Biology, Medicine, & Natural Product Chemistry
Volume 13, Number 2, October 2024 | Pages: 505-509 | DOI: 10.14421/biomedich.2024.132.505-509
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

The Effectiveness of Fragrant Pandan Leaves Ethanol Extract in Reducing Cholesterol Levels In High-Fat Diet-Induced Rats

Ni Made Wiasty Sukanty*, Farida Ariani, Lina Yunita
Undergraduate Nutrition Study Program, Faculty of Health, Universitas Bumigora,
Jl. Ismail Marzuki No.22, Cilinaya, Cakranegara, Mataram, Nusa Tenggara Barat 83127, Tel. +62-370-634498, Indonesia.

Corresponding author*
kanty@universitasbumigora.ac.id

Manuscript received: 28 August, 2024. Revision accepted: 19 September, 2024. Published: 28 October, 2024.

Abstract

Coronary heart disease was the primary cause of death in Asia in 2019. Indonesia is the country with the highest increase in cholesterol levels in Southeast Asia. One of the causes of coronary heart disease is consuming foods high in cholesterol. The commonly given therapy is simvastatin. However, long-term use of simvastatin can cause side effects, so it is necessary to use other alternatives. *Pandanus ammaryllifolius* also known as fragrant pandan is a plant that is often found in Indonesia and is used in the culinary industry. This study aims to test the effectiveness of fragrant pandan leaves ethanol extract (FPLEE) in reducing cholesterol levels in rats. A total of 30 rats were split into six groups (NC, C+, C-, T1, T2, and T3) and induced on a high-fat diet, except the NC group for 14 days. For the next 14 days, C+ was given a high-fat diet, C- was given simvastatin, T1, T2, and T3 were respectively given FPLEE doses of 8, 16, and 32 mg/200 g BW/day. Next, cholesterol levels were measured. The results of cholesterol levels were tested statistically using One-Way ANOVA and post hoc LSD tests with a = 0.05. There is no substantial difference between NC, C-, and T2. There is a significant difference between T1 and C+ with a higher T1 value. Meanwhile, for T3, there is no significant difference between T3 and NC, C-, and C+. Thus, the most effective dose for reducing cholesterol levels is 16 mg/200 g BW/day.

Keywords: Cholesterol; high-fat diet; *Pandanus ammaryllifolius*; simvastatin.

Abbreviations: Fragrant Pandan Leaves Ethanol Extract (FPLEE), high-fat diet (HFD), standard diet (SD), low-density lipoprotein (LDL).

INTRODUCTION

Cholesterol is one of the macronutrients for the body's metabolism. It plays a role in vitamin D, steroid hormones, and bile acids synthesis. Our body gets cholesterol in two ways. It is via the de novo mechanism and the food that we consume (Craig, Yarrarapu, and Dimri, 2023). However, continuous consumption of cholesterol-rich foods can increase the risk of coronary heart disease (Poli et al., 2021). This disease is the primary cause of death in Asia, namely around 35% of total deaths in Asia in 2019. This data is equivalent to 10.8 million (58%) of the 18.6 million deaths in the world. Indonesia is one of the countries in Asia with the highest increase in cholesterol levels in Southeast Asia (Zhao 2021).

Consuming foods that contain large amounts of cholesterol can cause hypercholesterolemia. Low-density lipoprotein (LDL) is one of the lipoproteins known as bad cholesterol. Cholesterol can accumulate and form plaque in the blood vessels, resulting in narrow blood vessels that disrupt blood flow (Upadhyay, 2023). If the narrowed blood vessels are the coronary arteries, the flow of oxygen-rich blood to the heart will be inhibited, causing coronary heart disease (National Heart, Lung, and Blood Institute, 2023).

