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Effectiveness Test of Ethanol Extract of Petai (*Parkia speciosa*) on the Number of Atherosclerotic Foam Cells in Male Wistar Rats (*Rattus norvegicus* strain Wistar) Atherosclerosis Model.

Ikhwan Handirosiyanto[1)], Seftiana Khaerun Nisa[2)], Indra Setiawan[3)], Dian Yuliartha Lestari[4)]
[1,2,3,4)] Fakultas Kedokteran, Universitas Muhammadiyah Malang

*Corresponding Author
Email : indra@umm.ac.id

Abstract

*Atherosclerosis is a chronic inflammatory disease that causes narrowing of the arterial lumen and is a dominant cause of cardiovascular diseases such as myocardial infarction (MI), heart failure, and stroke. Cardiovascular disease itself is the leading cause of death worldwide. Atherosclerosis has a complex pathophysiology, starting from endothelial dysfunction, thickening of the tunica intima, and the formation of atheromatous plaques. During its progression, foam cells are formed, which play a key role in the development of atherosclerosis. The ethanol extract of petai (Parkia speciosa) contains compounds such as flavonoids, saponins, and tannins that function to reduce the levels of Low-Density Lipoprotein (LDL). The purpose of this study was to determine the effectiveness of petai ethanol extract in reducing the number of atherosclerotic foam cells formed in male Wistar rats (*Rattus norvegicus strain Wistar) induced by lard. This study used a true experimental method with a post-test only control group design. A total of 25 rats were divided into five groups: one negative control group, one positive control group, and three treatment groups receiving petai ethanol extract at doses of 100, 200, and 400 mg/kg body weight. The formation of foam cells was observed using a microscope with 400× magnification. Data were analyzed using the One-Way ANOVA test and Post Hoc Tamhane's test. The results of the One-Way ANOVA test showed a significant difference among the groups ($p = 0.000$). The Post Hoc Tamhane's test between the positive control group and treatment groups 1, 2, and 3 indicated significant differences in foam cell formation due to the administration of petai ethanol extract compared to the positive control group ($p < 0.05$). In conclusion, the administration of petai ethanol extract was effective in reducing the number of atherosclerotic foam cells formed.*

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

INTRODUCTION

Atherosclerosis is a chronic inflammatory reaction of the blood vessel wall as a response to dyslipidemia and endothelial distress involving leukocyte inflammation and vascular cell activation (Luigi Riccioni and Enrico Sblendorio, 2012). Atherosclerosis is a dominant cause of cardiovascular diseases including myocardial infarction (MI), heart failure, stroke, and claudication (Jan Frostegård, 2013). Cardiovascular and cerebrovascular diseases are the leading causes of global mortality, making it important to identify all associated risk factors (Yao Yao et al., 2019). In 2016, the World Health Organization (WHO) reported that approximately 17.8 million (31.4%) deaths worldwide were caused by cardiovascular diseases (WHO, 2016). In Indonesia, the prevalence of heart disease is around 1.5%, including in East Java Province (Ministry of Health of the Republic of Indonesia, 2018).

Atherosclerosis has a complex pathophysiology, beginning with endothelial dysfunction, thickening of the tunica intima, and the formation of atheromatous plaques (Rohit Gupta, Asif Ali and Damanjit S. Sanghera, 2019). The main lesion in atherosclerosis is characterized by lipid deposition in arterial smooth muscle cells and the proliferation of fibrous matrix that develops into atherosclerotic plaques (Zhu Zhu et al., 2018). Based on the oxidative stress theory, atherosclerosis results from oxidative modification of Low-Density Lipoprotein (LDL) in the arterial wall by reactive oxygen species (ROS) (Kurniasari, 2018). Risk factors for atherosclerosis include high cholesterol and LDL levels, low High-Density Lipoprotein (HDL) levels, hypertension, smoking, diabetes mellitus, obesity, inflammation, unhealthy lifestyle, and aging (Mohammad Kopaei et al., 2014).

Petai (*Parkia speciosa*) is a food ingredient commonly consumed in Indonesia, but it is still rarely used for medicinal purposes, even though it has many potential benefits. Its extract has been reported to prevent and even reduce the occurrence of atherosclerosis, although further research is still required (Rianti et al., 2018; Kamisah et al., 2013). Previous studies have shown that the ethanol extract of petai seeds can increase High-Density Lipoprotein (HDL) levels and decrease Low-Density Lipoprotein (LDL) levels (Nanda, YAT., 2018). However, studies investigating the effect of petai ethanol extract on the number of foam cells have not been conducted previously.

