

Effect of Ashitaba Extract on Cholesterol in Wistar Rats Given a High-Fat Diet and Computational Lethal Dose Test

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ABSTRACT

High levels of cholesterol in the blood can cause various health problems, namely atherosclerosis, stroke, coronary heart disease, to hypertension. One drug option for someone with hypercholesterolemia is simvastatin. In addition to the use of the drug simvastatin, can take medicinal plants. A medicinal plant that can help lower high cholesterol levels is Ashitaba leaf extract (*Angelica keiskei*). One of the ingredients in ashitaba extract is xanthoangelol E, Ashitaba chalcones, which can reduce cholesterol synthesis. The purpose of this study was to determine the effect of ashitaba extract (*Angelica keiskei*) on the cholesterol levels of Wistar rats (*Rattus norvegicus*) given a high-fat diet. In this study, a computational lethal dose test was also carried out. The population in this study was male white rats of the Wistar strain with samples taken from as many as 30 heads. Data analysis of this study used the One Way Anova statistical test. The results showed that giving ashitaba extract 150mg/kg body weight, 300mg/kg body weight, and 600mg/kg body weight did not affect the cholesterol levels of Wistar rats given a high-fat diet. This is proven by the results of the ANOVA test obtained a significance value of $0.761 > 0.05$. Research on the toxic effects of ashitaba extract needs to be done for further research.

Kata-Kata Kunci: Ashitaba Extract, Cholesterol, Lethal Dosage

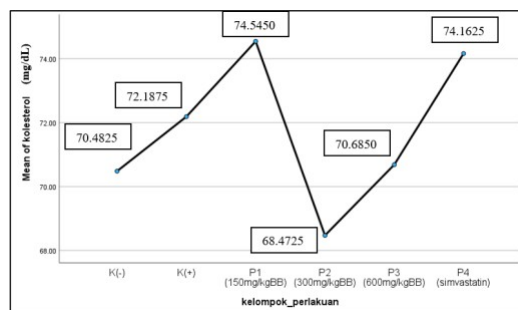
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INTRODUCTION

Obesity is a condition of increasing or excess fat in the body. (Russell-Mayhew et al., 2012). Excessive food intake will be stored as energy reserves in the form of fat which, if accumulated in the long term, will cause fat reserves in the body, causing obesity. In the body, fat will be stored as adipose tissue under the skin. (Sudargo et al., 2014). According to World Health Organization (WHO) data, the prevalence of obesity in various parts of the world is increasing rapidly, both in developed and developing countries. This increase nearly tripled from 1975 to 2016. (WHO, 2018). In Indonesia, obesity is one of the nutritional problems, as evidenced by the results of Basic Health Research in 2013, namely the prevalence of the

Indonesian population who are obese at 15.4%. Obesity cases in Surabaya City rank second highest in East Java Province with 98,344 cases. Riskesdas, 2013).

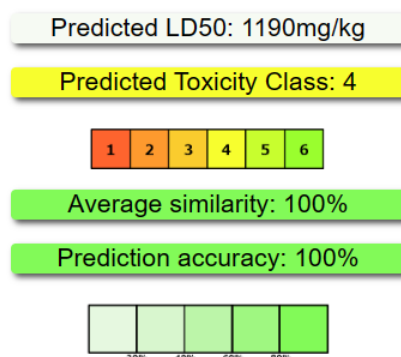
Individuals with obesity will adversely affect their health. Obesity will increase the risk of degenerative diseases, namely diabetes mellitus and hypertension, cardiovascular disease, dyslipidemia, and inflammatory response. (Medina-Remón et al., 2018). Components of dyslipidemia including high levels of total cholesterol, triglycerides, LDL, and low levels of HDL play an important role in the increase in cardiovascular disease and atherosclerosis. Total cholesterol is one of the indicators to determine the risk of cardiovascular disease.



Normally, cholesterol is produced by the body itself in the right amount, but cholesterol levels can increase due to the intake of foods derived from animal fats such as beef, mutton, duck, pigeon, gizzard, intestines, liver, lungs, to seafood such as squid, crabs, shrimp, shellfish and others. If cholesterol levels in the blood are sufficient and do not exceed the normal levels needed, then the regulation of cholesterol metabolism will run normally, but in someone with obesity, there is a disruption of fatty acid regulation that will increase cholesterol and triglyceride levels. (Listiyana et al., 2013). Normal levels of cholesterol in the blood range from 150-200 mg / dL. If cholesterol levels exceed this value, it is included in the category of hypercholesterolemia. (Ekayanti, 2020). In Wistar rats, normal blood cholesterol levels are 10-54 mg/dl (Smith & Mangkoewidjo, 1988). There are several efforts made to reduce high cholesterol levels to prevent and reduce the risk of disease due to high cholesterol. Among them meeting nutritional balance by changing diet, diligently exercising, and taking drugs, and medicinal plants.

