Volume 5, No. 10 October, 2024
p ISSN 2723-6927-e ISSN 2723-4339

Effect of Ashitaba Extract on Cholesterol in Wistar Rats Given a High-Fat Diet and Computationally Dosed Letal Test

Indah Widyaningsih[1], Natasya Nurulita Sugiharto[2], Febtarini Rahmawati[3], Fuad Amma[4], Dorta Simamora[5]
Universitas Wijaya Kusuma Surabaya
Email: indatamun@uwks.ac.id, natasyanurulita2018@gmail.com, fuad.ama22010@gmail.com, febtapatklin@gmail.com

ABSTRACT
High levels of cholesterol in the blood can lead to various health problems, including atherosclerosis, stroke, coronary heart disease, and hypertension. One treatment option for individuals with hypercholesterolemia is simvastatin. In addition to using simvastatin, herbal remedies can also be consumed. One herbal plant that can help lower high cholesterol levels is the extract of Ashitaba leaves (Angelica keiskei). One of the components in Ashitaba extract is xanthoangelol E, along with Ashitaba chalcones, which can reduce cholesterol synthesis. The aim of this study is to determine the effect of Ashitaba extract (Angelica keiskei) on the cholesterol levels of Wistar rats (Rattus norvegicus) that were given a high-fat diet. This study also conducted a computational lethal dose test. The population for this study consisted of male Wistar strain white rats, with a sample size of 30 individuals. Data analysis was performed using One Way ANOVA statistical tests. The results showed that administration of Ashitaba extract at doses of 150 mg/kg body weight, 300 mg/kg body weight, and 600 mg/kg body weight did not affect the cholesterol levels of Wistar rats on a high-fat diet. This was evidenced by the ANOVA test results, which yielded a significance value of 0.761, greater than 0.05. Further research on the toxic effects of Ashitaba extract is needed for future studies.

Keywords: Ashitaba Extract, Cholesterol, Lethal Dosage

Introduction

Obesity is a condition characterized by the accumulation or excess of fat in the body (Russell-Mayhew et al., 2012). Excessive food intake is stored as energy reserves in the form of fat, which, when accumulated over the long term, leads to fat deposits in the body, thus causing obesity. In the body, fat is stored as adipose tissue located beneath the skin (Freitag et al., 2014). According to data from the World Health Organization (WHO), the prevalence of obesity is increasing rapidly worldwide, both in developed and developing countries. This increase has nearly tripled from 1975 to 2016 (WHO, 2018). In Indonesia, obesity is a significant nutritional problem, evidenced by the 2013 Basic Health Research results showing that the prevalence of obesity among the Indonesian population is 15.4%. In Surabaya, obesity ranks as the second highest in East Java, with a total of 98,344 cases (Nainggolan et al., 2016). Individuals with

obesity face adverse health effects. Obesity increases the risk of degenerative diseases such as diabetes mellitus, hypertension, cardiovascular diseases, dyslipidemia, and inflammatory responses (Medina-Remón et al., 2018). Components of dyslipidemia, including high levels of total cholesterol, triglycerides, LDL, and low levels of HDL, play a crucial role in the rise of cardiovascular diseases and atherosclerosis. Total cholesterol is one of the indicators used to determine the risk of cardiovascular disease (Poss et al., 2011).

Normally, cholesterol is produced by the body in the right amounts, but cholesterol levels can increase due to dietary intake from animal fats, such as beef, goat meat, duck, pigeon, gizzards, intestines, liver, lungs, and seafood like squid, crab, shrimp, clams, and others (van der Wulp et al., 2013). When cholesterol levels in the blood are sufficient and do not exceed the normal range, cholesterol metabolism regulation functions properly. However, in individuals with obesity, there is a disruption in fatty acid regulation that increases cholesterol and triglyceride levels (Listiyana et al., 2013). Normal cholesterol levels in the blood range from 150 to 200 mg/dL. If cholesterol levels exceed this range, it falls into the category of hypercholesterolemia (Ekayanti, 2019). In Wistar strain rats, normal blood cholesterol levels are between 10 and 54 mg/dL (Smith & Mangkoewidjojo, 1988). Several efforts can be made to lower high cholesterol levels to prevent and reduce the risk of diseases related to high cholesterol. These include achieving nutritional balance by changing dietary patterns, engaging in regular exercise, taking medications, and using herbal remedies.

One treatment option for hypercholesterolemia is statin medications, one of which is simvastatin. In addition to using simvastatin, it can be accompanied by the consumption of herbal remedies. One herbal plant that can help lower cholesterol levels, particularly LDL, in the blood is the extract of Ashitaba leaves (Angelica keiskei) (Ernawati & Widjaja, 2018).

