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Effect of Extract *Piper nigrum* and *Cymbopogon citratus* on LDL and HDL Levels in *Rattus norvegicus* with Hypercholesterolemia

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Abstract

Dyslipidemia is the most common cause of noncommunicable diseases, which are a public health concern because they cause significant morbidity and mortality. It is characterized by elevated blood cholesterol, high LDL, and low HDL levels, which increase the risk of premature atherosclerotic cardiovascular disease. Black pepper (Piper nigrum) contains the active compound piperine, which may reduce cholesterol absorption. Lemongrass (Cymbopogon citratus) contains flavonoids that may lower cholesterol. The primary objective of this study is to investigate the impact of ethanol extracts of black pepper (Piper nigrum) and lemongrass (Cymbopogon citratus) on LDL and HDL levels in hypercholesterolemic white rats (Rattus novergicus). The research was conducted as a laboratory experimental study involving six groups of samples, with each group consisting of 5 mice. The sample groups included a negative control group (receiving only standard chow), a positive control group (receiving standard chow and high-fat chow), and treatment groups receiving standard chow, high-fat chow, and extract (group P1: black pepper extract at a dose of 200 mg/kgBW, group P2: black pepper extract at a dose of 500 mg/kgBW, group P3: lemongrass extract at a dose of 200 mg/kgBW, group P4: lemongrass extract at a dose of 500 mg/kgBW). The study's findings indicated that the ethanol extracts of black pepper and lemongrass effectively reduced LDL levels in hypercholesterolemic white rats (Rattus novergicus). The most effective dose of black pepper ethanol extract in reducing LDL levels was 500 mg/kgBB and the effective dose of lemongrass ethanol extract in reducing LDL levels was 200 mg/kgBW with a p-value = 0,013 < 0,05. This study showed that the ethanolic extract of black pepper and lemongrass did not increase HDL levels p-value = 0,665 > 0,05.

Keywords: black pepper extract, lemongrass extract, hypercholesterolemia, LDL, HDL

Introduction

Dyslipidemia occurs when blood cholesterol is excessive, LDL levels are too high and HDL levels are low (Azqinar et al., 2022). Dyslipidemia is often one of the causes of non-communicable diseases that are a health problem among people around the world because it can cause significant morbidity and mortality. Dyslipidemia can cause cardiovascular disease due to lipid deposits in the arterial blood vessel wall that support the process of atherosclerosis resulting in blockage of the arterial blood vessel wall. (WHO, 2018). According to WHO data in its 2019 report, the prevalence of dyslipidemia in the world is 45% and in Southeast Asia is 30%. Data from the Indonesian Ministry of Health in 2017 showed that the prevalence of dyslipidemia in Indonesia was 35% (Subandrate, 2019). This dyslipidemia condition can be treated with management both pharmacologically and non-pharmacologically (Handayani et al., 2020). Pharmacologically, dyslipidemia can be treated with several classes of drugs, one of which is the statin group (Sari, 2014). However, this statin group drug can have side effects on liver function, obstipation, has the effect of stomach discomfort, flushing, tachycardia, nausea, vomiting, itching, diarrhoea, and impaired liver function due to side effects from nicotinic acid content (Yuliantini et al., 2015). From some of the side effects that can be caused by long-term use of statin drugs, other alternatives are needed by providing natural supplements made from herbal plants that are widely available in Indonesia (Mustofa et al., 2022).

