Jurnal Saintika Medika
Jurnal Ilmu Kesehatan dan Kedokteran Keluarga
Faculty of Medicine UMM

Vol. 20 No. 1 June 2024
p-ISSN : 0216-759X e-ISSN : 2614-X476X
http://ejournal.umm.ac.id/index.php/sainmed

Effectiveness of Petai (Parkia speciosa) Ethanol Extract in Controlling Foam Cell Numbers in Atherosclerosis-Induced Male Wistar Rats

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Receive : May 21th2024. Revised : July 4th2024. Published: July 12th2024

DOI: https://doi.org/10.22219/sm.Vol20.SMUMM1.38652

ABSTRACT

Atherosclerosis is a chronic inflammatory disease that causes the narrowing of the arterial lumen, and it is considered the primary cause of cardiovascular diseases such as myocardial infarction (MI), heart failure, and stroke. Cardiovascular disease is the leading of death globally. Atherosclerosis has a complex pathophysiology, such as endothelial dysfunction, thickening of the tunica intima, and the formation of atheromatous plaques. After that process, foam cells will form, which is the key to the development of atherosclerosis. Petai ethanol extract contains antioxidants, which are flavonoids, saponins, and tannins, that can reduce the number of Low-Density lipoproteins (LDL). The purpose of this study was to evaluate the effect of petai ethanol extract on the number of atherosclerotic foam cells in male white rats (Rattus norvegicus strain wistar) induced by lard. The method of this study was a genuinely experimental and post-test-only control group design with 25 rats divided into five control groups, which are one negative control group, one positive control group, and three treatment groups with extract doses of 100mg/kgBW, 200mg/kgBW and 400mg/kgBW. Observation of foam cells was carried out using a microscope with 400x magnification. Data were analyzed using Oneway ANOVA and Post Hoc Tamhane's test. The results of the study using the Oneway ANOVA test showed that there were significant differences between groups (p: 0.000). Post Hoc Tamhane's test between the positive control group and treatment groups 1, 2, and 3 can be seen that there is a significant difference between the formation of foam cells due to the administration of petai ethanol extract compared to positive control ($p < 0.05$). This study concludes that Petai ethanol extract was effective in reducing the number of atherosclerotic foam cells formed.

Keywords: Atherosclerosis, Endothelial Dysfunction Foam Cells, Tunica Intima, Foam Cell, Petai Ethanol Extract

INTRODUCTION

Atherosclerosis is a chronic inflammatory reaction to the walls of blood vessels in response to states of dyslipidemia and endothelial distress involving inflammation of leukocytes and activation of vascular cells (Riccioni and Sblendorio, 2012). Atherosclerosis is the dominant cause of cardiovascular diseases, including *myocardial infarction* (MI), *heart failure*, stroke, and *claudication* (Frostegård, 2013). Cardiovascular and cerebrovascular diseases are the leading cause of death globally, so it is important to identify all the risks (Yao *et al.*, 2019). In 2016, *the World Health Organization* (WHO) reported that around 17.8 million (31.4%) deaths worldwide were caused by cardiovascular diseases (WHO, 2016). In Indonesia itself, the prevalence of heart disease is around 1.5%, as well as in the province of East Java (Ministry of Health of the Republic of Indonesia, 2018).

Atherosclerosis has a complex pathophysiology, ranging from endothelial dysfunction, thickening of the tunica intima, and the formation of atheomatous plaques (Gupta, Ali, and Sanghera, 2019). Primary lesions in atherosclerosis are characterized by the presence of lipid repositioning in arterial smooth muscle cells and the proliferation of fibrous matrix that develops into atherosclerosis plaques (Zhu *et al.*, 2018). Based on the theory of oxidative stress, atherosclerosis is an oxidative modification of LDL in the artery wall by reactive oxygen species (ROS) (Kurniasari, 2018). Risk factors for atherosclerosis include high cholesterol and LDL levels, low-density lipoprotein (HDL) levels, hypertension, smoking, diabetes mellitus, obesity, inflammation, unhealthy lifestyle, and age (Kopaei et al., 2014).