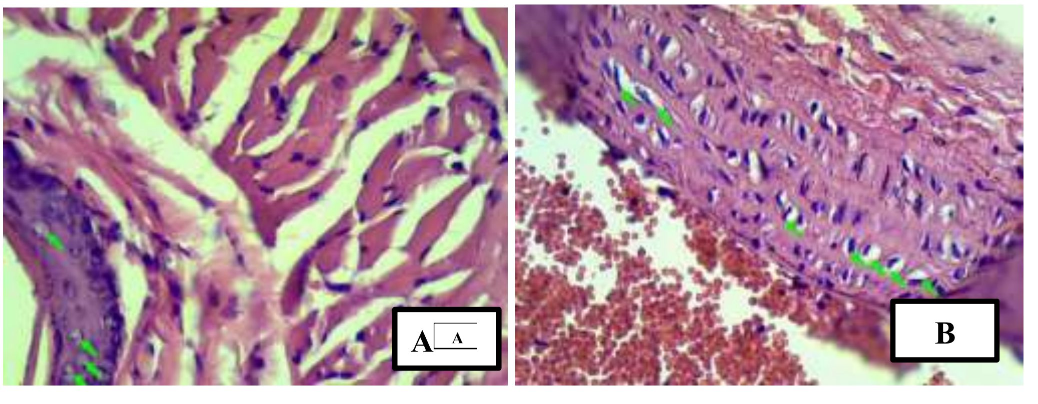
The selection of ethanol as the solvent was adjusted to the similarity in polarity between the solvent and the dissolved components, as the antioxidant compounds in petai have polarity compatible with 96% ethanol (Puspitasari et al., 2019). Petai contains various antioxidant compounds, including flavonoids, saponins, and tannins (Kamisah et al., 2013).

RESEARCH METHODS

This study was a true experimental study with a post-test only control group design. The experimental animals used were male white rats (Rattus norvegicus strain Wistar) with inclusion criteria of male rats, aged 2-3 months, and weighing 200-300 grams. A sample of 25 rats was divided into five (5) groups: one (1) negative control group fed only standard feed, one (1) positive control group fed standard feed supplemented with an atherogenic diet of lard (2 ml/day) for 14 days, and three (3) treatment groups fed standard feed, an atherogenic diet of lard (2 ml/day) and supplemented with petai ethanol extract at doses of 100 mg/kgBW/day, 200 mg/kgBW/day, and 400 mg/kgBW for 14 days. On the 22nd day, surgery was performed on the rats to remove the aortic arch and histopathological slides were prepared to observe the number of foam cells formed. Observations of foam cells were carried out using a microscope at 400x magnification with 10 different fields of view which would later be averaged.

RESULTS AND DISCUSSION

The results of histopathological observations of the atherosclerotic foam cells formed are as follows:



