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The Improvement of Heart Histopathology of Hypercholesterolemic Mice using Sidempuan Salacca (*Salacca sumatrana*) Vinegar

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Abstract

Hypercholesterolemic, a condition characterized by excess cholesterol content, can lead to coronary heart disease, atherosclerosis, and high-risk heart tissue degeneration. Current drugs, like simvastatin and pravastatin, focus on lowering cholesterol levels without addressing tissue degeneration. This study aimed to determine the effectiveness of sidempuan salacca vinegar in improving cardiac tissue degeneration in hypercholesterolemic mice. The experimental research involved two groups, the treatment group given different doses of sidempuan salacca vinegar and the control group. Histopathological observations showed improvement in cardiac tissue degeneration in hypercholesterolemic mice after treatment with salacca vinegar. The administration of salacca vinegar could serve as a reference for developing alternative hypercholesterolemic drugs that address both cholesterol levels and heart degeneration.

Keywords: Heart; Histology; Hypercholesterolemic; Sidempuan Salacca Vinegar.

INTRODUCTION

Hypercholesterolemia is characterized by cholesterol levels in the blood that exceed the level required by the body, which is more than 240 mg/dl in adults (Jempormase et al., 2016). Hypercholesterolemia has many harmful risk factors that will also affect the degeneration of heart and liver tissue, the digestive organs, and the storage of cholesterol in the body. The most common forms of degeneration are necrosis and inflammation in the heart tissue and steatosis in the liver. In more severe cases, hypercholesterolemia tends to initiate the onset of coronary heart disease, the number one killer in the world. When the body experiences hypercholesterolemia, it tries to balance cholesterol levels synthesizing cholesterol into gallic acid. This synthesis process will require oxygen and other supportive substances. This unnecessary oxidation will eventually result in free radicals (Duell et al., 2019).

If the formed free radicals attack the lipids in LDL, the oxidized LDL diffagositosis by macrophages forms foam cells, which are an early indication of the occurrence of atherosclerosis. Free radicals that attack LDL also produce lipid peroxide. Lipid peroxidation from a free radical oxidation reaction on the LDL-ox series with PUFA (polyunsaturated fatty acids) or lipids on the cell membranes of body tissue that can cause damage to the cells (Nanda, 2018). LDL, which is attacked by free radicals, also triggers an inflammatory response (Duell et al, 2019).

The inflammatory response to the heart associated with hypercholesterolemia will be visible in the endothelial cells and initiate immunocompetent cells, namely lymphocytes, monocytes, and macrophages. As the inflammation continues, the aorta will experience thickening and expansion of the arterial walls until the lumen loses its ability to limit it. Activating macrophages and lymphocytes causes the release of hydrolytic enzymes, cytokines, chemokines, and growth factors that can cause necrosis (Duell et al., 2019). This series of events will eventually change the picture of heart histopathology.

Treatment of hypercholesterolemia complications today is too focused on drugs with chemical compounds that have quite destructive side effects. Examples of drugs commonly used in patients with hypercholesterolemia are simvastatin, atorvastatin, fevastin, and pravastatin (Murray et al., 2018). Simvastatin itself has been known to cause quite bad side effects such as muscle dysfunction, abdominal pain, arrhythmia, tachycardia, and so on (Hariadini et al., 2020). With such a variety of negative side effects, researchers started looking for treatment alternatives that focused on herbal ingredients. In addition to being easy

to find, herbal ingredients are cheaper and are expected to have insignificant side effects. Many plants in Indonesia have been found to have the potential to prevent cholesterol disease and even have the potential to treat cholesterol. The traditional spices have serine of NTD-NPC1L1 for Cholesterol Binding with Compounds in Traditional Spicesis known to have the ability to interfere with cholesterol absorption at an early stage where there is a high binding affinity between Ezetimibe and these ligands interacting almost at the right location in the N-terminal domain. Through Ser_102 the N-terminal domain of NPC1L1 binds to ligands (Amelia et al., 2023). With its contents, Salacca sumatrana has the potential as an alternative herbal medicine to repair the degeneration of heart tissue that is damaged due to hypercholesterolemia. However, no further research has been conducted on the benefits of oatmeal vinegar in preventing organ degeneration due to hypercholesterolemia. For this reason, this research is important to open up new herbal treatment alternatives to hypercholesterolemia.