Statins are a drug that lowers cholesterol levels. The mechanism of action of statins is by reducing LDL levels and inhibiting the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, a catalysator of the conversion of 3-hydroxy-3-methylglutaryl-CoA into mevalonate in the cholesterol synthesis stage in the body (National Heart, Lung, 2023). Statins have pleiotropic effects, like influencing the immune response and increasing anti-inflammatory processes. This effect is utilized in various disease medications such as rheumatoid arthritis, multiple sclerosis, and COVID-19 (Hartonno and Sri, 2023). Apart from being affordable, statins also do not cause significant side effects. Regarding liver function for xenobiotic metabolism, long-term use of statins can cause hepatotoxicity. Apart from that, myopathy is the side effect of statin (Arfania et al., 2023; Liepinsh et al., 2024).

The Back to Nature campaign that is currently being promoted has had an effect on the choice of alternative medicine among the public, one of which is the use of herbal plants as a substitute for synthetic drugs (Endriyatno, 2021). Fragrant pandanus is a local plant that grows widely in tropical areas. Fragrant pandan leaves contain several active compounds, such as flavonoids, phenols, saponins, and tannins (Assauqi, Hafshah, and Latifah, 2023). Flavonoids can inhibit cholesterol synthesis, thereby minimizing plaque formation in blood vessels (Gupta et al., 2024).

The fragrant aroma of pandan leaves causes it to be used in the culinary world. Even though it has been widely used for daily needs, fragrant pandan leaves have not been used optimally for treatment. Several studies have proven that pandan has the potential effect as anti-inflammatory, antimicrobial, and antihyperglycemic. There is currently no research regarding the potential of fragrant pandan leaves ethanol extract (FPLEE) as an antihypercholesterolemia (Forestryana, Hayati, and Putri 2022; Wananggari and Oktavilantika 2024). It prompted researchers to analyze the effect of ethanol extract from fragrant pandan leaves in reducing cholesterol levels.

MATERIALS AND METHODS

Study Design

This research was conducted at the Al-Azhar Islamic University Research Laboratory in July-August 2024. The protocol has passed ethical tests with the research code of ethics 053/EC-04/FK-06/UNIZAR/VII/2024. The research design used in this research is quantitative experimental with a posttest-only control group design. The samples were 24 male white *Rattus norvegicus* rats aged 2-3 months with a body weight of 150-250 g. Rats were divided into standard control (NC), positive control (C+), negative control (C-), treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3).

Procedures

The production of fragrant pandan leaves ethanol extract (FPLEE)

Making fragrant pandan leaf ethanol extract is carried out using the maceration method which begins with drying the fragrant pandan leaves at a temperature of 40. The dried leaves are ground into powder and soaked in 96% ethanol in a ratio of 1:1 for three days. The soaking results are filtered using filter paper to separate the solution from the powder. The solution is then evaporated to separate the solvent from the fragrant pandan leaf extract.

The production of high-fat diet (HFD)

The composition of HFD is 15 g of 65% sucrose, 5 g of animal fat, and 80 g of duck egg yolk. HFD is given orally at 2 mL/200 g BW/day.

The production of simvastatin suspension

The amount of 20.6 mg simvastatin was disolved in 100 mL of distilled water. The suspension was administered to the rats was carried out using the sonde method at 0.206 mg/200 g BW/day.

The treatment of experimental animals

Before being given treatment, the rats were adapted in the laboratory for seven days by providing a standard diet (SD) and drinking water ad libitum. The random grouping was carried out into NC, C+, C-, T1, T2, and T3. The following is the treatment given to each group:

Table 1. The treatment of experimental animals.

Group	Days 1-14	Days 15-28
NC	SD	SD
C+	SD and HFD	SD and HFD
C-	SD and HFD	SD and 0.206 mg/200 g BW/day of simvastatin
T1	SD and HFD	SD and 8 mg/200 g BW/day of FPLEE
T2	SD and HFD	SD and 16 mg/200 g BW/day of FPLEE
T3	SD and HFD	SD and 32 mg/200 g BW/day of FPLEE

The cholesterol level measurement

Blood cholesterol levels in rats were measured using a Biosystem instrument after the administration of an ethanol extract of fragrant pandan leaves. Serum samples were taken by performing a heart puncture and collecting the blood in a red cap vacutainer tube.

