International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

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Macassar Fruit Extract (*Brucea javanica*) Ameliorates Liver and Pancreatic Histopathology of Mice Fed Cholesterol Diet

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Abstract

Hypercholesterolemia is a condition of increased blood cholesterol levels characterized by increased levels of Low Density Lipoprotein (LDL) exceeds normal limits and decreased levels of High Density Lipoprotein (HDL). Hypercholesterolemia causes oxidative stress that can damage the liver and pancreas. *Brucea javanica* (BJ) extract contains antioxidants that can repair cell damage. The purpose of this study was to determine the effect of Macassar fruit extract on liver and pancreatic histopathology of mice that were fed a cholesterol diet. This study is an experimental study with the design of pre-post test control group design. The study subjects used 30 male Swiss Webster mice (*Mus musculus*). The samples were grouped into P0 (negative control group), P1 (dietary cholesterol), P2 (dietary cholesterol and BJ 10mg/kgBW), P3 (dietary cholesterol and BJ 20mg/kgBW), P4 (dietary cholesterol and BJ 30mg/kgBW), and P5 (dietary cholesterol and simvastatin). Laparotomy in organ harvesting on the last day and then made histopathological preparations. The preparation of liver and pancreatic organ preparations is carried out to observe and assessed of the histopathology of the liver and pancreas using scoring method. The data were analyzed by Kruskal-Wallis test and Mann-Whitney test. The results of the Kruskal-Wallis test on the liver and pancreas showed that there were differences in pancreatic islets of Langerhans damage scores between groups with a significance value $p < 0.05$. The results of histopathological observations and statistical tests on the liver explained that the administration of Macassar fruit extract doses of 10mg/kg and 20mg/kg have not improved histopathological and statistical liver damage. The best results on liver histopathology were given a dose of 30mg / kgBB which gave a significant improvement effect histopathologically and statistically with a microscopic picture of the liver close to normal conditions (P0). Different results were shown in histopathological observations and statistical tests of pancreatic organs which explained that the administration of macassar fruit extract doses of 10mg/kgBW and 20mg/kgBW provide improvements in histopathological picture but have not shown statistically significant improvements. Administration of 30 doses of *Brucea javanica* extract gave excellent results that resembled normal conditions in Group P0.

Keywords: Macassar fruit (*Brucea javanica*), Cholesterol Diet, Liver Histopathology, Pancreatic histopathology

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1. Introduction

Hypercholesterolemia is a condition of increased blood cholesterol levels characterized by increased levels of Low Density Lipoprotein (LDL) exceeds normal limits and decreased levels of High Density Lipoprotein (HDL) [1]. High Density lipoproteins and Low Density lipoproteins are mostly produced by the liver, which functions as a cholesterol-carrying protein in the blood. High Density lipoproteins bind and remove cholesterol from the bloodstream to be returned to the liver, while Low Density lipoproteins carry cholesterol to all parts of the body. Cholesterol is produced by the body in normal amounts in many tissues and participates in the synthesis of all other steroids in the body, including corticosteroids, sex hormones, bile acids, and vitamin *D* [2]. In 2017, high non-HDL cholesterol was responsible for an estimated 3.9 million deaths worldwide, half of which occurred in east, southeast, and South Asia [3]. Basic Health Research in 2018 showed

that in Indonesia, total cholesterol levels in the population aged ≥ 15 years, 21.2% were at the threshold (200-239 mg/dl), and 7.6% were included in the high category (≥ 240 mg / dl) [4]. Liver and pancreas are organs that will be used in this study because it has many functions, namely as a regulator of the body's homeostasis, metabolism, biotransformation, synthesis, storage, and immunology [5]. The high concentration of LDL cholesterol in the blood causes Non-Alcoholic Fatty Liver Disease (NAFLD) where there is an increase in free radicals to oxidative stress in the liver in inducing changes in lipid, protein, and DNA content that cannot be repaired, causing disruption of the normal physiology of the liver [6]. The pancreas is also one of the organs that can be a place for fat accumulation. One of the diseases of the fatty pancreas is Non Alcoholic Fatty Pancreas Disease (NAFPD). NAFPD disease is an accumulation of pancreatic fat associated with a high-fat diet and obesity in the absence of significant alcohol consumption [7]. In NAFPD disease, the body's levels of free fatty acids increase significantly. The increase in free fatty acids causes the accumulation of fat in the pancreas, which triggers disturbances in the function of beta cells. Impaired beta cell function causes NAFPD condition to become diabetes mellitus (DM) .High-fat Diet is a risk factor for pancreatic fat accumulation, inflammatory cell infiltration, and apoptosis that can lead to type 2 diabetes mellitus [8].

