by inhibiting HMG CoA reductase, thereby inhibiting

liver cholesterol synthesis. It prevented the oxidative stress release, so there was no increase in endothelial permeability and oxidation of LDL to Ox-LDL, which could inhibit the increased size of the aortic wall thickness and foam cell formation.

The decrease in aortic wall thickness and the number of aortic foam cells in the fed soy milk groups was caused by antioxidant compounds in soy milk such as isoflavones consisting of genistein, daidzein, and glycitein. Isoflavones reduced the levels of total cholesterol, LDL, and triacylglycerols [11]. Based on previous studies, soy milk at animal dose of 2.25 g/200 g BW and 4.5 g/200 g BW could reduce LDL levels [18]. This statement was supported by previous clinical trial research, that soy consumption of 25-30 g/day is effective in reducing LDL cholesterol by 4-8% [12]. Reduced LDL levels also lowered the amount of oxidized LDL which stimulated smooth muscle cell proliferation and inhibited the aortic wall thickening because isoflavones reduced LDL cholesterol levels in the circulation. This mechanism was thought to reduce the risk of endothelial dysfunction and Ox-LDL formation. It also reduced the risk of monocyte adhesion to the endothelium and the endocytosis activity of macrophages through the scavenger receptors against Ox-LDL, which ultimately prevents foam cells formation. The risk of stimulating smooth muscle cell proliferation was also reduced so that the thickening of the aortic wall in rats treated with soy milk can be prevented.

Conclusion

Soy milk with a dose of 12.5 g and 25 g affected the histopathological view of the aorta in reducing the aortic wall thickness and foam cells.

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