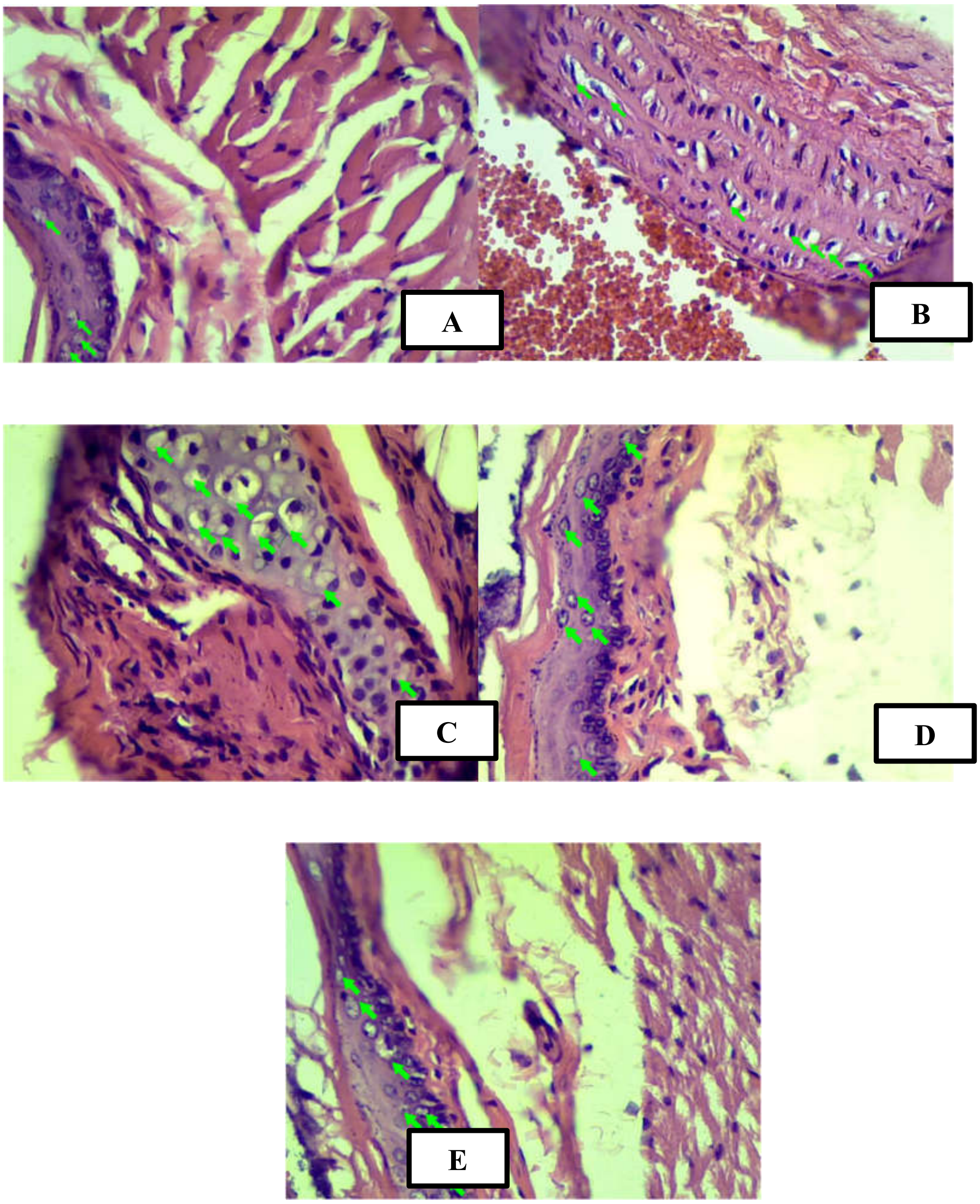
Petai is a food ingredient that is often consumed in Indonesia. However, it is still rarely used in medicine where the benefits are very many, including the extract can prevent or even reduce the incidence of atherosclerosis, but further research is still needed (Rianti *et al.*, 2018) (Kamisah *et al.*, 2013). Based on previous evidence, it has been proven that petai seed ethanol extract can increase *High-Density Lipoprotein* (HDL) levels and reduce *Low-Density Lipoprotein* (LDL) levels (Nanda, YAT. 2018). Meanwhile, research on petai ethanol extract on the number of foam cells has never been done before. The selection of ethanol solvents is adjusted to the same level of polarity between the solvent and the dissolved components, where the antioxidant content of petai itself is polarized by 96% ethanol (Puspitasari *et al.*, 2019). In retail, there are many antioxidants, including flavonoids, saponins, and tannins (Kamisah *et al.*, 2013).