Currently, therapeutic treatment of high cholesterol levels or hypercholesterolemia can be done with lifestyle modifications such as structured exercise and proper diet [7]. Synthetic drugs such as simvastatin are reported to improve the condition of Hypercholesterolemia in NAFLD and NAFPD. Although it can be a therapeutic option, these synthetic drugs have side effects on the body. Therefore, replacement therapy in the form of herbal plants containing natural components is required in the treatment of the disease. One of the herbs that can be used is the Macassar fruit (*Brucea javanica*). Macassar fruit (*Brucea javanica*) is found in many Asian countries, one of which is Indonesia. Macassar fruit grows in wild forests or in the yard of the house for hedges and is widely used as a traditional medicine for digestive problems and lowering cholesterol levels [9]. In addition, Macassar fruit has also been studied to have anticancer effects on the pancreas and liver organs [10]. Macassar fruit contains flavonoids, phenols, tannins, antioxidant capacity and vitamin C. Antioxidants have an influence on reducing the atherogenic index. The content of flavonoids is an antioxidant that can break the chain of unsaturated fatty acids so as to prevent oxidative stress [11]. It is this antioxidant effect that helps in limiting inflammation, reducing cholesterol levels, and avoiding the adverse effects of free radicals to lower the risk of certain diseases.

2. Materials and methods

The design of this study using experimental study design pre post test control group using experimental animals to determine the effect of Macassar fruit extract (*Brucea javanica*) on hepatic histopathology of mice (Mus musculus) fed a cholesterol diet. Samples were grouped into negative control group (P0), positive control group (P1, dietary cholesterol), group P2 (dietary cholesterol and BJ 10mg/kgBB), group P3 (dietary cholesterol and BJ 20mg/kgBB), group P4 (dietary cholesterol and BJ BJ 30mg/kgBB), and P5 group (cholesterol diet and simvastatin). This group of subjects will have their weight measured to meet the inclusion criteria and cholesterol levels measured before and after treatment. The data obtained from the observation of histopathology on all samples, mouse weight, and liver weight were processed statistically using Statistical program Service Solution (SPSS). Weight of mice univariate test data using the normality test with the saphiro-Wilk test to determine the distribution of data and test the homogeneity of the Levenne test variants. The distribution of the data is said to be normal and the variance of the data is said to be homogeneous if the value of p>0.05. Next will be the calculation of central tendency using descriptive statistics. Bivariate test for mouse data and liver weight is done by one way ANOVA parametric test with the condition that the data is normally distributed and homogeneous variants, but if the data distribution is not normal then it will do the data transformation so that the data distribution becomes normal. If the distribution is not normal even had done the transformation, then, as an alternative is to use a non-parametric test Kruskal-Wallis continued with the Mann-Whitney Post-Hoc test. Furthermore, the picture data will be done correlation test to see the difference between the histopathology of hepatic Mus musculus between treatment groups by using Spearman correlation test.

3. Results and Discussion

3.1. Cholesterol Levels

The results of cholesterol levels in all groups will be tested for normality with the Shapiro-Wilk test. Normality test results obtained $p > 0.05$ so it is concluded that the data is distributed normally. In the homogeneity test with Levene's test, the result is $p = 0.004$ ($p < 0.05$) which shows inhomogeneous data variants. The data cannot be tested with One Way ANOVA but tested with an alternative test in the form of Kruskal-Wallis. Then proceed with the Mann-Withney post hoc test to see meaningful differences between treatment groups. On the first day, the initial cholesterol level was checked, then the cholesterol level was checked again on the 14th day to check whether the cholesterol had exceeded >128mg/dL so that the mice were said to have hypercholesterolemia, and the last cholesterol level was checked again on the 28th day to see if there was a decrease in cholesterol levels after being given Macassar fruit extract (*Brucea javanica*) and simvastatin. Acclimatization of mice would be done before the administration of cholesterol treatment on 7 days before. After acclimatization, the mice were grouped into 6 groups that each group consisting of 5 tails, then given induction of high cholesterol diet for 14 days in groups P1, P2, P3, P4, and P5. Then for 14 days given Macassar fruit extract (*Brucea javanica*) for group P2, P3, and P4 and simvastatin administration in Group P5.