Ashitaba has the scientific name Angelica, which means "angel," and "keiskei" is used to honor the 19th-century Japanese botanist Ito Keisuke, who discovered the plant. The physical appearance of ashitaba resembles that of celery, but ashitaba is larger and taller than celery. Ashitaba (Angelica keiskei) contains chalcone compounds, which have effects such as acting as antioxidants, anti-tumor agents, and reducing cholesterol levels, among others (Swarayana et al., 2012). Previously, a study by (Ernawati & Widjaja, 2018) examined "The Utilization of Ashitaba Leaf Infusion with Simvastatin in Reducing LDL Levels in Male Wistar Strain White Rats." According to the results of that study, the benefits of ashitaba leaves as a companion to simvastatin were not proven to lower LDL levels. The insignificant difference in LDL levels may be due to the competitive nature of ashitaba and simvastatin, which may block each other. There was no difference between groups, but the average LDL values indicated that the group given ashitaba and the combination of ashitaba with simvastatin had lower LDL values than the control group (Zhou & Martirosyan, 2024).

Computational testing to obtain data on the predicted lethal dose (LD50) of this extract is conducted using computer applications, specifically Swis ADME and Protox. In these applications, the LD50 value will be determined, which indicates that if this dose is administered to test animals, a mortality rate of 50% is expected. The level of toxicity can also be classified into six categories according to the Protox application:

Class 1: lethal (LD50 ≤ 5)
Class 2: fatal if ingested (5 < LD50 ≤ 50)
Class 3: toxic if ingested (50 < LD50 ≤ 300)
Class 4: dose (300 < LD50 ≤ 2000)
Class 5: dose (2000 < LD50 ≤ 5000)
Class 6: non-toxic (LD50 > 5000)

Research Methods

Research Design

The study on "Effect of Ashitaba Extract (Angelica keiskei) on Cholesterol Levels of Wistar Rats (Rattus novergiccus) Given a High-Fat Diet" included laboratory experimental research conducted in the laboratory using the Post Test Only Control Group Design. Data collection was carried out at the end of the study after the treatment was carried out and then compared the results in the treated group with the group that was not treated.

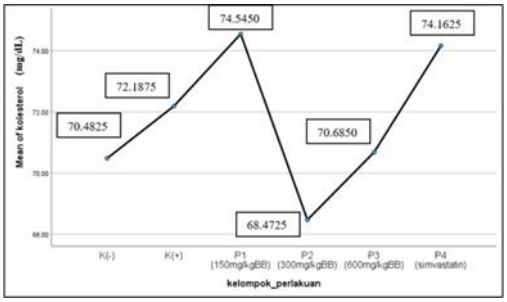
Population and Sample

The population in this study is male rats of the wistar strain (Rattus novergiccus) who are 2-3 months old with a body weight of ± 100-200 grams (average weight 150 grams).

Data Analysis

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

Results and Discussion



Information:

K- : Healthy wistar rats 70.4825
K+ : Wistar rats fed a high-fat diet 72.1875
P1 : Wistar rats fed a high-fat diet and ashitaba extract 150mg/kgBB 74.5450
P2 : Wistar rats fed a high-fat diet and ashitaba extract 300mg/kgBB 68.4725
P3 : Wistar rats fed a high-fat diet and ashitaba extract 600mg/kgBB 70.6850
P4 : 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 average

cholesterol level is highest in the P1 group or the wistar rat group who were given a high-fat diet and ashitaba extract with a dose of 150mg/kgBB, which was 74.5450. Meanwhile, the lowest average cholesterol level was found in the P2 group or the group of wistar rats who were given a high-fat diet and ashitaba extract with a dose of 300mg/kgBB, 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 the cholesterol measurement data had a p-value of 0.200 > 0.05. This means that the cholesterol level measurement data has a normal distribution.

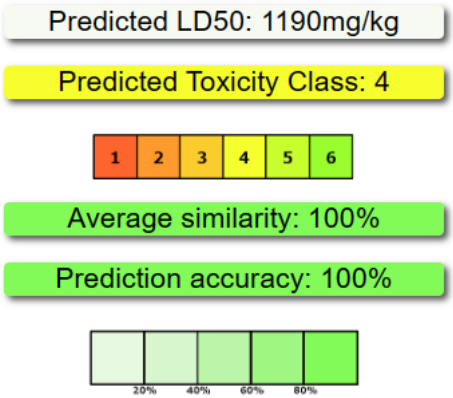
Homogeneity Test Homogeneity Test

The variance homogeneity test (Levene's Test) aims to determine whether the data group has a homogeneous variance or not. The cholesterol level measurement data had a normal distribution, then it was continued by conducting the Levene's Test, the results were obtained that the cholesterol level had a p-value of 0.084 > 0.05. This means that the variance of cholesterol data is homogeneous, so that testing whether there is a difference between groups using the ANOVA test.

ANOVA Test

To see if there is a difference between treatment groups, the One-Way ANOVA test is used. The results of the ANOVA test on the measurement of cholesterol levels showed a significance of p-value 0.761 > 0.05 so that there was no significant difference between the treatment groups.