METHODS

This study is an actual experimental research with a post-test-only control group design. The sample used male white rats (Rattus norvegicus strain wistar) with the criteria of male rat inclusion, age 2-3 months, body weight 200-300 grams. The number of samples of 25 rats was divided into five (5) groups with one (1) negative control group only given standard feed, one (1) positive control group given standard feed plus a 2ml/day lard atherogenic diet for 14 days, three (3) treatment groups were fed standard feed, 2m/day lard atherogenic diet and added with petai ethanol extract at a dose of 100mg/kgBB/day, 200mg/kgBB/day, and 400mg/kgBB for 14 days. On the 22nd day, rat surgery was carried out to take the rat aortic arcus, and a histopathological preparation was made to observe further the number of foam cells formed. Observation of foam cells is carried out with a 400x magnification microscope with 10 different fields of view, which will later be averaged.

RESULTS AND DISCUSSION

The results of histopathological observation of atherosclerosis foam cells formed are as follows:



(Data primer, 2021)

Figure 1. Foam cells (HE painting, 400x magnification) in rat aortic arcus in various Groups. A. Arcus aorta of rat K-; B. Arcus aorta of rat K+; C. Arcus aorta of rat P1; D. Arcus aorta of rat P2; E. Arcus aorta of rat P3. Descriptively, the number of foam cells in the K+ group was the highest compared to other groups, and the number of foam cells in the P3 group was the lowest.

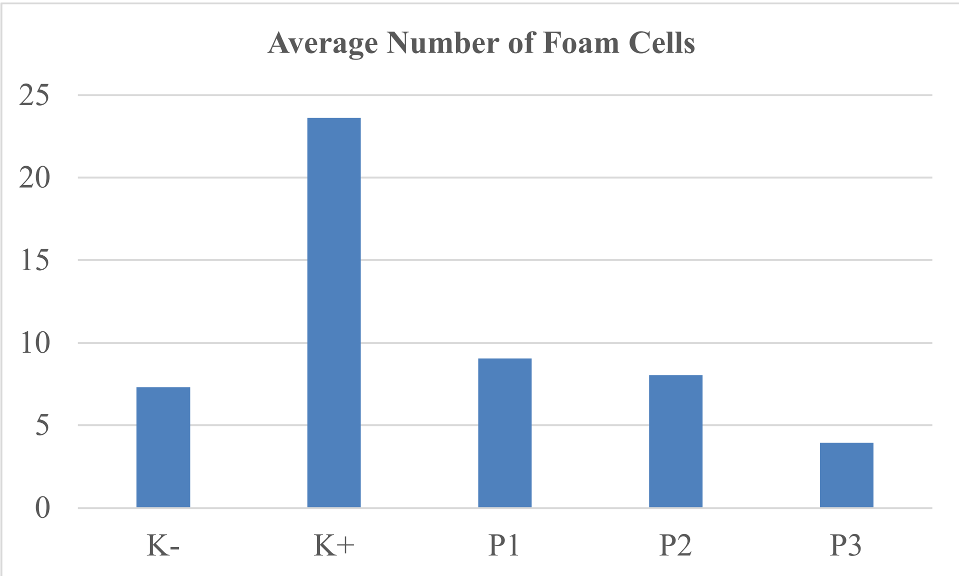
The green arrow indicates the foam cells that are formed.

The results of the calculation of the number of foam cells in the aortic arcus of mice are shown in Table 1 and Figure 2.