MATERIALS AND METHODS

Study area

This research is based on True Experimental Design: Pre and Post Test with Control Group Design. 25 male mice were divided into 5 groups. There were normal group (K-), hypercholesterolemic group (K+), hypercholesterolemic + sidempuan salacca vinegar 0,2 mL group (P1), hypercholesterolemic+ sidempuan salacca vinegar 0,4 mL group (P2), hypercholesterolemic + sidempuan salacca vinegar 0,8 mL group (P3).

Procedures

Mice were given an oral and egg yolk diet to create hypercholesterolemia in mice treated with K+, P1, P2 and P3 for 14 days. Cholesterol levels are measured three times, i.e., before high cholesterol nutrition and after high cholesterol nutrition and after treatment with sidempuan salacca vinegar using easy touch. Histological heart observations were carried out after administering sidempuan salacca vinegar for 14 days to hypercholesterolemic mice.

Data analysis

To determine the effect on the histopathology of the heart administered with the sidempuan salacca vinegar, a descriptive analysis of the qualitative data of the histopathology of hypercholesterolemia was conducted. Quantitative data analysis is also carried out with the Kruskal-Wallis test, with the observed variables being necrosis and inflammation. Each sample was given damage scores for the categories of necrosis and inflammation. Score 1 is given if there is mild necrosis or inflammation in the heart. (vocal). Score 2 is given to indicate the presence of necrosis or inflammation in the heart of a moderate nature. (multivocal). Score 3 is given for necrosis or inflammation in the severe category (diffuse) (Pratama., et al 2018). If the results of the Kruskal-Wallis test show a p significance value of 0.05, which means there are significant histopathological differences, then the analysis will be continued with the Mann-Whitney test to determine the difference in efficacy between treatment doses.

RESULTS AND DISCUSSION

Result

The blood cholesterol levels of treated mice during 4 weeks are presented in Figure 1. The result of this research show that all mice had hypercholesterolemia by dietary offal and egg yolk as marked in the blue bar graph, then there was a decrease in cholesterol levels after giving of sidempuan salacca vinegar (orange bar graph). Mice are said to be hypercholesterol if the cholesterol level in their body reaches 130 mg/dl. Giving salacca vinegar tends to reduce blood cholesterol levels in male mice. The highest reduction was in the P3 treatment, namely 86.6 mg/dL and the lowest was in the K- treatment, namely -1 mg/dL.

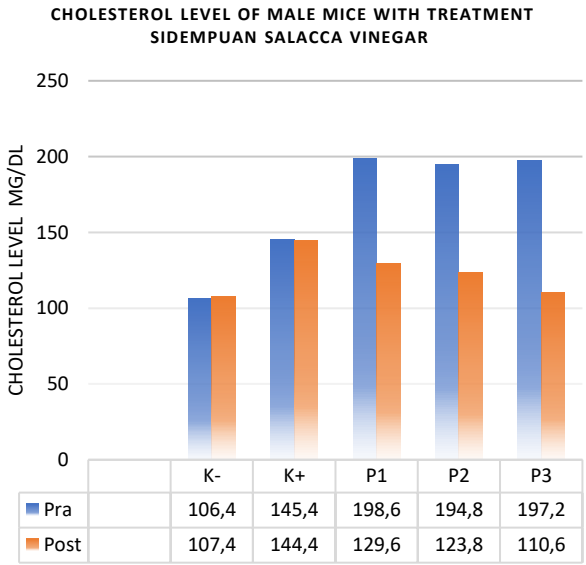

Figure 1. Comparison of CholesterolLevel of Male Mice Pra and Post Treatment Sidempuan Salacca Vinegar.

The histopathological picture of the heart of hypercholesterolemic male mice after treatment is shown in the following picture. Necrotic degeneration and inflammation could be observed and the negative control samples showed normal routine histopathology.