Lethal dose test



DISCUSSION

The research results indicate that based on the average, the negative control group (K-) or the group given standard feed has a lower cholesterol level of 70.4820 compared to the positive control group (K+) or the group given a high-fat diet with a level of 72.1880. The increase in cholesterol levels after being given a high-fat diet occurs because the fats in the food form monoglycerides and fatty acids. Once these two compounds are digested, they reassemble with proteins in the intestinal cells, and subsequently, they are excreted into the lymphatic system as lipoproteins known as chylomicrons (Guyton and Hall, 2011). These compounds are metabolized and incorporated into tissue lipids or oxidized for energy, with some being further oxidized into acetyl CoA, which is a precursor for cholesterol formation (Heriansyah, 2013).

The study shows that there is no significant difference in cholesterol levels between the group of test animals given a high-fat diet and the group given a high-fat diet along with ashitaba extract and simvastatin. However, based on the averages, the group on a high-fat diet had a higher cholesterol level of 72.1880 compared to the group given a high-fat diet and 300 mg/kg body weight of ashitaba extract (P2) for 14 days, which had a cholesterol level of 68.4725, and the group given a high-fat diet and 600 mg/kg body weight of ashitaba extract (P3) for 14 days, which had a level of 70.6850. This indicates a reduction in cholesterol levels after the administration of ashitaba extract.

The results of the cholesterol level study statistically showed a significance of p-value 0.761 > 0.05. H0 received showed that there was no effect of ashitaba extract administration on cholesterol levels in wistar rats fed a high-fat diet could be caused by several factors.

The results of this study showed that there was another effect besides the administration of ashitaba extract in lowering cholesterol levels in wistar rats fed 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 with a dose of 600 mg/kgBB (P3) for 14 days, which was 70.6850. From these results, it can provide an idea that the administration of ashitaba extract at a dose of 600 mg/kgBB for 14 days can reduce cholesterol levels in wistar rats given a high-fat diet.

The decrease in cholesterol levels in high-fat diet rats given ashitaba extract was due to the content contained in ashitaba extract, namely chalcones compounds. Chalcones contain two flavonoid compounds, namely xanthoangelol and 4-hydrooxyderricin. Flavonoids are natural phenolic compounds whose one 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 the toxic effects of the presence of free radicals (Sumardika, I Wayan and Jawi, 2014). In a person 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-methyl-glutaril-CoA (HMG-CoA) reductase. (Arief et al., 2012).

Conclusion

In the study entitled "Effect of Ashitaba Extract (Angelica keiskei) on Cholesterol Levels of Wistar Rats (Rattus novergiccus) Given a High-Fat Diet", the results of the ANOVA test showed a significance of p-value 0.761>0.05 so 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. On average, the group given a high-fat diet had a higher cholesterol value of 72.1880 when compared to the group given a high-fat diet and a dose of 600 mg/kgBB (P3) extract for 14 days, which was 70.6850. This shows a decrease in cholesterol levels after the administration of ashitaba extract. In the prediction of the lethal test using a computer, the dose obtained was 1190mg/kgbb with a prediction of the safety level of class 4

REFERENCE

Ekayanti, I. G. A. S. (2019). Analisis kadar kolesterol total dalam darah pasien dengan diagnosis penyakit kardiovaskuler. *International Journal of Applied Chemistry Research*, *1*(1), 6–11.

Ernawati, E., & Widjaja, T. R. (2018). Pemanfaatan Seduhan Daun Ashitaba dengan Simvastatin dalam Menurunkan Kadar LDL Tikus Putih Jantan Strain Wistar. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, *7*(1), 31–37.

Freitag, H., Rosiyani, F., Kusmayanti, N. A., & Sudargo, T. (2014). *Pola Makan dan Obesitas*. Yogyakarta: Gadjah Mada University Press.

Listiyana, A. D., Mardiana, M., & Prameswari, G. N. (2013). Obesitas sentral dan kadar kolesterol darah total. *Jurnal Kesehatan Masyarakat*, *9*(1), 37–43.

Nainggolan, O., Hapsari, D., & Indrawati, L. (2016). Pengaruh akses ke fasilitas kesehatan terhadap kelengkapan imunisasi baduta (analisis riskesdas 2013). *Media Litbangkes*, *26*(1), 15–28.

Poss, J., Custodis, F., Werner, C., Weingartner, O., Bohm, M., & Laufs, U. (2011). Cardiovascular disease and dyslipidemia: beyond LDL. *Current Pharmaceutical Design*, *17*(9), 861–870.

Russell-Mayhew, S., McVey, G., Bardick, A., & Ireland, A. (2012). Mental health, wellness, and childhood overweight/obesity. *Journal of Obesity*, *2012*(1), 281801.

Smith, J. B., & Mangkoewidjojo, S. (1988). *Pemeliharaan, pembiakan dan penggunaan hewan percobaan di daerah tropis*. Penerbit Universitas Indonesia.

van der Wulp, M. Y. M., Verkade, H. J., & Groen, A. K. (2013). Regulation of cholesterol homeostasis. *Molecular and Cellular Endocrinology*, *368*(1–2), 1–16.

Zhou, J., & Martirosyan, D. (2024). Functional foods for cholesterol management: A comparison between the United States and Japan. *Functional Food Science-Online ISSN: 2767-3146*, *4*(6), 228–250.