Data Analysis

Data from measuring cholesterol levels were analyzed using SPSS version 23 software by first testing for normality and homogeneity, then differences in results in each group were tested using the One-Way ANOVA followed by the Post Hoc LSD test with a confidence level of 95% (α= 0.05).

RESULTS AND DISCUSSION

Cholesterol levels in each group were carried out on the 29th day. The results of measuring cholesterol levels are shown in Figure 1. Group T1 is the group with the highest cholesterol levels, while T2 is the group with the lowest cholesterol levels. The results of the One-Way ANOVA test showed p-value = 0.006, which means there were at least two groups that had significant differences in cholesterol levels, so the test was continued using the Post Hoc LSD test to determine the differences in each group.

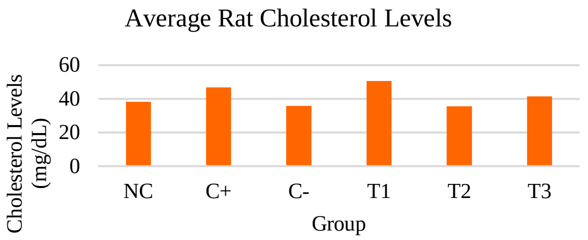

Figure 1. Average cholesterol levels after treatment.

The NC and C- groups had lower cholesterol levels than C+ with a p-value <0.05. Group P1 is the group with the highest cholesterol levels. The Post Hoc LSD test results showed a p-value < 0.05 when compared with C+, thus giving FPLEE at a concentration of 8 mg/200 g BW/day could not reduce the cholesterol levels of rats. Group T3 is a group that has cholesterol levels between NC, C-, T2, and K+, T1. The Post Hoc LSD test results only show p < 0.05 when compared with T1, thus concluding that T3 cannot reduce cholesterol levels in rats. When compared with other treatment groups, T2 had the lowest cholesterol levels. The LSD test results for the T2 group showed that there was no significant difference between T2 and NC and C-. It indicates that administration of FPLEE at a concentration of 16 mg/200 g BW/day was able to reduce the cholesterol levels of rats to close to the cholesterol levels of normal rats and rats treated with simvastatin.

Discussion

Cholesterol is a macronutrient that plays a role in vitamin D, steroid hormones, and bile acid synthesis (Craig, Yarrarapu, and Dimri, 2023). The results of measuring cholesterol levels show a difference in cholesterol levels between NC and C- compared with C+, which means giving high-fat feed can increase the blood cholesterol levels of rats. Foods rich in saturated fatty acids from HFD containing duck egg yolk and animal fat can raise blood cholesterol levels, especially LDL. This mechanism involves a decrease in LDL receptor mRNA expression and LDL receptor activity that allows a reduction in LDL absorption by the liver so that LDL levels in the blood increase. Apart from food, the liver synthesizes cholesterol and distributes it to all tissues. The synthesis of bile acids also needs cholesterol in the absorption of lipids in the small intestine (Houttu et al., 2023).

The cholesterol levels in the C- group showed that administration of simvastatin reduced the cholesterol levels of HFD-induced rats to reach levels like those in the NC group. Simvastatin is a drug commonly used to diminish cholesterol levels. The mechanism of action of simvastatin is by inhibiting the action of the HMG-CoA reductase, a catalisator for HMG-CoA conversion to mevalonic acid in the de novo cholesterol synthesis process. The inhibition of this enzyme can reduce cholesterol levels in the blood (Hartonno and Sri, 2023).

The cholesterol levels in the T1 group did not decrease. It is thought to be caused by low FPLEE concentrations. Thus, the antioxidants contained in FPLEE were unable to reduce rats's cholesterol levels. In animal models of hyperlipidemia, continuous administration of HFD without balance with sufficient antioxidant intake causes the formation of free radicals. As a result, oxidative stress occurs and leads to an increase in cholesterol levels (Adhitama, Kuswanti, and Khaleyla, 2023).