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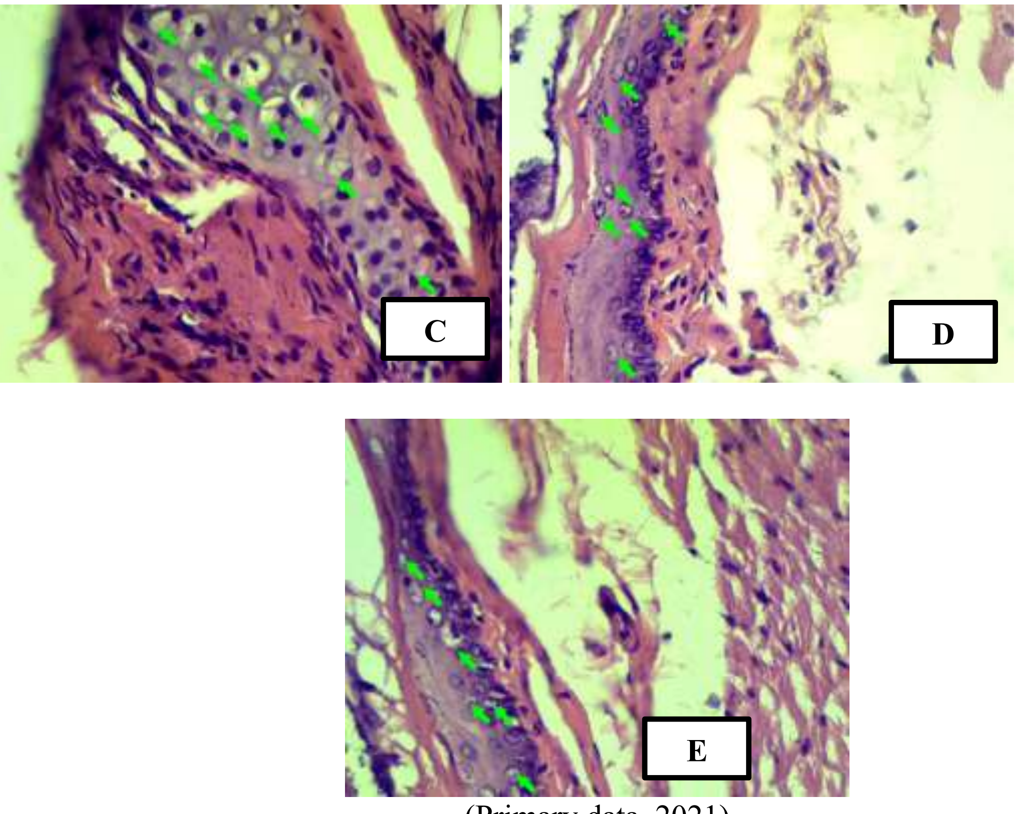


(Primary data, 2021)

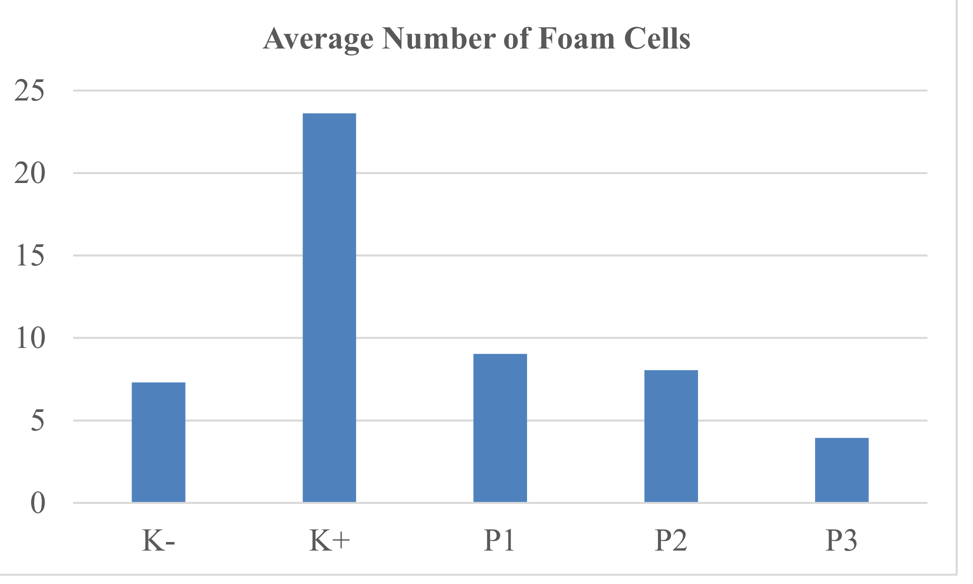
Figure 1. Foam cells (HE staining, 400x magnification) in the aortic arch of rats in various groups. A. Aortic arch of K- rats; B. Aortic arch of K+ rats; C. Aortic arch of P1 rats; D. Aortic arch of P2 rats; E. Aortic arch of P3 rats. Descriptively, the number of foam cells in the K+ group was the highest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

The green arrow indicates the foam cells formed. The results of the foam cell count in the aortic arch of rats are shown in Table 1 and Figure 2.