One of the drug options for hypercholesterolemia is statin drugs, one of which is simvastatin. In addition to the use of the drug simvastatin, it can also be accompanied by taking medicinal plants. Medicinal plants that can help lower cholesterol levels, especially LDL in the blood, are Ashitaba leaf extract (*Angelica keiskei*). (Ernawati & Widjaja, 2018).

Ashitaba has a Latin name *Angelica* which means angel and *keiskei* is used to honor a Japanese botanist in the 19th century named Ito Keisuke who became the discoverer of the plant. The physical shape of the ashitaba is similar to the celery plant, but the size of the ashitaba is larger and taller than the celery plant. Ashitaba (*Angelica keiskei*) contains chalcones compounds, which have effects as antioxidants, and anti-tumors, can lower cholesterol levels, and others. (Swarayana et al., 2012). Previously, there was a study by (Ernawati & Widjaja, 2018) on "Utilization of Ashitaba Leaf Steeping with simvastatin in Reducing LDL Levels of Male White Rats Wistar Strain". The results of the study showed that the benefits of ashitaba



leave as a companion to the drug simvastatin were not proven to lower LDL levels. The result of this insignificant difference in LDL levels may be due to ashitaba with simvastatin being competitive or blocking each other. There was no difference between groups, but the average LDL value showed that the group given ashitaba and the combination of ashitaba with simvastatin had lower LDL values than the control group.

Computational tests to obtain data on lethal dose prediction (LD50) from this extract using applications on computers using Swiss ADME and Protox. In this application will get LD50, which means that if the dose is given to experimental animals, there will be death by 50%. Levels of Toxicity can also be classified into 6 i.e. (protox applications).

1. Class 1: death ($LD50 \leq 5$)
2. Class 2: burdensome if ingested ($5 < LD50 \leq 50$)
3. Class 3: Toxic if ingested ($50 < LD50 \leq 300$)
4. Class 4: Dosage ($300 < LD50 \leq 2000$)
5. Class 5: Dosage ($2000 < LD50 \leq 5000$)
6. Class 6: nontoxic ($LD50 > 5000$)

METHOD

Research Design

Research on "The Effect of Ashitaba Extract (*Angelica keiskei*) on Cholesterol Levels of Wistar Rats (*Rattus norvegicus*) Given a High-Fat Diet" included laboratory experimental research conducted in the laboratory using the Post Test Only Control Group Design research design. Data collection was carried out at the end of the study after treatment was carried out and then compared the results in the treated group with the untreated group.

Population and Sample

The population in this study was male rats of the Wistar strain (*Rattus norvegicus*) aged 2-3 months with a body weight of ± 100 -200 grams (average body weight 150 grams).

Data Analysis

The primary data obtained were carried out with statistical tests, namely normality tests using the Kolmogorov-Smirnov test and homogeneity tests using the Levene test. If both tests show normal and homogeneous data ($p > 0.05$), then a parametric statistical test is carried out with One-Way ANOVA and then continued with the Least Significant Difference (LSD) test with a degree of significance $p < 0.05$ ($\alpha = 5\%$). However, if the normality and homogeneity test is not met, then use the Kruskal-Wallis test. Statistical analysis was performed using SPSS software version 29.0 for Windows.

RESULTS AND DISCUSSION

Information:

1. K-: Healthy Wistar rats amounted to 70.4825
2. K+: Wistar rats fed a high-fat diet 72.1875
3. Q1: Wistar rats given a high-fat diet and ashitaba extract 150mg/kgBB 74.5450
4. P2: Wistar rats given a high-fat diet and ashitaba extract 300mg/kgBB 68.4725
5. Q3: Wistar rats given a high-fat diet and ashitaba extract 600mg/kgBB 70.6850
6. Q4: Wistar rats fed a high-fat diet and simvastatin 74.1625

Graph 1 shows the average cholesterol levels of group K(-) of 70.4825, K(+) of 72.1875, P1 of 74.5450, P2 of 68.4725, P3 of 70.6850, and P4 of 74.1625. It can be seen that the highest average cholesterol levels were found in the P1

group or Wistar rat group given a high-fat diet and ashitaba extract at a dose of 150 mg/kg body weight, which is 74.5450. The lowest average cholesterol levels were found in the P2 group or wistar rat group given a high-fat diet and ashitaba extract at a dose of 300 mg/kg BB, which was 68.4725.

Normality Test

This test is needed to compare the distribution of cholesterol level measurement data with the standard normal distribution. The test results showed that cholesterol measurement data had a p-value of $0.200 > 0.05$. This means that the cholesterol level measurement data has a normal distribution.

Homogeneity Test

The homogeneity test of variance (Levene's Test) aims to determine whether the data group has homogeneous variance or not. Data measuring cholesterol levels have a normal distribution, then continued by conducting the Levene's Test, it was found that cholesterol levels had a p-value of $0.084 > 0.05$. This means that the variance of cholesterol data is homogeneous, so testing whether there are differences between groups using the ANOVA test.