A unique phenomenon occurred in T3. Although T3 is the group given the highest concentration of FPLEE, there was no reduction in cholesterol levels. It shows that antioxidants that are too high cannot reduce cholesterol levels. The mechanism that can occur in T3 is that the antioxidants contained in high concentrations of FPLEE disrupt lipid metabolism, undergo auto-oxidation, and become pro-oxidants, thereby increasing free radicals and causing oxidative stress and increased cholesterol levels (Amida et al., 2021; Sukanty and Saputra, 2023).

The T2 had the same cholesterol levels as NC and C-. It indicates that administration of FPLEE at a concentration of 16 mg/200 g BW/day decreased cholesterol levels in rats until it reached the same cholesterol level as NC. FPLEE contains flavonoids, phenols, saponins, and tannins. This compound has antioxidant activity that can capture radicals. This ability is higher than commercial vitamin E (Suryani et al., 2018).

There are several roles of antioxidants in reducing cholesterol levels. Oxidizing LDL by free radicals is one of the factors causing atherosclerosis. Therefore, with the presence of antioxidants that can capture free radicals, the formation of oxidized LDL can be prevented (Hasim et al., 2018). Flavonoid antioxidants can increase the number of LDL receptors both in hepatocytes and in extrahepatic tissue, thereby increasing cholesterol uptake by hepatocytes and extrahepatic tissue. It results in a decrease in cholesterol levels in the blood (Mutia, Fauziah, and Thomy, 2018). Apart from that, flavonoids can also inhibit the activity of the HMG-CoA reductase enzyme in hepatocytes, thereby reducing cholesterol production (Hasim et al., 2018). Another mechanism of flavonoids is to reduce free fatty acids, thereby reducing the buildup of cholesterol in the blood (Adhitama, Kuswanti, and Khaleyla, 2023).

Tannin, another antioxidant in FPLEE, has a mechanism for decreasing blood cholesterol levels. It focuses on cholesterol absorption in the intestine. Tannins will bind to pancreatic lipase protein and coat the intestinal walls so that the lipid absorption process is impeded. The identical mechanism is shown in phenol. It binds to cholesterol-carrying proteins that function in cholesterol absorption in the intestine, thereby reducing cholesterol absorption. Another mechanism is that phenol reduces the secretion of apolipoprotein B, a protein involved in lipid transport in the blood (Mutia, Fauziah, and Thomy, 2018). Saponin functions to inhibit the activity of the lipase enzyme in the pancreas. Low lipase activity results in a decrease in triacylglycerol. Saponins can also speed up cholesterol metabolism (Viviandhari et al., 2020). Thus, the reduction in cholesterol levels in the T2 was caused by the antioxidant content in FPLEE that inhibits cholesterol synthesis and absorption, so the cholesterol levels in the T2 were the same as those in the NC and C- groups. Thus, of the three existing FPLEE concentrations, 16 mg/200 g BW/day is the most effective dose for reducing rat cholesterol levels.

CONCLUSIONS

Induction of fragrant pandan leaves ethanol extract was able to reduce cholesterol levels in rats induced by a high-fat diet. The optimum concentration of fragrant pandan leaves ethanol extract is used to decrease cholesterol levels and reach concentrations like those of the standard group and the opposing group, which is 16 mg/200 g BW/day. Further research needs to be carried out to determine changes in liver function and the histology of liver cells in hypercholesterolemic rats treated with fragrant pandan leaf ethanol extract.

Acknowledgements: The author would like to thank the Ministry of Education, Culture, Research and Technology (Kemdikbudristek), Bumigora University, and all colleagues who helped in carrying out this research.

Authors' Contributions: Ni Made Wiasty Sukanty designed the study. Ni Made Wiasty Sukanty, Farida Ariani, and Lina Yunita carried out the laboratory work. Ni Made Wiasty Sukanty analyzed the data and wrote the manuscript. All authors have read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: This research was funded by the Ministry of Education, Culture, Research and Technology (Kemdikbudristek) through the Research and Community Service (BIMA) Program in 2024.