Table 1. Average calculation of the number of foam cells

Group	Number of Foam Cells (*Foam Cell*)					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

(Primary data, 2021)



(Primary data, 2021)

Figure 2. Graph of the Average Number of Foam Cells in the Aortic Arch of Rats in Each Group

Description:
K−: Group that received only a normal diet for 14 days.
K+:Group induced with an atherogenic diet of lard 2 ml/day for 14 days.
P1 : Group induced with an atherogenic diet of lard 2 ml/day for 14 days and given petai ethanol extract at a dose of 100 mg/kg body weight.
P2 : Group induced with an atherogenic diet of lard 2 ml/day for 14 days and given petai ethanol extract at a dose of 200 mg/kg body weight.
P3 : Group induced with an atherogenic diet of lard 2 ml/day for 14 days and given petai ethanol extract at a dose of 400 mg/kg body weight.

Based on the data shown in Figure 2, the negative control group was used as a reference to determine the normal value of the average number of foam cells formed, which was 7.3 cells. In the positive control group, the average number of foam cells was higher than in the negative control group, reaching 23.62 cells. Meanwhile, in groups P1, P2, and P3, the average number of foam cells decreased compared with the positive control group. In group P1, the average number of foam cells formed was 9.04 cells; in group P2 it was 8.4 cells; and in group P3 the average number of foam cells formed was 3.94 cells.

The normality test results showed a value of $p > 0.05$, indicating that the data were normally distributed. The analysis was therefore continued with a homogeneity test, which showed that the data were not homogeneous because the value obtained was $p = 0.002$. A One-Way ANOVA test was then performed, yielding a value of $p = 0.000$, indicating a significant difference in the number of foam cells formed due to the administration of petai ethanol extract. Because the data were not homogeneous, the analysis was continued with the Post Hoc Tamhane's test to determine which dose produced a significant difference in the number of foam cells. The significance values obtained were 0.008 for treatment 1, 0.027 for treatment 2, and 0.000 for treatment 3 ($p < 0.05$).

Furthermore, a linear regression test was conducted to determine the magnitude of the effect of petai ethanol extract dosage. The R-square value obtained was 0.557, meaning that the administration of petai ethanol extract contributed 55.7% to the reduction in the number of foam cells formed. In addition, it was found that administration of a dose of 1 mg/kg body weight/day could reduce foam cell formation by 0.042, while a dose of 270.952 mg/kg body weight/day was required to achieve a condition similar to that of the negative control group.

Based on observations of the number of foam cells formed, the group of rats fed an atherogenic diet consisting of 2 ml/day of lard was proven to trigger foam cell formation. These results are consistent with the research conducted by Maramis, Kaseke, and Tanudjaja (2014), in which foam cells were identified as a marker of atherosclerosis. The positive control group had the highest average number of foam cells compared with the other groups.

In this study, the treatment groups showed a decrease in the number of foam cells formed after administration of petai ethanol extract (*Parkia speciosa*) at doses of 100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight, with the most effective dose observed in treatment group three at 400 mg/kg body weight. The dose determination in this study was based on research by Nanda (2018), which showed that petai ethanol extract could increase High-Density Lipoprotein (HDL) levels and decrease Low-Density Lipoprotein (LDL) levels in rats. A reduction in LDL levels will certainly affect the number of atherosclerotic foam cells formed. When LDL levels decrease or remain within normal conditions, hyperlipidemia can be avoided. Without hyperlipidemia, the levels of reactive oxygen species (ROS) produced will also decrease. ROS are responsible for endothelial injury, which increases endothelial permeability, allowing monocytes and LDL to migrate into the vascular wall. When this condition is prevented, monocytes will not differentiate into macrophages, and LDL will not undergo oxidation to form oxidized LDL, which would otherwise be phagocytosed by macrophages and lead to foam cell formation.

This effect is attributed to the high antioxidant content in petai, including flavonoids, saponins, and tannins. Flavonoids act as inhibitors of the HMG-CoA reductase enzyme, which plays a role in cholesterol biosynthesis. When cholesterol is transported from the intestine to the liver, inhibition of the HMG-CoA reductase enzyme disrupts the conversion of acetyl-CoA into mevalonate, thereby reducing the synthesis of cholesterol by the liver. Flavonoids also increase HDL cholesterol levels by promoting cholesterol efflux from macrophages and enhancing the expression of ATP-binding cassette (ABC) A1 as well as apolipoprotein A1, which are essential components in HDL formation.