Indonesia as the second richest country in the world, has

tropical forests that are rich in flora that hold a lot of potential, namely plants that can be processed as food and as a basis for medicine (Robi et al., 2019). One of them is the black pepper plant (*Piper nigrum*) which is used as a spice in food (Mohammed et al., 2016). Black pepper contains piperine, an active compound that can reduce cholesterol absorption by inhibiting cholesterol transport proteins (Duangjai et al., 2014). Piperine can also prevent the absorption of oxidized low-density lipoprotein (LDL) in macrophages, prevent lipid peroxidation, lipid droplet formation, adhesion of inflammatory cells to the endothelial monolayer, and increase cholesterol removal from macrophages. Therefore, the piperine content in black pepper can prevent or treat dyslipidemia, hypercholesterolemia, hypertension, thrombosis, stroke, and cardiovascular disease (Wang et al., 2021). In addition, lemongrass (*Cymbopogon citratus*) is a plant usually used as a spice and medicinal ingredient (Ibrahim et al., 2021). Lemongrass has the main content of polyphenols which can also reduce cholesterol levels by reducing cholesterol absorption in the intestine through micellar breakdown (Da Ressurreição et al., 2022). Lemongrass leaves also contain secondary metabolites, namely flavonoids, which have antioxidant properties and can cause a decrease in total cholesterol numbers (Ahire et al., 2022). A study conducted by Bandi et al. (2021) found that there was a significant effect on lowering cholesterol by giving lemongrass leaf ethanol extract to white rats. This study reinforces the potential for lemongrass leaf ethanol extract to lower total cholesterol in the body. Therefore, the potential between black pepper and lemongrass is expected to be an alternative to natural medicine by using it as a supplement to lower cholesterol levels more effectively. This study aims to determine the effect of ethanol extract of black pepper and lemongrass on LDL and HDL levels in hypercholesterolemic white rats.

Methods

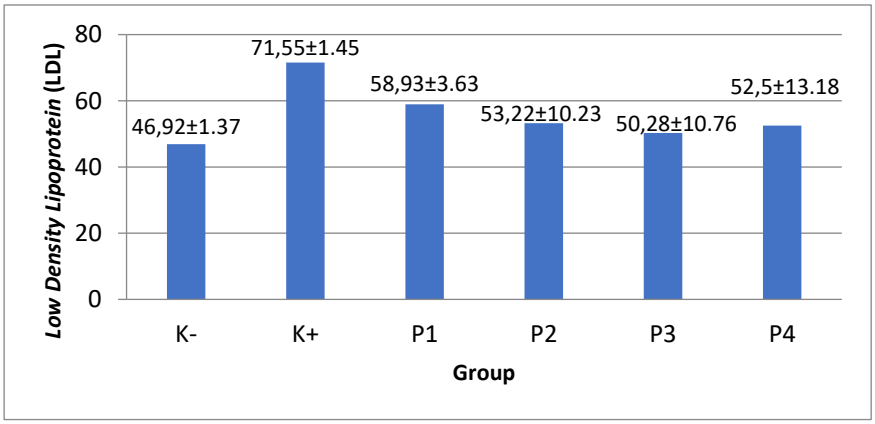
This type of research was laboratory experimental research with a true experimental design approach, with a post-test-only controlled group design. This study uses six groups that have been randomized. Each group had five rats. The sample group consisted of negative control group K- (only given standard feed), positive control group K+ (given standard feed and high fat feed only), treatment group given standard feed, high fat feed and extracts (Group P1 was given a dose of 200 mg/kgBW black pepper extract, Group P2 was given a dose of 500 mg/kgBW black pepper extract, Group P3 was given a dose of 200 mg/kgBW lemongrass extract, Group P4 was given a dose of 500 mg/kgBW lemongrass extract). Examination of LDL and HDL levels in rat blood using enzymatic spectrophotometer. Measurement of LDL levels was performed with heparin (100,000 U/L) and sodium citrate (64 mmol/L) reagents. HDL measurements were made with phospotungstic acid (1.4 mmol/L) and magnesium chloride (8.6 mmol/L) reagents. To ensure that rats are in hypercholesterolemia condition, total cholesterol examination is carried out by terminating one rat in each group, then blood is taken through the heart and checked with an enzymatic spectrophotometer. Then the K- and K+ groups were only given standard feed while the P1, P2, P3, and P4 treatment groups were treated according to the dose of extracts. After 7 days of treatment, LDL and HDL levels were measured in all rats. Data from the examination of LDL and HDL levels were analysed quantitatively using SPSS. Statistical tests were performed with Kruskall Wallis and One Way Anova. To determine further differences between treatments, Mann Whitney Post Hoc Test was conducted. To determine the effect of giving black pepper extract at a dose of 200 mg/kgBW and a dose of 500 mg/kgBW and lemongrass at a dose of 200 mg/kgBW and a dose of 500 mg/kgBW on reducing LDL levels and increasing HDL levels.