REFERENCES

Adhitama, Shendy, Nur Kuswanti, and Firas Khaleyla. 2023. "Pengaruh Ekstrak Daun Kedondong Terhadap Penurunan Kadar Kolesterol Total Dan Berat Badan Mencit Diabetes Melitus Tipe II." *LenteraBio: Berkala Ilmiah Biologi* 12, no. 3: 354–62. https://doi.org/10.26740/lenterabio.v12n3.p354-362.

Amida, N, Lisdiana Lisdiana, W Christijanti, and R S Iswari. 2021. "Efek Ekstrak Black Garlic Terhadap Kualitas Spermatozoa Tikus Setelah Dipapar Asap Rokok." *Prosiding Seminar Nasional Biologi* 9: 298–303. https://proceeding.unnes.ac.id/index.php/semnasbiologi/article/view/799.

Arfania, Maya, Dhavid Twua Mangunsong, Renita Hamjah, and Wipena Fariza. 2023. "Efektivitas Terapi Obat Golongan Statin Terhadap Pasien Dislipidemia." *Jurnal Ilmiah Wahana Pendidikan* 9, no. 16: 550–54. https://doi.org/10.5281/zenodo.8251366.

Assauqi, Niamul Faza, Mutista Hafshah, and Rais Nur Latifah. 2023. "Penentuan Nilai Konsentrasi Hambat Minimum (KHM) Dan Konsentrasi Bunuh Minimum (KBM) Ekstrak Etanol Daun Pandan (Pandanus Amaryllifolius Roxb) Terhadap Bakteri Streptococcus Mutans." *JC-T (Journal Cis-Trans): Jurnal Kimia Dan Terapannya* 7, no. 1: 1–9. https://doi.org/10.17977/um0260v7i12023p001.

Craig, Micah, Siva Naga S. Yarrarapu, and Manjari Dimri. 2023. "Biochemistry, Cholesterol." Treasure Island (FL): StatPearls. 2023. https://www.ncbi.nlm.nih.gov/books/NBK513326/.

Endriyatno, Nur Cholis. 2021. "Potensi Bahan Alam Sebagai Antihiperkolesterolemia." *Jurnal Sudut Pandang* 2, no. 7: 21–25. https://doi.org/10.55314/jsp.v2i7.183.

Forestryana, Dyera, Annisa Hayati, and Aristha Novyra Putri. 2022. "Formulation and Evaluation of Natural Gel Containing Ethanolic Extract of Pandanus Amaryllifolius R. Using Various Gelling Agents." *Borneo Journal of Pharmacy* 5, no. 4: 345–56. https://doi.org/10.33084/bjop.v5i4.1411.

Gupta, Lovlish, Monika Chauhan, Ajay Kumar, Diwakar Chauhan, and Pragati Saini. 2024. "Flavonoids and Cardiovascular Diseases." In *Advances in Flavonoids for Human Health and Prevention of Diseases*, edited by Raj K. Keservani Nisha Sharma, Deepika Saini, Rajesh K. Kesharwani, Prakash Chandra Gupta, 1st ed., 304. Boca Raton: CRC Press.

Hartonno, A. P, and Adi Sumiwi Sri. 2023. "Review: Penggunaan Statin Sebagai Obat Off-Label." *Farmaka* 21, no. 3: 342–49. https://doi.org/10.24198/farmaka.v21i3.46895.

Hasim, Hasim, Qomariah Hasanah, Dimas Andrianto, and Didah Nur Faridah. 2018. "Aktivitas Antioksidan Dan Antihiperkolesterolemia In Vitro Dari Campuran Ekstrak Angkak Dan Bekatul." *Jurnal Teknologi Dan Industri Pangan* 29, no. 2: 145–54. https://doi.org/10.6066/jtip.2018.29.2.145.

Houttu, Veera, Aldo Grefhorst, Danny M Cohn, Johannes H M Levels, Jeanine Roeters Van Lennep, Erik S G Stroes, Albert K Groen, and Tycho R Tromp. 2023. "Severe Dyslipidemia Mimicking Familial Hypercholesterolemia Induced by High-Fat, Low-Carbohydrate Diets: A Critical Review." *Nutrients* 15, no. 4: 962. https://doi.org/10.3390/nu15040962.

