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ARTIKEL PENELITIAN

Effectiveness Test of Petai Seed Methanol Extract Against Cholesterol of Wistar Strain Rats Induced by High Fat Feed

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Abstrak

Tujuan: Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak metanol biji petai terhadap kadar kolesterol tikus galur wistar yang diinduksi diet tinggi lemak. **Metode:** Penelitian true eksperimental dengan desain post test only control group design dengan menggunakan 25 ekor tikus yang dibagi menjadi 5 kelompok yaitu kelompok kontrol (negatif dan positif) dan kelompok perlakuan (perlakuan 1, 2 dan 3). Diet tinggi lemak selama 30 hari diberikan pada kelompok K+, P1, P2, dan P3. Kemudian dilanjutkan dengan pemberian ekstrak metanol biji petai pada kelompok perlakuan dengan dosis masing-masing 100 mg/kgBB, 200 mg/kgBB, dan 400 mg/kgBB selama 10 hari. Kadar kolesterol rata-rata diperiksa menggunakan metode CHOD-PAP dan data dianalisis menggunakan uji nonparametrik Kruskal-Wallis dan Post-Hoc Mann-Whitney. **Hasil:** Hasil penelitian menunjukkan pemberian ekstrak metanol biji petai dosis 100 mg/kgBB, 200 mg/kgBB, dan 400 mg/kgBB terdapat perbedaan bermakna dengan kelompok kontrol positif ($p<0,05$). **Kesimpulan:** Penelitian ini menunjukkan ekstrak metanol biji petai efektif menurunkan kadar kolesterol pada tikus galur wistar yang diinduksi diet tinggi lemak.
Kata kunci: fitokimia; ekstrak metanol; biji petai; kolesterol

Abstract

Objective: This study aims to determine the effectiveness of the methanol extract of petai seeds on the cholesterol levels of Wistar strain rats induced by a high-fat diet. **Methods:** True experimental research with post test only control group design using 25 rats which were divided into 5 groups, namely the control group (negative and positive) and the treatment group (treatments 1, 2 and 3). High-fat diet for 30 days was given to groups K+, P1, P2, and P3. Then continued with the administration of methanol extract of petai seeds in the treatment group with each dose of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW for 10 days. The mean cholesterol levels were checked using the CHOD-PAP method and data were analyzed using the Kruskal-Wallis and Post-Hoc Mann-Whitney nonparametric tests. **Results:** The results showed that the administration of petai seed methanol extract at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW had a significant difference with the positive control group ($p<0.05$). **Conclusion :** This study showed the methanol extract of petai seeds had effectiveness in lowering cholesterol levels in wistar strain rats induced by high fat diet.
Keywords**:** *phytochemical; methanol extract; petai seeds; cholesterol*

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INTRODUCTION

Cholesterol is a fat-like substance because it has similar physical and chemical properties and is a sterol compound that does not contain fatty acids.[1] This substance is synthesized naturally in the body. Besides being synthesized in the body, cholesterol is also supplied in animal products such as meat, chicken, eggs and dairy products. This substance is needed in the body to protect nerves, make cell membranes and produce certain hormones.[2] Steroid hormones such as estrogen, progesterone, testosterone and vitamin D are formed by cholesterol as its precursor.[3]

Cholesterol in normal amounts is beneficial for the body, but if the amount is excessive it will be bad for the body. If the intake of cholesterol is excessive, there will be an increase in cholesterol in the blood called hypercholesterolemia. A person who is declared hypercholesterolemic if he has a total cholesterol level in the blood of more than 240 mg/dL.[4] This condition if it continues continuously will cause atherosclerosis, namely the formation of plaque on the walls of blood vessels which will progressively narrow the lumen of the blood vessels.[5] Atherosclerosis will cause cardiovascular disease (heart disease and stroke), stroke occurs when the plaque builds up in the blood vessels of the brain, and coronary heart disease when the plaque builds up in the coronary arteries and clogs that area.[6]