Table 1. Average calculation of the number of foam cells

Group	Number of Foam Cells					Average	Standard Deviation
	1	2	3	4	5		
K-	3.3	7.8	3.8	3.6	18	7.3	±6,258594
K+	21.5	27.7	24.5	22.8	21.6	23.62	±2.582053
P1	13.8	4.2	9.3	14.4	3.5	9.04	±5.137412
P2	15.8	15.2	4.1	5.6	1.3	8.4	±6.665958
P3	3.2	3.3	6.2	4.5	2.5	3.94	±1.453616

(Data primer, 2021)



(Data primer, 2021)

Figure 2. Graph of the average number of rat aortic arcus foam cells in each group

Information :

K-: Group that was fed only regular feed for 14 days

K+: Group given 2 ml/day of lard atherogenic induction for 14 days of treatment

P1: The group that was given 2 ml of lard atherogenic induction per day for 14 days of treatment was given 100mg/kgBB of petai ethanol extract

P2: The group that was given 2 ml of lard atherogenic induction per day for 14 days of treatment was given 200mg/kgBB of petai ethanol extract

P3: The group that received 2 ml of lard atherogenic induction per day for 14 days of treatment received a petai ethanol extract of 400 mg/kgBB.

Based on the data obtained in Figure 2, the negative control group is used as a reference to determine the expected value of the average number of foam cells formed, which is 7.3 pieces. In the positive control group data, the average number of foam cells was more than that of the negative control group, which was 23.62 pieces. Meanwhile, in the P1, P2, and P3 groups, the average number of foam cells formed decreased compared to the positive control group. In the P1 group, the average number of foam cells formed was 9.04 pieces; in the P2 group, the average number of foam cells was 8.4 pieces; and in the P3 group, an average of 3.94 foam cells were obtained.

The results of the normality test obtained a value (p) > 0.05, meaning that the data had a normal distribution, so continued in the homogeneity test, the data obtained in this study was not homogeneous because the value (p) = 0.002, and the Oneway ANOVA test was carried out with a value of p = 0.000 (p > 0.05). Namely, there was a difference in the administration of petai eranol extract from the number of foam cells formed. Because the data are not homogeneous, it will be continued with the post hoc Tamhane's test to find out which dose provides a significant difference in the number of foam cells; a significance value was obtained in treatment 1 of 0.008, treatment 2 of 0.027 and treatment 3 of 0.000) (p < 0.05). Furthermore, a linear regression test was carried out to determine how much effect the administration of petai ethanol extract dose; an R *square value* of 0.557 was obtained, meaning that the administration of petai ethanol extract had an influence of 55.7% on the incidence of a decrease in the number of foam cells. In addition, it is also known that the administration of a dose of 1mg/kgBB/day can reduce the formation of foam cells by 0.042 with a dose of 270,952 mg/kgBB/day required to achieve conditions such as negative control.

Based on the observation of the number of foam cells formed, the group of mice given an atherogenic diet in the form of 2 ml/day of lard was proven to trigger the formation of foam cells. This result is in line with research conducted by Maramis, Kaseke, and Tanudjaja (2014). Where foam cells are a sign of atherosclerosis. The positive control group had the highest average number of foam cells formed compared to the other groups.

In this study, the treatment group showed a decrease in the number of foam cells formed after the administration of petai ethanol extract with doses of 100mg/kgBB, 200mg/kgBB, and 400mg/kgBB, with the most effective dose in the third treatment group, which was 400mg/kgBB. The dosage was determined in this study by Nanda (2018), who found that petai ethanol extract can increase HDL levels and reduce LDL levels in rats. The decrease in LDL will undoubtedly affect the number of atherosclerosis foam cells that will be formed. Where when LDL levels decrease or even under normal conditions, the condition of hyperlipidemia will be avoided. When there is no hyperlipidemia condition, the level *of reactive oxygen species* (ROS) that is formed will decrease. ROS is the cause of endothelial injury, which, if this condition occurs, will cause an increase in endothelial permeability so that monocytes and LDL will be able to migrate into the vessel. If this condition arises, the monocytes will not differentiate into macrophages, nor will LDL turn into oxidized LDL, which will later be phagocytes by macrophages, causing the formation of foam cells (Ziegler *et al.*, 2019).