The black pepper in this study was obtained from Muara Dua Kisam, South OKU, South Sumatra. Lemongrass used in this study came from Gunung Timba village, East Denggen village, Selong, East Lombok, West Nusa Tenggara. Black pepper and lemongrass are made into extracts through maceration process with the addition of 96% ethanol.

Ethical Approval No. 52/SLE/FK/UWKS/2024
Ethical approval in the use of experimental animals is used as a research protocol as a research subject that can be considered relevant to human health guided by the principles of ethical animal research for health research using experimental animals.

Result

This research was conducted at the Laboratory of Animal Experiments, Faculty of Medicine, Wijaya Kusuma University Surabaya and the manufacture of ethanol extracts of black pepper and lemongrass was carried out at the Biochemistry Laboratory, Wijaya Kusuma University Surabaya.



Picture 1. Graph of Mean LDL Level in white rats *(Rattus norvegicus)*

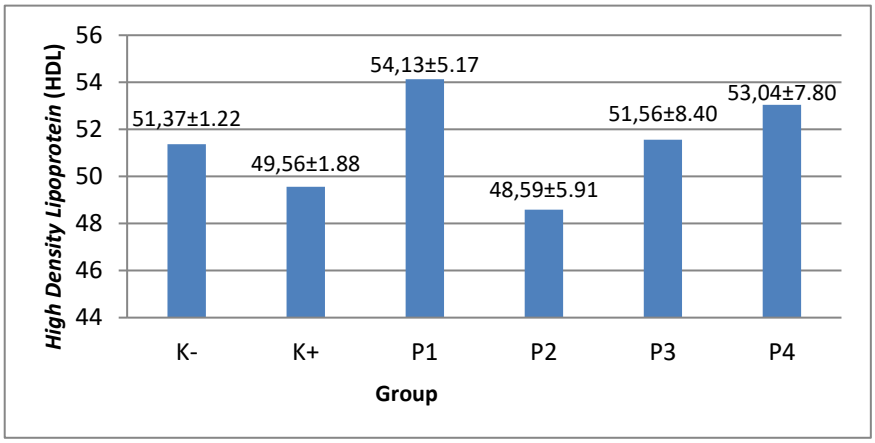
The graph above, show that the highest average LDL level value is in the K+ group, the positive control group (rats given high-fat feed) at 71.55 mg/dL. In contrast, the lowest LDL level value is in the K- group, the negative control group that is only given standard feed with an LDL level value of 46.92 mg/dL.

Based on the normality test results with Kolmogorov-Smirnov, the p-value = 0.290 means that the data is normally distributed. Then continued the homogeneity test with the Levene Test, the p-value = 0.000, which means the data is not homogeneous so the results of the difference test were carried out with the Kruskall Wallis test. The test results showed significant differences between treatment groups with a p-value = 0.013 between each treatment group. The results of the Mann Whitney Post Hoc Test analysis showed that the group of rats given a high-fat diet alone (K+) with the group given black pepper ethanol extract (*Piper nigrum*) at a dose of 200 mg / kgBW (P1) and a dose of 500 mg / kgBW (P2) had a significant difference. In addition, between the group of rats given a high-fat diet alone (K+) and the group given lemongrass ethanol extract (*Cymbopogon citratus*) at a dose of 200 mg / kgBW (P3) and a dose of 500 mg / kgBW (P4) also showed significant differences.

Table 1. Results of Post Hoc Test Mann-Whitney Analysis of LDL Levels in White Rats

Treatment Group	LDL Levels
K(-)	46,92 ± 1,37[b]
K(+)	71,55 ± 1,45[c]
P1	58,93 ± 3,63[a]
P2	53,22 ± 10,23[ab]
P3	50,28 ± 10,76[ab]
P4	52,50 ± 13,18[ab]

Notes: Superscripts a,b, and c with the same letter in the column of research variables mean there is no significant difference (p> 0.05).