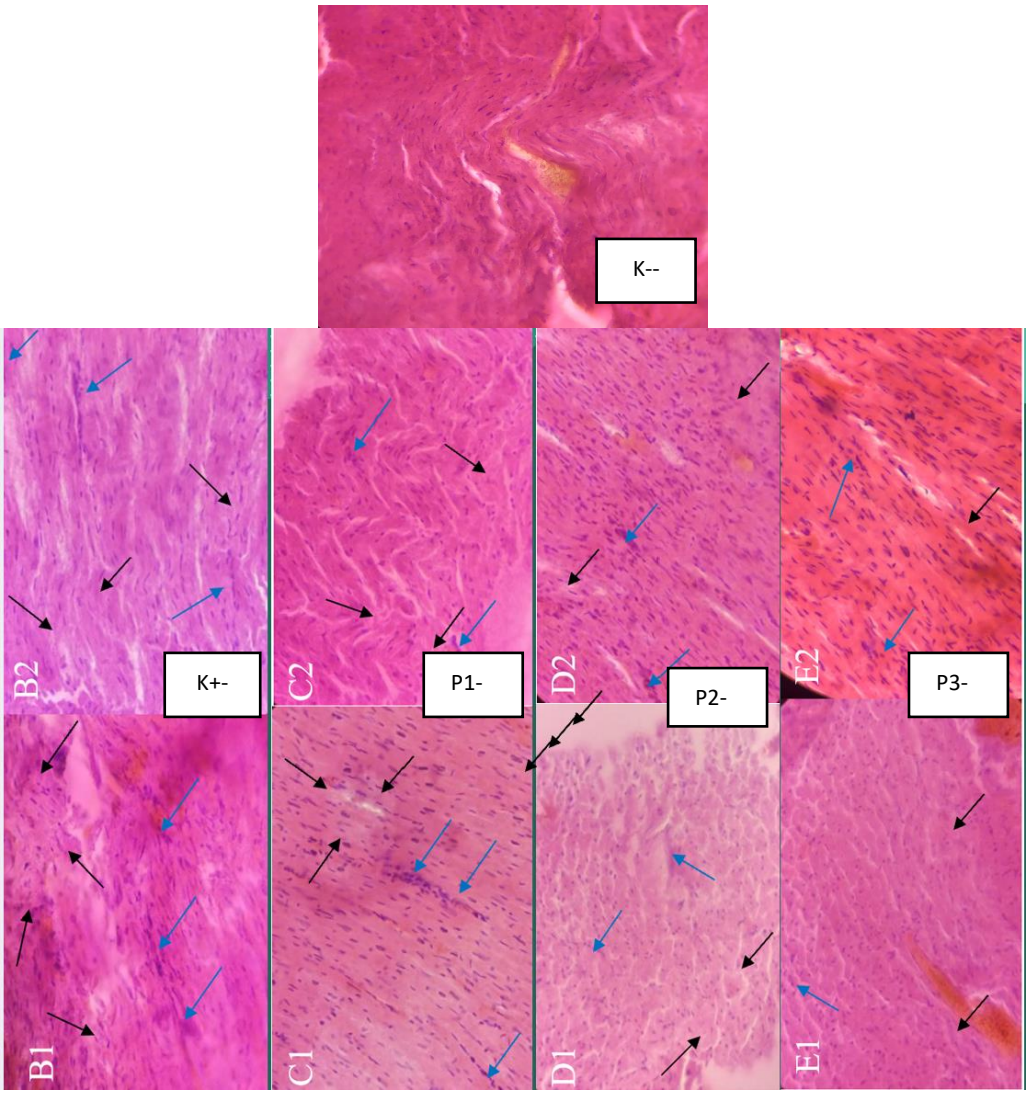

Figure 2. Histopathology of Heart (400x magnifications). K- = normal diet, K+ = high Cholesterol diet without salacca vinegar, P1 = high Cholesterol diet + salacca vinegar dose of 0.2 ml, P2 = high Cholesterol diet + salacca vinegar dose of 0.4 ml, P3 = high Cholesterol diet + salacca vinegar dose of 0.8 ml, black arrow = necrosis, blue arrow = imflamation.