Cases of cardiovascular disease show a high rate. WHO states that this disease is the number 1 disease causing death globally compared to other diseases, namely 17.9 million people died in 2019, with a percentage of 32% of deaths worldwide. 85% of these deaths are caused by heart attacks and strokes.[7] In low- and middle-income countries, deaths from cardiovascular disease are more than three-quarters.[7] The prevalence of stroke according to WHO 15 million people suffer from stroke every year worldwide with the number dying 5 million people and 5 million people with disabilities.[8] In Indonesia alone, people with coronary heart disease who had been diagnosed by a doctor in 2013 were 0.5% or 883,447 people while those diagnosed with symptoms were 1.5% or 2,650,340 people.[9] In the province of West Sumatra, 0.6% or 20,587 people diagnosed with coronary heart disease, while 1.2% or 41,113 people diagnosed with coronary heart disease.[9]

Based on the epidemiological data above, the best efforts are needed to prevent atherosclerosis associated with risk factors for hypercholesterolemia. The management of hypercholesterolemia is divided into 2, namely pharmacological (drugs) and non-pharmacological.[10] For pharmacological treatment, the drugs most often used to lower cholesterol are statin drugs, these drugs work by inhibiting the action of the HMG-CoA enzyme. reductase which reduces the formation of cholesterol in the liver.[10,11] This drug has side effects of myopathy and an increase in liver enzymes. People with acute and chronic liver disease are not allowed to take this drug because it is an absolute contraindication to statins.[4]

Non-pharmacological therapy acts as supportive therapy, such as maintaining an ideal body weight, doing regular physical activity for at least 30 minutes every day, not smoking, not consuming alcohol, maintaining a low-fat and high-fiber diet, such as eating fruits and vegetables so that cholesterol levels in the blood remains stable, and can also use herbal

medicines.[12,13] The use of these herbal medicines has increased rapidly in the last three decades about 80% of people around the world use them as part of health care.[14]

Petai with the scientific name Parkia speciosa Hassk. is one of the herbal plants that has long been used. This plant is widely found in tropical areas such as Malaysia, Indonesia, Thailand and the Philippines.[15,16] Petai seeds are traditionally used to treat inflammation, liver failure, kidney disease, edema, hypertension, headaches, diabetes and intestinal worms.[17,18] Petai has polyphenolic compounds, phytosterols and flavonoids which are antioxidants that are good for the body.[17] Petai is also a typical food that is often consumed. Petai processing is also carried out in various ways such as fried, boiled or sugared. In 2019, petai production in Indonesia was quite large, reaching 310,103 tons.[19]

In Desy Purnamawati's research (2018) on the test of ethanol extract of petai seeds on cholesterol levels at a dose of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, the best results were obtained at a dose of 100 mg/kgBW.[20] one method is the extraction method. Extraction is the process of separating materials from their mixture using a suitable solvent. Obtaining levels of active substances from the extracted plant can be influenced by the solvent used. Solvents are polar and soluble in water, such as methanol, ethanol, and acetone. In this study, we will use methanol as a solvent to compare the ethanol solvents that have been studied previously. In addition, according to previous research, petai seed methanol extract has a higher total phenolic content than ethanol extract.[18] Methanol is a compound with the chemical formula CH3OH and is the simplest form of alcohol.[21,22] Apart from differences in the type of solvent used , the difference in the sampling area of the plant also affects the phytochemistry of the plant, this is related to environmental conditions (light intensity, temperature, rainfall) and agricultural practices (fertilizer, irrigation, harvesting, post-harvest).[23] Based on the description above, the researcher is interested in to examine the effectiveness of methanol extract of petai seeds grown in West Sumatra on total cholesterol levels in Wistar strain rats induced by high-fat diet.

METHODS

The type of research is true experimental research. The design used is the Posttest Only Control Group design. The population used in this study was male wistar rats (Rattus novergicus L.). Samples that met the inclusion criteria were male Wistar rats, 2-3 months old, weighing 200-250 grams, without any anatomical defects and actively moving. For the criteria for the execution of wistar strain rats who appeared sick (behaved abnormally, lost weight drastically, had disease or physical injury) or died during the study. The sampling technique is simple random sampling. The determination of the sample size needed in this study was based on general guidelines for methodologies on research and evaluation of traditional medicine, namely 5 (five) wistar rats for each treatment group..[24] To prevent drop outs during the study because the rats died or were sick, the sample size was corrected using the formula.[25] $n' = \frac{n}{1-f}$ n' : sample size after correction n : number of samples based on previous estimates f : prediction of sample drop out percentage It is estimated that the subject dropped out or did not comply with the protocol by 10%