Picture 2. Graph of mean HDL levels in white rats (*Rattus novergicus*)

The graph above, show that the highest average HDL level value is in group P1, which is the group given black pepper ethanol extract with a composition of 200mg/kgBW at 54.13 mg/dL, while the lowest HDL level value is in group P2, which is the black pepper ethanol extract treatment group with a composition of 500mg/kgBW with an HDL level value of 48.59 mg/dL.

Based on the the Kolmogorov-Smirnov normality test results, the p-value = 0.458 indicates that the HDL level data has normal data. Then continued with the homogeneity test (Levene Test) was obtained with p-value = 0.128, indicating that HDL levels have homogeneous data. Analysis of the difference test with One-Way ANOVA on High-Density Lipoprotein (HDL) levels found no statistically significant difference between treatment groups with a p-value of 0.665 > 0.05. So, post-hoc testing was not carried out because there was no significant difference between treatment groups.

Discussion

The administration of black pepper ethanol extract at 200 mg/kgBW (P1) and 500 mg/kgBW (P2) doses resulted in a significant reduction in LDL levels in hypercholesterolemic rats. This is attributed to the presence of piperine, the main active compound in black pepper, which can decrease cholesterol levels by inhibiting the activity of the HMG-KoA reductase enzyme in tissues, enhancing the activity of the lipoprotein lipase enzyme, and increasing bile acid excretion (Setyowati, 2018). Piperine also effectively hinders lipoprotein accumulation by regulating the lecithin-cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL) enzymes. These combined effects of piperine prevent the build-up of plasma lipids and lipoproteins in hypercholesterolemic mice, thereby reducing LDL levels (Stojanović et al., 2019).

The analysis of the Mann-Whitney Post Hoc Test results also indicated a significant distinction between the rats given a high-

fat diet alone (K+) and those administered lemongrass ethanol extract at 200 mg/kg body weight (P3) as well as 500 mg/kg body weight (P4) with a p-value of <0.05. This can be attributed to lemongrass's high polyphenol content, which reduces cholesterol absorption in the intestine through micellar breakdown (Da Ressurreição et al., 2022). Additionally, the polyphenols can lower LDL levels by inhibiting the enzyme β-HMG-CoA reductase (Ekpenyong et al., 2015) and impacting LDL α-tocopherol during oxidative stress due to their potent antioxidant properties that inhibit plasma and LDL oxidation in vitro (Sun et al., 2021).

The results of this study indicate that rats given black pepper ethanol extract have been unable to reduce normal LDL levels in rats. This could be due to factors from the standard feed of rats, namely feed 511 which contains 4% crude fat (Fadli, 2015). This content can affect the increase in LDL levels in rats, which can be proven in the group of rats that are only given standard feed (K-), which shows an increase in LDL exceeding the normal LDL levels of rats. Normal LDL levels in rat blood are 7 - 27.2 mg/dl (Riesanti et al., 2015). However, the group of rats given a dose of 500 mg/dL black pepper ethanol extract can reduce LDL levels close to the group of rats that are only given standard feed (K-). This is evidenced by the Mann Whitney Post Hoc Test analysis that there is no significant difference between the K- and P2 groups with a significance value of p-value> 0.05.

The one-way ANOVA analysis test indicated that providing black pepper ethanol extract at 200 mg/kgBW or 500 mg/kgBW did not impact on the increase in HDL levels in hypercholesterolemic white rats (Rattus novergicus). This is supported by the one-way ANOVA difference test results, which showed a significance value of p-value of 0.665 > 0.05. It was also observed that the average HDL levels between groups K- and K+ with P1 and P2 were nearly identical at 48.59 - 54.13 mg/dL. Rampengan (2015) noted that boosting HDL levels is not as straightforward as lowering LDL levels, which is echoed in this study due to the brief duration of the 7-day treatment because of the limited extract quantity. Vijayakumar et al. (2014) demonstrated that black pepper ethanol extract could significantly elevate HDL levels when administered to rats for 10 weeks. Furthermore, the study's outcomes may have been influenced by the plant's quality and limitations during the extract maceration process, which might have led to the incomplete extraction of the active substance and reduced the potential of piperine in black pepper.