Saponins are able to inhibit pancreatic lipase activity, an enzyme responsible for the hydrolysis of long-chain fatty acids into free fatty acids and 2-monoacylglycerol. In intestinal epithelial cells, these compounds normally recombine through enzymatic reactions to form triacylglycerol. Therefore, inhibition of pancreatic lipase activity can reduce triacylglycerol levels. In addition, saponins inhibit cholesterol absorption in micelles, the reabsorption of bile acids, and cholesterol synthesis. When cholesterol absorption in the intestine is inhibited, unabsorbed cholesterol will be excreted with feces. Inhibition of lipid absorption is an important strategy developed by the pharmaceutical industry to reduce fat absorption after consumption. When fat absorption decreases, the condition of hyperlipidemia can be avoided.

Tannins, on the other hand, function by binding lipids in the digestive tract through interaction with mucosal proteins and intestinal epithelial cells, thereby inhibiting intestinal fat absorption. Tannins can also precipitate mucosal proteins on the surface of the small intestine, reducing cholesterol absorption activity. Furthermore, tannins help reduce cholesterol absorption by regulating lipoprotein hydrolysis and metabolic processes in several tissues.

The regression test results in this study showed an R-square value of 0.557, indicating that the administration of petai ethanol extract had an effectiveness of 55.7% in reducing the number of foam cells formed in the aortic arch of male white rats, while the remaining 44.3% was influenced by other factors not examined in this study. These factors may include endogenous and exogenous influences.

The ANOVA test results showed a significance value of $p = 0.000$, indicating a significant difference in the number of foam cells formed between the positive control group and treatment groups P1, P2, and P3 after administration of petai ethanol extract. The subsequent Post Hoc Tamhane's test demonstrated that all treatment doses produced significant reductions in foam cell formation compared with the positive control group.

Endogenous factors that may have influenced this study include the physical and mental condition of the rats during the experiment. Stress can increase total cholesterol, triglyceride, and LDL levels while decreasing HDL levels in the blood. Genetic factors may also influence the results, for example if some rats had already experienced hyperlipidemia prior to the experiment due to inheritance from their parents, which could not be detected by the researchers due to study limitations.

Exogenous factors may include inflammatory reactions during the gavage procedure. When inflammation occurs, various pro-inflammatory factors are released, inducing the formation of reactive oxygen species (ROS). Additionally, each rat may adapt differently to the experimental conditions. Petai extract itself also contains compounds other than flavonoids, saponins, and tannins, such as protein, fat, carbohydrates, fiber, calcium, iron, phosphorus, and vitamin C. Moreover, because this study used a post-test only control group design, any abnormalities present in the rats before treatment could not be identified, which may have introduced bias.

CONCLUSION

The conclusions that can be drawn based on the results and discussion of this study are:

1. Administering an atherogenic diet induction using 2 ml of lard/day was proven to induce the formation of atherosclerotic foam cells in the aortic arch of male white rats throughout the study.
2. The most effective dose capable of reducing the number of foam cells formed during the study was the dose in treatment group 3, at 400 mg/kgBW/day.
3. Administration of petai (Parkia speciosa) ethanol extract was proven to be effective in reducing the number of atherosclerotic foam cells formed in the aortic arch of male white rats throughout the study.

REFERENCES

Artha, C., Mustika, A. and Sulistyawati, S. W. 2017. Pengaruh Ekstrak Daun Singawalang terhadap Kadar LDL Tikus Putih Jantan Hiperkolesterolemia Singawalang Leaf Extract Effects on LDL Levels of Hypercholesterolemic Male Rats', 5(2), pp. 105–109.

Ekananda, N. A. 2015. [Artikel Review] Bay Leaf in Dyslipidemia Therapy', *Dyslipidemia Therapy J MAJORITY* |, 4, p. 64.

Frostegård, J. 2013. Peripheral Neuropathy and HIV - VA National HIV/AIDS Website', pp. 1–8. Available at: http://www.biomedcentral.com/1741-7015/11/117%0Apapers3://publication/uuid/D0C88CBA-A315-4DF2-8BF5 CD34F24146B2.

Gupta, K. K., Ali, S. and Sanghera, R. S. 2019. Pharmacological Options in Atherosclerosis: A Review of the Existing Evidence. *Cardiology and Therapy*, 8(1), pp. 5–20. doi: 10.1007/s40119-018-0123-0.

Kamisah, Y., Othman, F., Qodriyah, M. S. et al. 2013. Parkia speciosa Hassk.: Apotential phytomedicine. Evidence-based Complementary and Alternative Medicine, 2013. doi: 10.1155/2013/709028.