(f = 0.1) so that it was obtained: $n' = \frac{n}{1-f} = \frac{5}{1-0,1} = 5,556\ ekor \sim 6\ ekor$ So, in this study, the sample size for each treatment was at least six wistar rats, so the total number of wistar rats needed was 30 wistar rats. Each group was given a different treatment with the following details: The control group was negative (K-), with samples of wistar strain rats being given standard feed ad libitum Positive control group (K+), with samples of wistar strain rats given standard feed and high fat diet (MDTL) Treatment group 1 (P1), with samples of wistar strain rats given a standard diet, given MDTL, and given petai seed extract 100 mg/kgBW Treatment group 2 (P2), with a sample of wistar strain rats given a standard diet, given MDTL, and given petai seed extract 200 mg/kgBW Treatment group 3 (P3), with samples of wistar strain rats given a standard diet, given MDTL, and given petai seed extract 400 mg/kgBW The data obtained were analyzed statistically using a computer program that has a 95% confidence interval and a significance of 0.05 (p=0.05). The data was tested for normality by Shapiro Wilk. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out, if a significant difference is obtained, then it is continued with the Post Hoc statistical test (Tukey HSD). If the results of the data distribution are not normal and not homogeneous (or one of them), then a non-parametric Kruskal Wallis statistical test is carried out in order to find out there is a difference, and if from the results of the statistical test there is a significant difference, then proceed with the Mann-Whitney statistical test. The goal is to see if there are differences between treatment groups. The administration of petai seed methanol extract was carried out for 10 days after the rats were induced by high-fat diet for 30 days. High-fat diet food is made with a mixture of 1.5 kg of beef fat, 3 kg of standard feed, and 0.5 egg yolks. Cholesterol levels of rats were measured using the CHOD-PAP enzymatic calorimetric test method. This research has passed the ethical review with the ethical review permit number 565/UN.16.2/KEP-FK/2022 issued by the research ethics commission of the Faculty of Medicine, Andalas University.

RESULTS AND DISCUSSION

1. Research Data

Research on the effectiveness of methanol extract of petai seeds on cholesterol levels of Wistar rats induced by high-fat diet was conducted at the Biochemistry Laboratory, Faculty of Medicine, Andalas University and Pharmacology Laboratory, Faculty of Pharmacy, Andalas University. This research was conducted for 47 days.

The results of weighing rats were carried out 3 times, namely after acclimatization for 7 days, after administration of MDTL for 30 days, and after administration of petai seed methanol extract for 10 days. The results are obtained as in table 1.

Table 1. Average Rat Body Weight

Group	n	BW average 1 (g)	BW average 2 (g)	BW average 3 (g)
K-	5	226,8	240,2	243,4
K+	5	237,0	307,2	309,0
P1	5	224,2	313,2	309,6
P2	5	223,8	317,2	315,0

P3	5	216,4	316,2	315,0
Average		225,6	298,8	298,4

Table 1 shows the average body weight of wistar rats. The first stage in the acclimatization stage the average of the five groups was 225.6 grams. The second stage after being given a high-fat diet the average was 298.8 grams. The third stage after being given the petai seed extract the average was 298.4 grams. There was an increase in body weight in each experimental group after being given a high-fat diet. The most weight gain of rats after being given a high-fat diet was in treatment group 2 with an average of 317.2 grams. Overall, after administration of petai seed extract, the body weight of the mice decreased slightly.

Measurement of serum cholesterol levels of wistar rats was carried out once in the Biochemistry Laboratory after all treatments were completed. From the data obtained, the average cholesterol levels between groups in this study were as follows:

Tabel 2. Average Cholesterol Levels in Rats

Group	Average cholesterol (mg/dL) ± SD	Decrease (%)
K-	60,76 ± 6,65	-
K+	74,26 ± 9,16	-
P1	62,54 ± 3,16	15,78
P2	59,10 ± 2,62	20,41
P3	56,98 ± 2,92	23,26

Table 2 The highest mean cholesterol levels of rats were in the positive control group who only ate a high-fat diet, which was 74.26 mg/dl. Treatment group 3 which was given petai seed extract at a dose of 400 mg/kgBW had the lowest mean cholesterol compared to other groups, which was 56.98 mg/dl. In the treatment group that was given a high-fat diet and given petai seed extract with doses of 100 mg/kgBW, 200 mg/kgBW and 400 mg/kgBW, the average cholesterol levels were 62.54 mg/dl, 59.10 mg/dl. dl, and 56.98 mg/dl. When compared with the positive control, the largest decrease was in the treatment group 3, which was 23.26%.