Table 1. Swiss Webster (Mus musculus) Pre and Post Cholesterol Data

	Cholesterol Levels		
Group	Day 0 (mg/dL) Mean	Day 14th (mg/dL) Mean	Day 28th (mg/dL) Mean
P0	106,6 ± 3,13	107 ± 5	108,6 ± 3,20
P1	107,4 ± 5,03	155,4 ± 14,46	-
P2	109,6 ± 4,03	150,8 ± 7,62	110,8 ± 5,16
P3	106,4 ± 4,33	144,8 ± 12,39	110 ± 3,08
P4	108,2 ± 3,63	146,8 ± 13,98	105,2 ± 2,77
P5	106,8 ± 3,34	144,2 ± 8,04	110,2 ± 2,86

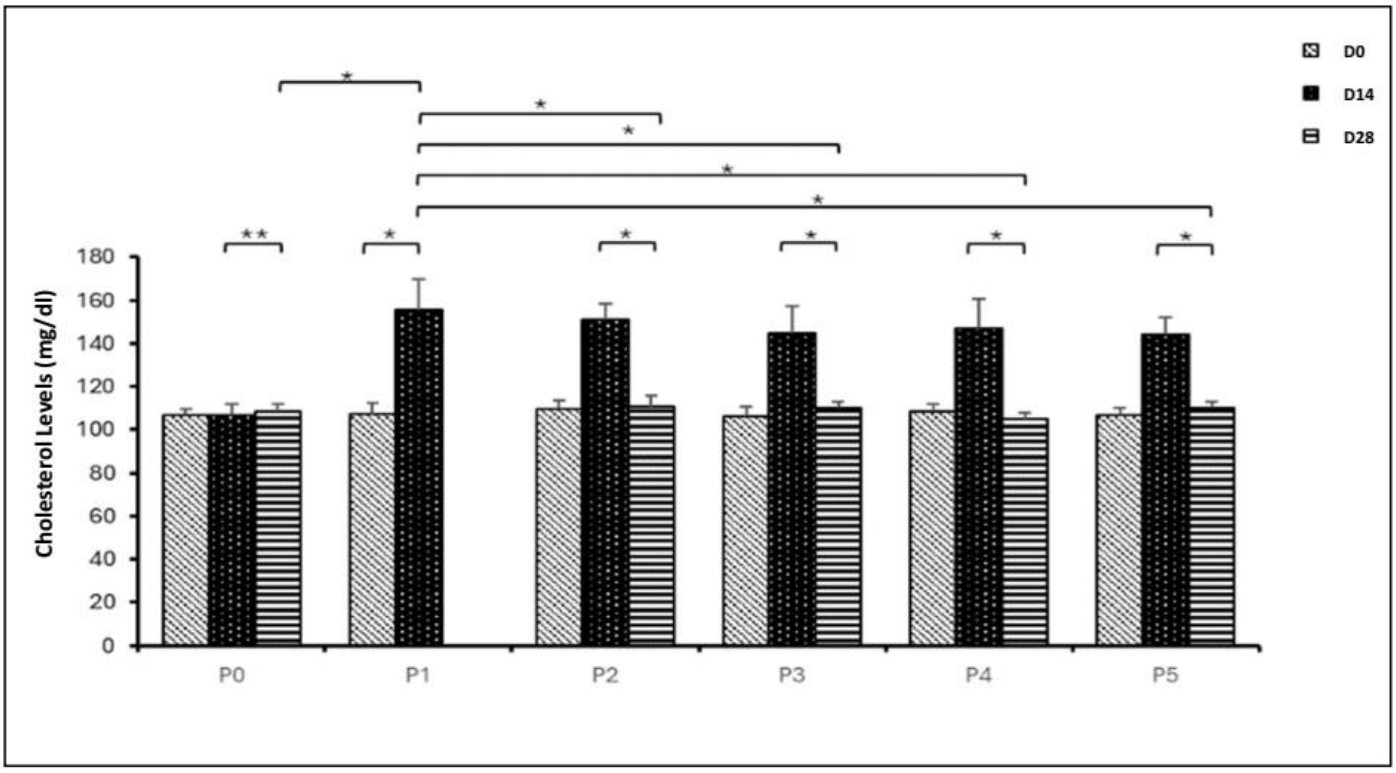
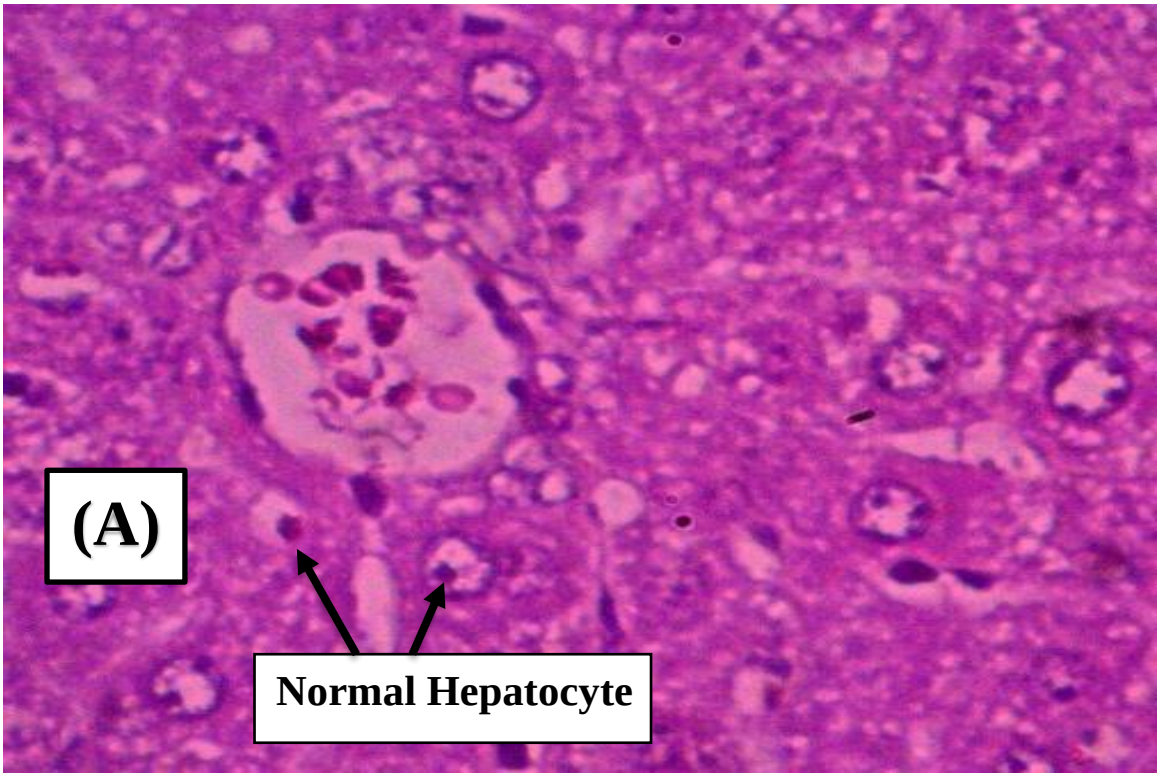
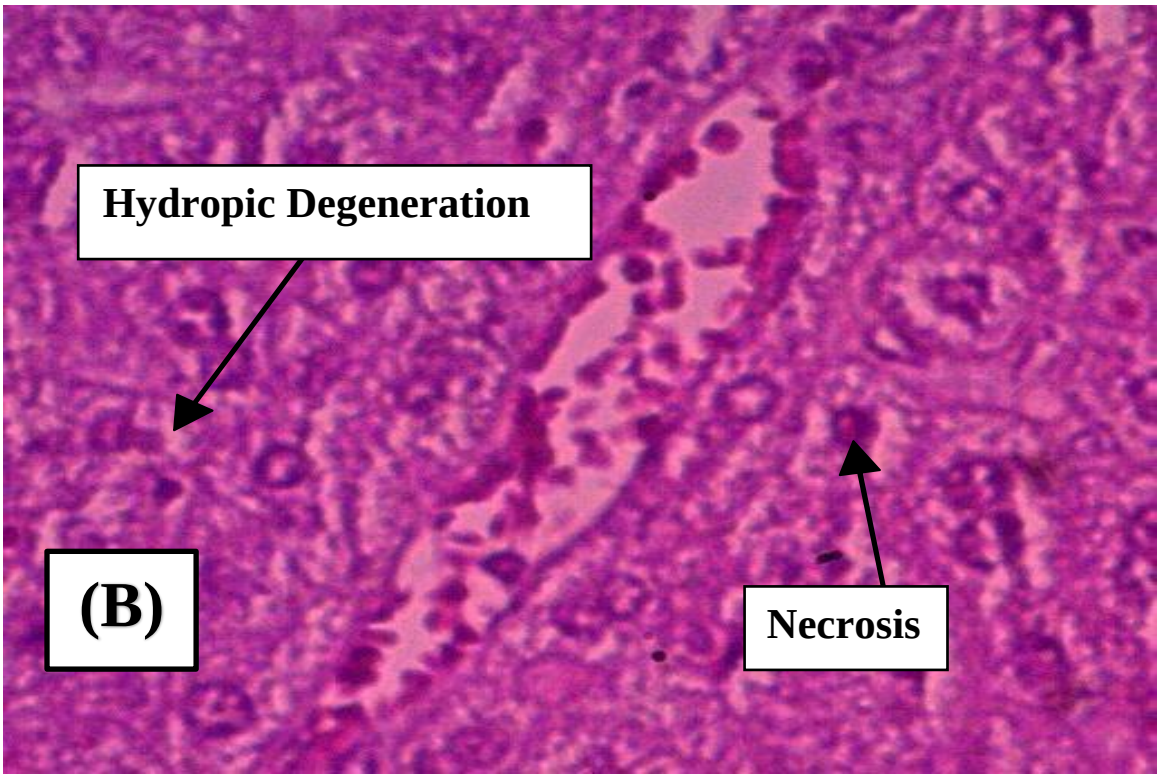