Liepinsh, Edgars, Liga Zvejniece, Laura Clemensson, Melita Ozola, Edijs Vavers, Helena Cirule, Stanislava Korzh, et al. 2024. "Hydroxymethylglutaryl-CoA Reductase Activity Is Essential for Mitochondrial β-Oxidation of Fatty Acids to Prevent Lethal Accumulation of Long-Chain Acylcarnitines in the Mouse Liver." *British Journal of Pharmacology* 181, no. 16: 2750–73. https://doi.org/10.1111/bph.16363.

Mutia, Sri, Fauziah, and Zairin Thomy. 2018. "Pengaruh Pemberian Ekstrak Etanol Daun Andong (Cordyline Fruticosa (L.) A. Chev) Terhadap Kadar Kolesterol Total Dan Trigliserida Darah Tikus Putih (Rattus Norvegicus) Hiperkolesterolemia." *Jurnal Bioleuser* 2, no. 2: 29–35. https://jurnal.usk.ac.id/bioleuser/article/view/14883/11193.

National Heart, Lung, and Blood Institute. 2023. "What Is Coronary Heart Disease?" National Heart, Lung, and Blood Institute. 2023. https://www.nhlbi.nih.gov/health/coronary-heart-disease.

Poli, Andrea, Franca Marangoni, Alberto Corsini, Enzo Manzato, Walter Marrocco, Daniela Martini, Gerardo Medea, and Francesco Visioli. 2021. "Phytosterols, Cholesterol Control, and Cardiovascular Disease." *Nutrients* 13, no. 8: 2810. https://doi.org/10.3390/nu13082810.

Sukanty, Ni Made Wiasty, and I Putu Bayu Agus Saputra. 2023. "Efektivitas Ekstrak Etanol Tauge (Vigna Radiata) Dalam Meningkatkan Kadar Glutation Peroksidase Pada Tikus Putih (Rattus Norvegicus) Yang Diinduksi Toluena." *Ganec Swara* 17, no. 4: 2079–84. https://doi.org/10.35327/gara.v17i4.673.

Suryani, Chatarina Lilis, Siti Tamaroh Cahyono Murti, Agusta Ardiyan, and Astuti Setyowati. 2018. "Aktivitas Antioksidan Ekstrak Etanol Daun Pandan (Pandanus Amaryllifolius) Dan Fraksi-Fraksinya." *Agritech* 37, no. 3: 271–79. https://doi.org/10.22146/agritech.11312.

Upadhyay, Ravi Kant. 2023. "High Cholesterol Disorders, Myocardial Infarction and Its Therapeutics." *World Journal of Cardiovascular Diseases* 13, no. 08: 433–69. https://doi.org/10.4236/wjcd.2023.138040.

Viviandhari, Daniek, Rini Prastiwi, Elva Fitriani Puspitasari, and Pegi Perdianti. 2020. "Aktivitas Fraksi Ekstrak Etanol Luffa Acutangula (L.) Roxb. Terhadap Penurunan Kolesterol Pada Hamster Hiperlipidemia." *Jurnal Jamu Indonesia* 5, no. 2: 45–55. https://doi.org/10.29244/jji.v5i2.171.

Wananggari, Larasati Ayuni, and Dina Melia Oktavilantika. 2024. "Formulasi, Evaluasi, Dan Uji Aktivitas Antibakteri Clay Mask Ekstrak Daun Pandan Wangi (Pandanus Amaryllifolius Roxb.) Terhadap Bakteri Propionibacterium Acnes." *Jurnal Farmasi Dan Farmakoinformatika* 2, no. 1: 63–75. https://doi.org/10.35760/jff.2024.v2i1.9729.

Zhao, Dong. 2021. "Epidemiological Features of Cardiovascular Disease in Asia." *JACC: Asia* 1, no. 1: 1–13. https://doi.org/10.1016/j.jacasi.2021.04.007.