2. Analisis Data Penelitian Bivariat

The results of rat cholesterol levels in the research group will be analyzed statistically. The data was tested for normality using the Shapiro-Wilk test, it was found that the data were normally distributed ($p>0.05$), then homogeneity test was carried out using the Levene Statistic test, the data obtained were not homogeneous ($p<0.05$). So it was continued to use the Kruskal-Wallis nonparametric test to assess the significance of cholesterol levels in each study group. The results of the analysis showed that the value of $p = 0.018$ means that there is a significant difference ($p <0.05$) in cholesterol levels in each research group. Then to find out the difference in significance in the study group, the Mann-Whitney test was carried out. The Mann-Whitney analysis is obtained in the following table.

Table 3. Mann-Whitney analysis results

Group	K-	K+	P1	P2	P3
K-	-	0,047	0,754	0,754	0,465
K+	0,047	-	0,047	0,016	0,009
P1	0,754	0,047	-	0,076	0,047
P2	0,754	0,016	0,076	-	0,175
P3	0,465	0,009	0,047	0,175	-

Based on table 3 shows negative control has a significant difference with positive control with p = 0.047, positive control has a significant difference with treatment group 1 (p = 0.047), treatment group 2 (p = 0.016), and treatment group 3 (p = 0.009) , and treatment group 1 had a significant difference with treatment group 3 with p = 0.047.

Effects of MDTL and Petai Seed Methanol Extract on Rat Body Weight

The group of mice that consumed MDTL experienced more weight gain than the group of mice that consumed the standard diet. This is related to the consumption of a high-fat diet which will result in an increase in the amount of fat embedded in adipose tissue in the abdominal cavity and under the skin which will have an impact on weight gain.[26] This is supported by research by Choirun Nissa, et al. which was given MDTL was significantly higher than the mice fed the standard diet.[27] This is also in accordance with the research of Iva et al. that a high-fat diet can increase the body weight of the mice.[26]

The group of rats that were given petai seed extract experienced a slight decrease in treatment groups 1, 2 and 3. In accordance with Luthfiana's 2018 research which stated that the results of processing body weight data at doses of 100 m/kgBW, 200 mg/kgBW and 400 mg/kgBW did not show a significant difference with the smallest increase occurred at a dose of 100 mg/kgBW.[28]

Effects of MDTL on Cholesterol Levels in Rats

Mice were said to be hypercholesterolemic if their cholesterol levels were >54 mg/dL.[29] The negative control group had hypercholesterolemia with a mean of 60.76 mg/dL. The increase in cholesterol in the negative control group was probably caused by stress and also the presence of fat content in the standard feed (BRII feed contains 5% fat). Stress in rats can be caused by actions taken during the study, namely weighing, cleaning cages, and individual cages. Stress will increase free fatty acid levels thereby increasing the secretion of VLDL by the liver involving cholesterol and triacylglycerol into the circulation.30 This is related to research by Crisdina S who found an increase in cholesterol in negative controls..[31]

The rats fed a high-fat diet (MDTL) ad libitum had higher total cholesterol levels than the other groups, namely 74.26 mg/dL. The high-fat diet (MDTL) induced in mice was made from a mixture of 3 kg of standard feed, 1.5 kg of beef fat and 0.5 kg of egg yolk. This increase in cholesterol levels is because beef fat contains 130 mg/10 g of cholesterol and egg yolks contain 2000 mg/10 g of cholesterol.32 Cholesterol in the body can come from food intake or from cholesterol synthesis by the body, resulting in high cholesterol levels. Consumption of cholesterol can increase cholesterol levels in the blood. Then, saturated fatty acids will increase

cholesterol levels by decreasing the synthesis and activity of LDL receptors.[30]

This is in accordance with Reni Haryeni's study which induced rats with a high-fat diet using a mixture of beef fat, cooking oil, egg yolk and standard feed for 30 days. high-fat diet and standard diet for 4 weeks using lard and duck egg yolk caused the rats to experience hypercholesterolemia. It also causes an increase in LDL, triacylglycerol and a decrease in HDL.[34] Another study by Sara et al showed an increase in cholesterol levels after being given chicken egg yolk, sucrose, beef fat and standard feed..[35]