Kementerian Kesehatan RI. 2018. Laporan Riskesdas 2018. *Laporan Nasional RIskesdas 2018*, 53(9), pp. 181–222. Available at: http://www.yankes.kemkes.go.id/assets/downloads/PMK No. 57 Tahun 2013 tentang PTRM.pdf.

Kopaei, M.R. Setorki, M. Doudi, M. *et al*., 2014. Atherosclerosis : Process , Indicators , Risk Factors and New Hopes. , 5(8), pp.927–946.

Kurniasari, F. N. 2018. Indonesian Journal of Human Nutrition', *Indonesian Journal of Human Nutrition*, 1(1), pp. 14–22. doi: 10.21776/ub.ijhn.2016.003.Suplemen.5.

Marrelli, M., Conforti, F., Araniti, F., et al. 2016. Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity, Molecules, 21(10). doi: 10.3390/molecules21101404.

Nanda, YAT. 2018. Pengaruh Ekstrak Etanol Biji Petai (Parkia speciosa hassk.) Terhadap Kadar High Density Lipoprotein dan Low Density Lipoprotein Pada Tikus Wistar Jantan Yang Diinduksi Pakan Tinggi Lemak. Dspace UII

Nuralifah, N. Wahyuni, W. Parawansah, P. *et al*. 2019. Uji Aktivitas Antihiperlipidemia Ekstrak Etanol Daun Notika (Arcboldiodendron calosericeum Kobuski) Terhadap Kadar Kolesterol Total Tikus (Rattus norvegicus) Jantan Galur Wistar. *Journal Syifa Sciences and Clinical Research*, 2(1), pp. 1–10. doi: 10.37311/jsscr.v2i1.2704.

Puspasari, A. F., Agustini, S. M. and Illahika, A. P. 2016. Pengaruh Ekstrak Daun Kersen (Muntingia Calabra L.) Terhadap Profil Lipid Mencit Putih (Mus Musculus) Jantan Yang Diinduksi Minyak Jelantah. *Saintika Medika*, 12(1), p. 49. doi: 10.22219/sm.v12i1.5260.

Puspitasari, A. D., Anwar, F. F. and Faizah, N. G. A. 2019. AKTIVITAS ANTIOKSIDAN, PENETAPAN KADAR FENOLIK TOTAL DAN FLAVONOID TOTAL EKSTRAK ETANOL, ETIL ASETAT, DAN n-HEKSAN DAUN PETAI (Parkia speciosa Hassk.). *Jurnal Ilmiah Teknosains*, 5(1), p. 1. doi: 10.26877/jitek.v5i1.3490.

Rianti, A. Parassih, E. K., Novenia, A. E. et al. 2018. Potential of Petai (Parkia speciosa) as An Antioxidant Source. Jurnal Dunia Gizi, 1(1), pp. 10–19

Riccioni, G. and Sblendorio, V. 2012. Atherosclerosis: From biology to pharmacological treatment. *Journal of Geriatric Cardiology*, 9(3), pp. 305–317. doi: 10.3724/SP.J.1263.2012.02132.

World Health Organization. 2016. GHE2016_Deaths_Global_2000_2016. *2018*. Available at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/.

Wurdianing, I., Nugraheni, S. and Rahfiludin, Z. 2014. Efek ekstrak daun sirsak (Annona muricata Linn) terhadap profil lipid tikus putih jantan (Rattus Norvegicus). *Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)*, 3(1), pp. 7–12. doi: 10.14710/jgi.3.1.96-101.

Yao, BC. Meng, L. Hao, M. *et al*. 2019. Chronic stress: a critical risk factor for atherosclerosis. Journal of International Medical Research, 47(4), pp. 1429–1440. doi: 10.1177/0300060519826820.

Zhu, Y. Xian, X. Wang, Z. *et al*. 2018. Research progress on the relationship between atherosclerosis and inflammation. *Biomolecules*, 8(3), pp. 1–11. doi: 10.3390/biom8030080.

Ziegler, T., Rahman, F. A., Jurisch, V. et al. 2019. Atherosclerosis and the Capillary Network; Pathophysiology and Potential Therapeutic Strategies. Cells, 9(1), pp. 1–13. doi: 10.3390/cells9010050.