Uji ANOVA

To see whether there are differences between treatment groups, the One-Way ANOVA test is used. ANOVA test results on cholesterol level measurement showed a significance p-value of $0.761 > 0.05$ so there was no significant difference between treatment groups.

Uji Lethal dose

Computational test results with Swiss Adme and Protox were obtained.

The results showed that based on the average negative control group (K-) or the group given standard feed had lower cholesterol levels of 70.4820 when compared to the positive control group

(K+) or the group given a high-fat diet of 72.1880. Increased cholesterol levels after being given a high-fat diet occur because the fat contained in food will form monoglyceride compounds and fatty acids. After both compounds are digested, they will recombine with proteins in intestinal cells, then will be excreted into the lymphatic system and lipoproteins called chylomicrons. (Guyton and Hall. 2011). These compounds are metabolized and incorporated into tissue lipids or oxidized as energy and some will be oxidized to acetyl CoA which is a precursor to cholesterol formation. (Heriansyah, 2013).

The study showed that there was no significant difference in cholesterol levels between the group of experimental animals given a high-fat diet and the group given a high-fat diet and ashitaba extract and simvastatin. However, based on the average group given a high-fat diet had higher cholesterol levels of 72.1880 when compared to the group given a high-fat diet and ashitaba extract dose of 300 mg/kg body weight (P2) during 14 days amounted to 68.4725 and the group given an high-fat diet and ashitaba extract dose of 600 mg/kg BB (P3) for 14 days amounted to 70.6850. This shows a decrease in cholesterol levels after the administration of ashitaba extract.

The results of the study of cholesterol levels statistically showed the significance of p-values of $0.761 > 0.05$. The accepted H_0 showed no effect of ashitaba extract on the cholesterol levels of Wistar rats given a high-fat diet could be caused by several factors.

The results of this study showed that there were other influences besides giving ashitaba extract in lowering the cholesterol levels of Wistar rats given a high-fat diet. This is evidenced by the results of the average value of cholesterol levels for each treatment group.

Based on the average negative control group (K-) of 70.4820, the lowest average cholesterol level was found in the group with a high-fat diet and ashitaba extract at a dose of 600 mg/kg BB (P3) for 14 days, which was 70.6850. From these results, it can give an idea that giving an ashitaba extract dose of 600 mg/kg body weight for 14 days can reduce cholesterol levels of Wistar rats given a high-fat diet.

The decrease in cholesterol levels in high-fat diet rats given ashitaba extract was caused by the content contained in ashitaba extract, namely chalcones compounds. Chalcones contain two flavonoid compounds, xanthoangelol, and 4-hydroxyderricin. Flavonoids are natural phenolic compounds whose function is as an antioxidant. The mechanism of action of flavonoids as antioxidants is by donating hydrogen ions, so that results will be obtained in the form of neutralizing toxic effects from the presence of free radicals (Sumardika, I Wayan and Jawi, 2014). In someone with high cholesterol levels (hypercholesterolemia), flavonoids will decrease cholesterol synthesis. The mechanism of action in reducing cholesterol synthesis is by inhibiting the activity of the enzyme 3-hydroxyl-methylglutaril-CoA (HMG-CoA) reductase. (Arief et al., 2012).

CONCLUSION

In a study entitled "The Effect of Ashitaba Extract (*Angelica keiskei*) on Cholesterol Levels of Wistar Rats (*Rattus novergicus*) Given a High-Fat Diet" ANOVA test results showed a significance p-value of $0.761 > 0.05$ so that there was no significant difference in cholesterol levels between the experimental animal group given a high-fat diet and the group given a high-fat diet and ashitaba extract. On average, the group given a high-fat diet had a cholesterol value of 72.1880 higher when compared to the group given a high-fat diet and ashitaba extract dose of 600 mg / kg BB (P3) for 14 days, which was 70.6850. This shows a

decrease in cholesterol levels after giving ashitaba extract. In the prediction of lethal doses test using a computer, it was found to be 1190mg / kgbb with a prediction of a safety level of class 4.

In order to improve understanding of the potential use of ashitaba extract in lowering cholesterol levels, it is recommended to carry out further research by considering the "dose effect relationship" in Wistar rats given a high-fat diet in dose variations. The study can provide a more comprehensive picture related to the effect of giving ashitaba extract on reducing cholesterol levels. In addition, additional research is needed to clarify the factors that play a role in increasing and decreasing cholesterol levels, so as to provide deeper insight into the mechanism of action of ashitaba extract. Measurements of the activity of the enzyme 3-hydroxyl-methylglutaril-CoA (HMG-CoA) reductase in mice fed a high-fat diet also need to be further explored to understand the role of the enzyme in cholesterol regulation. Finally, research on the effects and toxicity of ashitaba extract in rats needs to be carried out before its application in humans, to ensure the safety and efficacy of its use.

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