Histological observations of the heart were carried out to see the effectiveness of sidempuan salacca vinegar on its effect on repairing heart tissue. The results of the study showed that there was improvement in heart tissue along with increasing the dose of sidempuan salacca vinegar given to hypercholesterolemic mice.

Table 1. Heart Histopathology Degeneration Score in Hypercholesterolemia Mice After Treatment Salacca Vinegar.

Degeneration	Group of Treatment	N	Score			
			0	1	2	3
Necrosis	K-	5	5	-	-	-
	K+	5	-	-	2	3
	P1	5	-	1	3	1
	P2	5	-	2	2	1
	P3	5	-	4	1	-
Inflammation	K-	5	5	-	-	-
	K+	5	-	-	1	4
	P1	5	-	1	2	2
	P2	5	-	2	3	-
	P3	5	-	4	1	-

In the study, the negative control group (K-) showed no signs of hypercholesterolemia or degenerative forms of necrosis or inflammation. However, the K+ group with high cholesterol nutrition treatment without salacca vinegar administration experienced diffuse degeneration with an average necrosis score of 2.6 and an average inflammation score of 2.8. Treatment groups P1, P2, and P3 were treated with high cholesterol and salacca vinegar as an antithesis with different doses per treatment, improving tissue degeneration. The P1 treatment group with a dose of 0.2 mL salacca vinegar showed better histopathological degeneration results than the K+ group. In the K+ group, 3/5 of the samples had a necrosis score of 3, meaning they had diffuse or severe necrosis, and 2/5 were indicated as multifocal. This score was better than the inflammation score in the K+ group, where 4/5 had diffuse inflammations and 1/5 included multifocal inflammations.

Table 2. Kruskall-Wallis test results for necrosis and inflammation.

Degeneration	N	*p-value*	H	*Error*	*Hypothesis acceptance*
Necrosis	5	0,002	17,3	0,16%	Accepted
Inflammation	5	0,001	18,9	0,082%	Accepted

The Kruskal-Wallis test results showed a significant difference in the dependent variable between treatment groups for necrosis and inflammation. The p values of 0.001638 (P(x17.3693) = 0.9984) and 0.001, respectively, indicate a 0.16% probability of rejecting the hypothesis. This suggests that the administration of sidempuan salacca vinegar may improve the histopathology of male hypercholesterolemia. The Mann-Whitney test, which was conducted for P1 and P2, yielded a p value of 0.5067 (p(xZ) = 0.7466), indicating a 50.67% probability of error to accept the hypothesis. The results suggest that the administration of sidempuan salacca vinegar may have potential benefits in improving male hypercholesterolemia histopathology.

Table 3. Mann Whitney Intergroup Treatment Test.

Degeneration	N	*p-value*	Z	*Error*	*Hypothesis acceptance*
Inflammation	P1 with P2	0,73	0,34	73,37%	Denied
	P1 with P3	0,08	1,73	8,33%	Denied
	P2 with P3	0,23	1,19	23,2%	Denied
Necrosis	P1 with P2	0,25	1,13	25,68%	Denied
	P1 with P3	0,06	1,81	6,96%	Denied
	P2 with P3	0,27	1,1	27,03%	Denied

Discussion

After carrying out the one-way ANOVA test, it showed that there was a significant effect of salacca vinegar on the cholesterol levels of mice, so it was continued with a further DMRT test. Based on the results of further DMRT tests, it showed that on the 14th day after being treated with salacca vinegar, blood cholesterol levels between the treatment groups (P1, P2, and P3) were significantly different (α=0,05) compared to mice suffering from hypercholesterolemia without treatment with sidempuan salacca vinegar (K+) and mice that did not suffer from hypercholesterolemia without treatment with sidempuan salacca vinegar (K-). Blood cholesterol levels in the group of mice given sidempuan salacca vinegar were lower compared to the group of hypercholesterolemic mice without treatment given sidempuan salacca vinegar. In mice treated with sidempuan salacca vinegar (P1, P2, P3), group P3 was significantly different from groups P1 and P2, while groups P1 and P2 were not significantly different.