This is because petai itself relies on many antioxidants, such as flavonoids, saponins, and tannins (Kamisah *et al.*, 2013). Flavonoids play a role as inhibitors of the reducing HMG-CoA enzyme, which is an enzyme used for cholesterol biosynthesis. When cholesterol is transported from the intestine to the liver, the inhibited HMG-CoA reductase enzyme will cause the process of converting acetyl-coA to mevalonate to be disrupted so that the cholesterol synthesis products by the liver are reduced (Ekananda, 2015) (Nuralifah *et al.*, 2019). Flavonoids have a mechanism to increase the amount of HDL cholesterol by increasing the release of cholesterol from macrophages and increasing the expression of *ATP-binding cassette* (ABC) A1 as well as increasing apolipoprotein A1, which is the basis for the formation of HDL (Puspasari *et al.*, 2016).

Saponins themselves can inhibit the activity *of pancreatic lipase,* which plays a role in the hydrolysis of fatty acids from all long chains to produce free fatty acids and 2-monoacylglycerol. When in intestinal epithelial cells, these fatty acids and 2-monoacylglycerol will recombine through an enzymatic reaction to form triacylglycerol. So, inhibition of *pancreatic lipase* activity will reduce triacylglycerol levels (Wurdianing *et al.*, 2014). In addition, saponins also inhibit the absorption of cholesterol in the micellar, the reabsorption of bile acids, and the synthesis of cholesterol. The absorption of cholesterol that is inhibited in the intestines will cause unabsorbed cholesterol to be excreted along with feces (Puspasari *et al.*, 2016). Blocking its activities is one of the important strategies developed by the pharmaceutical industry to reduce fat absorption after consumption. When fat absorption decreases, the condition of hyperlipidemia can be avoided (Marrelli *et al.*, 2016)

As for tannin compounds, they work by binding lipids to the digestive tract by binding to mucosal proteins and intestinal epithelial cells, which causes inhibition of intestinal fat absorption. In addition, tannins can precipitate mucosal proteins on the surface of the small intestine so that cholesterol absorption activity will be reduced (Artha *et al.*, 2017). Tannins also work to reduce cholesterol absorption by controlling the hydrolysis activity of lipoproteins and metabolic processes in several tissues (Puspasari *et al.*, 2016).

The regression test value in this study was R squared of 0.557, which means that the administration of petai ethanol extract effectively reduced the number of foam cells formed in the aortic arcus of male white rats by 55.7%. In comparison, 44.3% was another factor that was not studied. Other factors that have not been studied are endogenous factors and exogenous factors.

The results of the ANOVA test that has been carried out obtained a significance value (p) = 0.000, indicating a significant difference in the number of foam cells formed against the administration of petai ethanol extract in the positive control group and the P1, P2, and P3 groups. After continuing with Tamhane's post hoc test to find out which dose gave a significant difference in the number of foam cells formed compared to the positive control group, the results were obtained that all the treatment doses given were able to provide significant results in reducing the number of foam cells formed.

Endogenous factors that can influence this study can be the physical and mental condition of each rat during the survey. Stress can increase the level of total cholesterol, triglycerides, and LDL and lower HDL levels in the blood. In addition, genetic factors from the mice themselves are influential, such as mice have experienced hyperlipidemia conditions from the beginning of the study because they are inherited from their parents, which cannot be known by researchers due to research limitations (Puspasari *et al.*, 2016).

Exogenous factors can be in the form of an inflammatory reaction during sonde treatment. When an inflammatory response occurs, many pro-inflammatory factors come out and induce the formation of free radicals (ROS), and the adaptation process of each mouse is different. In addition, petal extract itself contains other than flavonoids, saponins, and tannins, such as protein, fat, carbohydrates, fiber, calcium, iron, phosphorus, and vitamin C. In addition, because this study is a study with *a post-test-only control design*, the abnormalities that occur in rats before harlequin cannot be known and can cause bias.

CONCLUSION

The study concluded that an atherogenic diet induced with 2 ml/day of lard effectively stimulated the formation of atherosclerosis foam cells in the aortic arcus of male Wistar rats. Among the tested doses, the administration of 400 mg/kgBW/day of petai ethanol extract (Parkia speciosa) was identified as the most effective in reducing the number of foam cells formed. This finding demonstrates the potential efficacy of petai ethanol extract in mitigating atherosclerotic development, providing valuable insights into its therapeutic application for cardiovascular health.

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