This study also showed that there was no effect of lemongrass ethanol extract administration at a dose of 200 mg/kgBW or a dose of 500 mg/kgBW on the increase in HDL levels in hypercholesterolemic white rats (Rattus novergicus). This could be due to the quality of the plant, the lack of extract maceration time, and the extract evaporation process that experienced obstacles so that the active substance content of the kitchen lemongrass extract did not come out. This constraint can also affect the potential of polyphenol content in raising HDL levels. In addition, in this study, lemongrass extract was treated for 7 days due to the limited extract obtained. In previous research conducted by Widaryanti and Linda Tripramatasari (2021), the length of treatment for giving kitchen lemongrass extract that can effectively raise HDL levels is 14 days. Therefore, it is necessary to do longer treatment so that the administration of kitchen lemongrass extract can affect in raising HDL levels.

Group K+ is a group that was given a high-fat diet for 3 weeks without administration of the extract. The high-fat diet was carried out with the composition of used cooking oil: beef fat: and duck egg yolk with a composition ratio of 20%: 10% : 20%. The high-fat diet was given to white rat animals orally as much as 5 ml and given once a day (Purwaningsih, 2020) for 2 weeks and a high-fat diet with a composition of duck egg yolk: quail egg yolk: lard: butter: standard feed with a composition of 5%: 5 % : 10 % : 5% : 75% (Untari & Pramukantoro, 2020) for 1 week. High-fat diet intake in rats will trigger the formation of chylomicrons, which are synthesized in the intestinal mucosa during the absorption process of liver products. In the liver, the fat will be converted into cholesterol with the help of the enzyme HMG-KoA reductase so that cholesterol in the blood increases (Indahsari et al., 2020). The liver also releases triglycerides into the blood plasma through VLDL (Very-Low-Density Lipoprotein Lipase). Once in the plasma, VLDL will be converted into LDL (Low-Density Lipoprotein). This is why a high-fat diet can increase LDL levels in plasma (Pirahanchi et al., 2023). However, in this study, the provision of a high-fat diet has not succeeded in reducing the HDL levels of rats below normal. Normal HDL levels of rats are > 54 mg/dL (Riesanti, et al., 2015). A study conducted by Teuku Heriansyah (2018) showed that the duration of high-fat diet had an effect on significantly reducing HDL levels at a duration of 8 weeks. Therefore, it is necessary to have a longer, high-fat diet in order to reduce HDL levels.

Conclusion

Based on the research results, it was concluded that:

1. Ethanol extract of black pepper (*Piper nigrum*) significantly reduces LDL levels in hypercholesterolemic rats when administered at 200 mg/kgBW and 500 mg/kgBW.
2. The administration of black pepper ethanol extract (*Piper nigrum*) at 200 mg/kgBW and 500 mg/kgBW did not increase in HDL levels in rats with high cholesterol.
3. The administration of lemongrass (*Cymbopogon citratus*) ethanol extract at 200 mg/kgBW and 500 mg/kgBW resulted in a notable reduction in LDL levels in hypercholesterolemic rats.
4. The administration of lemongrass (*Cymbopogon citratus*) ethanol extract at 200 mg/kgBW and 500 mg/kgBW doses did not increase in HDL levels in rats with high cholesterol.

Conflict of Interest

The researchers have stated that there are no conflicts of interest in this study.

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Author contribution

N.P.K.A.P. designed the research concept, collected data, analysed data, wrote and revised the final manuscript. N.K.I. and O.H. contributed to reviewing the research concept, research data and analyses, revising the manuscript, and approving the final manuscript.

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