The increase in total blood cholesterol levels of mice in the K+, P1, P2, P3 treatments was due to the high fat content in chicken egg yolks, a chicken egg contains 31.8-35.5 fat (Pratama et al., 2018). Giving mice egg yolk for 14 days increased the mice's total cholesterol levels to 152.80 mg dL-1. This is in accordance with the theory which states that egg yolk can increase total cholesterol levels in the blood because the fat content in eggs is quite large (Pratama et al., 2018; Lai et al., 2010). The decrease in cholesterol levels in mice after giving sidempuan salacca vinegar was due to salacca vinegar contains ingredients that can inhibit the increase in cholesterol levels in the blood. Salacca vinegar is vinegar from salacca which has high functional capabilities and contains natural compounds. Research shows that the test results of salacca vinegar contain phenolic compounds, tannins, flavonoids, antioxidants, vitamin C, and acetic acid (Aisah, 2021). This is in line with research that reported active flavonoid compounds have many benefits for the body. One of them is that flavonoids can be used to lower cholesterol. In the body, flavonoids are able to

erode cholesterol deposits on the walls of coronary blood vessels (Widyaningsih et al., 2010; Karta et al., 2008; Nalole et al., 2009).

Improvements in tissue degeneration were also observed in the P2 and P3 treatment groups with an additional dose of salacca vinegar. Treatment group P2 received a dose of 0.4 mL of salacca vinegar, while treatment group P3 received 0.6 mL of salacca vinegar. This resulted in a decrease in the average degeneration score for both categories and a reduction in the incidence rate to a lower rate of degeneration. In the P2 group, no more samples with diffuse degeneration were found for the category of inflammation, and the average necrose degeneration score in P2 was lower than in P1.

A drastic decrease in the average degeneration score occurred with the administration of 0.6 ml of salacca vinegar, with no diffuse degeneration observed for either category. Only one sample had moderate-category degeneration, and four others indicated focal degeneration, with an average of 1.2 for both categories. The histopathological picture of the heart-scratched male hypercholesterolemia after treatment is seen in the following picture: degenerative necrosis and inflammation can be clearly observed, and samples from negative controls show a picture of normal heart histopathology.

The obtained histopathological picture is then analyzed to give a degeneration score. The scores obtained were then tested with the Kruskall-Wallis test to find out the significance of the correctional effects of the degeneration of the heart tissue in the male hypercholesterolemic given salacca vinegar. As a result, the p value is 0,005, which means there is significant improvement in the degeneration of heart tissue for both categories of necrosis and inflammation.

The Kruskall-Wallis trial's significance requires an advanced Mann-Whitney test to determine the significance between treatment groups of sidempuan salacca vinegar. The Mann-Whitney test for P1 and P2 yielded a p value of 0.5067, with a probability of error of 50.67%. The Mann-Whitney test for degenerative necrosis and inflammation showed p > values, indicating that the administration of salacca vinegar effectively repairs degenerative heart tissue and hypercholesterolemia. When the body experiences hypercholesterolemia, it will carry out additional metabolism to digest the excess cholesterol. This additional metabolism requires additional energy and resources. This inappropriate metabolism then forms ROS which leads to degeneration of the heart as an organ for digesting cholesterol. This is where salacca vinegar, which is known to be rich in antioxidants, works. The antioxidants in salacca vinegar will work as additional energy and resources so that even if there is additional cholesterol metabolism, ROS will not be formed which will initiate any form of degeneration in heart tissue. The difference in dose administration in this research did not significantly lower the average degeneration score. The treatment of both P1, P2, and P3 showed the same effect in improving the degeneration of male hypercholesterolemia. This is in accordance with research by Atifah and Diana (2024) which stated that giving sidempuan salacca vinegar can improve kidney degeneration in hyperuricemic mice.

CONCLUSIONS

Sidempuan salacca (*Salacca sumatrana*) vinegar effectively improves heart tissue degeneration, as demonstrated by histopathological data and histopathological observations. The effect increases with dose, indicating its effectiveness in reducing heart tissue degeneration.

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

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