Effects of Petai Seed Methanol Extract on Cholesterol Levels in Rats

Extraction is the process of separating materials from the mixture by using a suitable solvent. Obtaining levels of active substances from the extracted plant can be influenced by the solvent used. Solvents that are polar and soluble in water, such as methanol, ethanol, and acetone. In a previous study, it was found that the methanol extract of petai seeds had a higher total phenolic content than the ethanolic extract of petai seeds.[18]

Petai seed methanol extract was administered for 10 days to mice given MDTL. The doses given to each treatment were 100 mg/kgBW, 200 mg/kgBW and 400 mg/kgBW. The decrease in cholesterol levels in each treatment group when compared to the positive control was 15.78%, 20.41%, and 23.26%. This shows that the larger the dose given, the lower the cholesterol levels in the treatment group. The results of statistical analysis got a significant mean decrease ($p<0.05$) in group P1, group P2 and group P3.

The occurrence of a decrease in cholesterol levels in this study because the petai seed extract contains phytochemicals that can lower cholesterol, namely, flavonoids, tannins, phytosterols, alkaloids and saponins. Flavonoids work by inhibiting the activity of HMG-CoA reductase so that cholesterol synthesis in the liver decreases, inhibits cholesterol absorption so that less is absorbed in the blood vessels and increases LDL receptors so that cholesterol in LDL binds to LDL receptors. absorption of fat because tannins react with cell proteins and intestinal epithelial cell mucosa.[38] Phytosterols have a structure similar to cholesterol which is different from the side of the skeleton. Therefore, when consumed by the body, phytosterols and cholesterol will compete for absorption so that cholesterol levels decrease. [39] Alkaloids can inhibit lipase enzyme activity so that fat cannot be broken down which results in increased fat secretion through feces, so that the inhibition of fat absorption by the liver causes it to be irreversible. Saponins as hypocholesterolemic agents work by inhibiting the absorption of cholesterol in the intestine by forming insoluble complex bonds with cholesterol, saponins also form bonds with bile acids to form micelles, and increase cholesterol binding by fiber.[38]

The results of this study are in accordance with previous studies regarding plant extracts in lowering cholesterol. Research by Sijani Prahastuti et al about the effect of bay leaf infusion which contains flavonoids, tannins, and saponins that have an effect in lowering cholesterol. [41] Then research by Helmice Afriyeni et al on the effectiveness of antihypercholesterolemic ciplukan plants containing flavonoids, alkaloids, and saponins have effectiveness as hypolipidemics. 35 Research on the ethanolic extract of petai seeds by Desy

Purnamawati also found a decrease in cholesterol levels.[20] and by Nicky Wahyuni at a dose of 398mg/200g BW it could significantly reduce cholesterol.[42] Another plant extract used was chickpeas. This research was carried out by Sri Wahjuni et al. Beans contain phytochemical alkaloids, saponins, tannins, flavonoids, and phytosterols. The results of his research that beans can lower cholesterol, LDL, and increase HDL.[43]

The results of the analysis using the Kruskal-Wallis nonparametric test found that there were significant differences between negative control, positive control, treatment group 1, treatment group 2, and treatment group 3 with a significance of p=0.018 (p<0.05). Then the results of the Mann-Whitney test showed that the treatment group 1 did not have a significant difference with the negative control and treatment group 2, but had a significant difference with the positive control group and the treatment group 3. Then the treatment group 2 did not have a significant difference with the negative control. and treatment group 3, but had a significant difference with the positive control. Furthermore, the treatment group 3 did not have a significant difference with the negative control but had a significant difference with the positive control. From the results of this analysis, it can be seen that treatment group 3 with a dose of 400 mg/KgBW had a higher effectiveness in lowering rat cholesterol than other treatment groups.

CONCLUSION

Based on the results of research conducted on the effectiveness of giving methanol extract of petai seeds (Parkia speciosa Hassk.) to the cholesterol of Wistar strain rats induced by high-fat diet, it was concluded: The average cholesterol level in the negative control group (which was given standard feed) was 60.76 mg/dL. The average cholesterol level in the positive control group (which was given MDTL) was 74.26 mg/dL. The mean cholesterol levels in the treatment group 1, treatment group 2 and treatment group 3 when compared with the positive control group there was a significant difference.

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CONFLICT OF INTEREST

There are no conflicts of interest in conducting this research.

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