Figure 1. Mann-Withney Test Results on Cholesterol Levels

Description: *= Mann-Whitney test, there is a significant difference, $p < 0.05$; **= Mann-Whitney test shows no significant difference, $p > 0.05$.


(A)
Normal Hepatocyte


Hydropic Degeneration
(B)
Necrosis

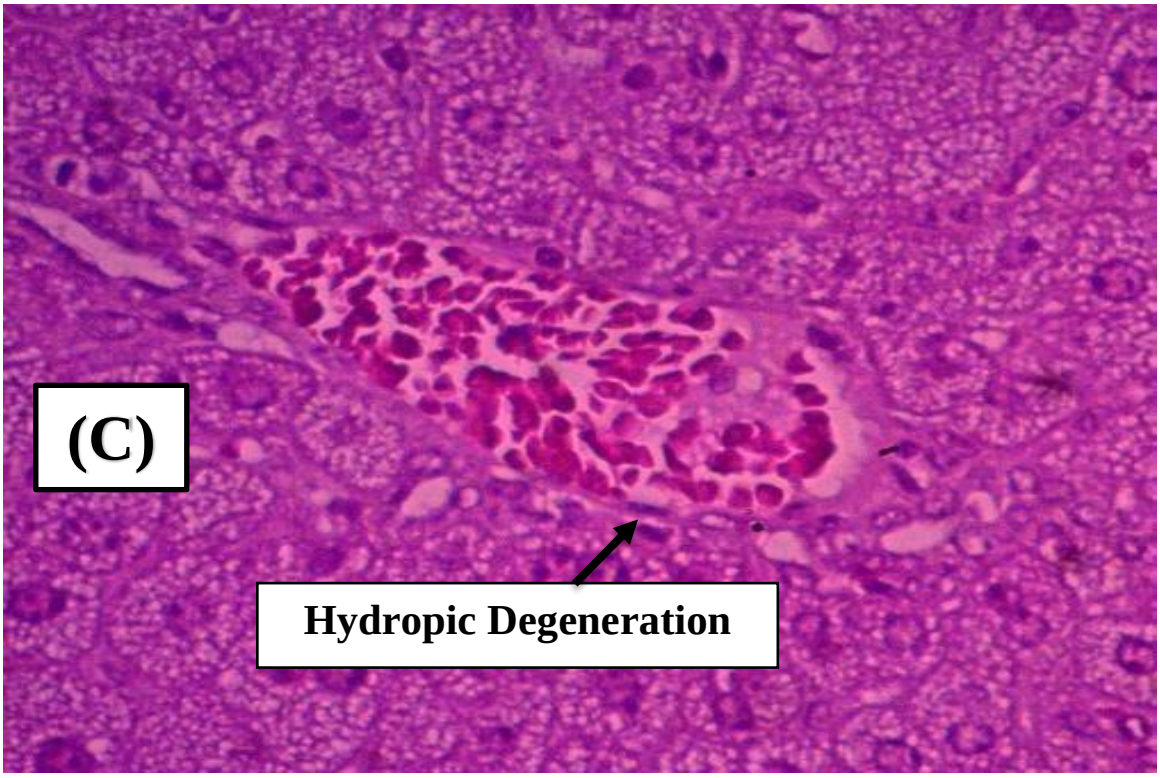


Figure 2. A: Normal Liver Cell; B: Degeneration and Necrosis Liver Cell; C: Degeneration Liver Cell

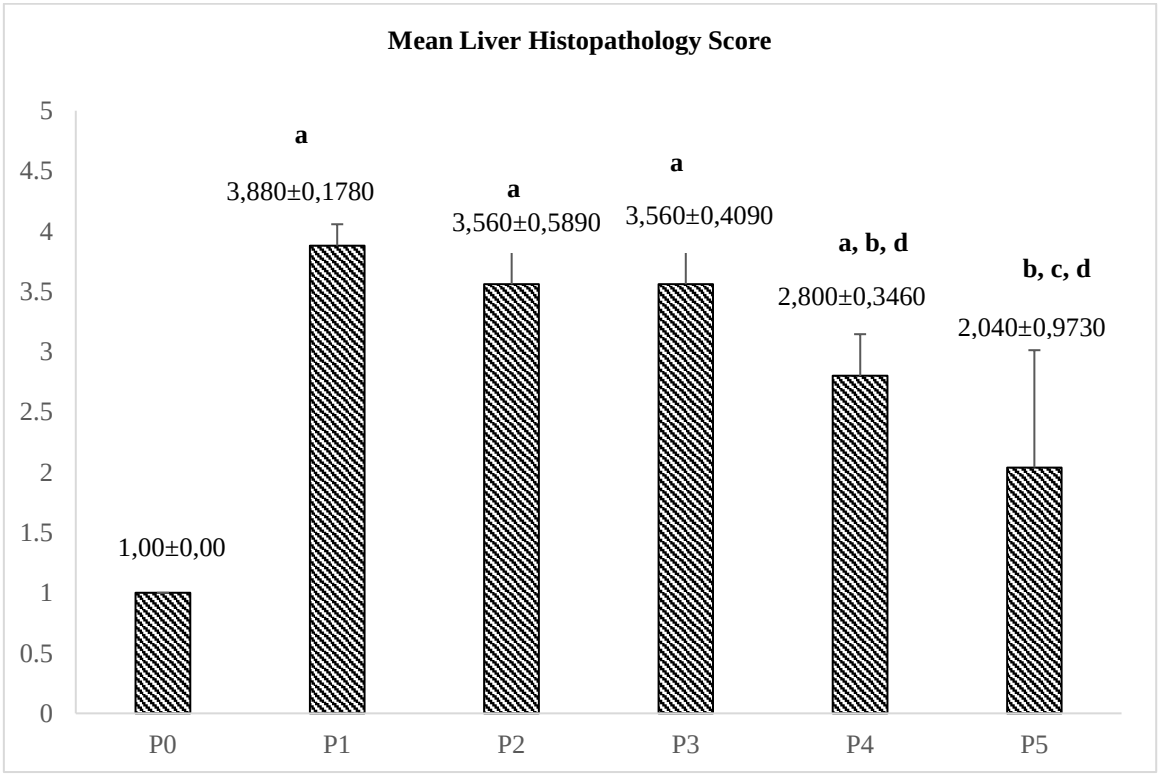
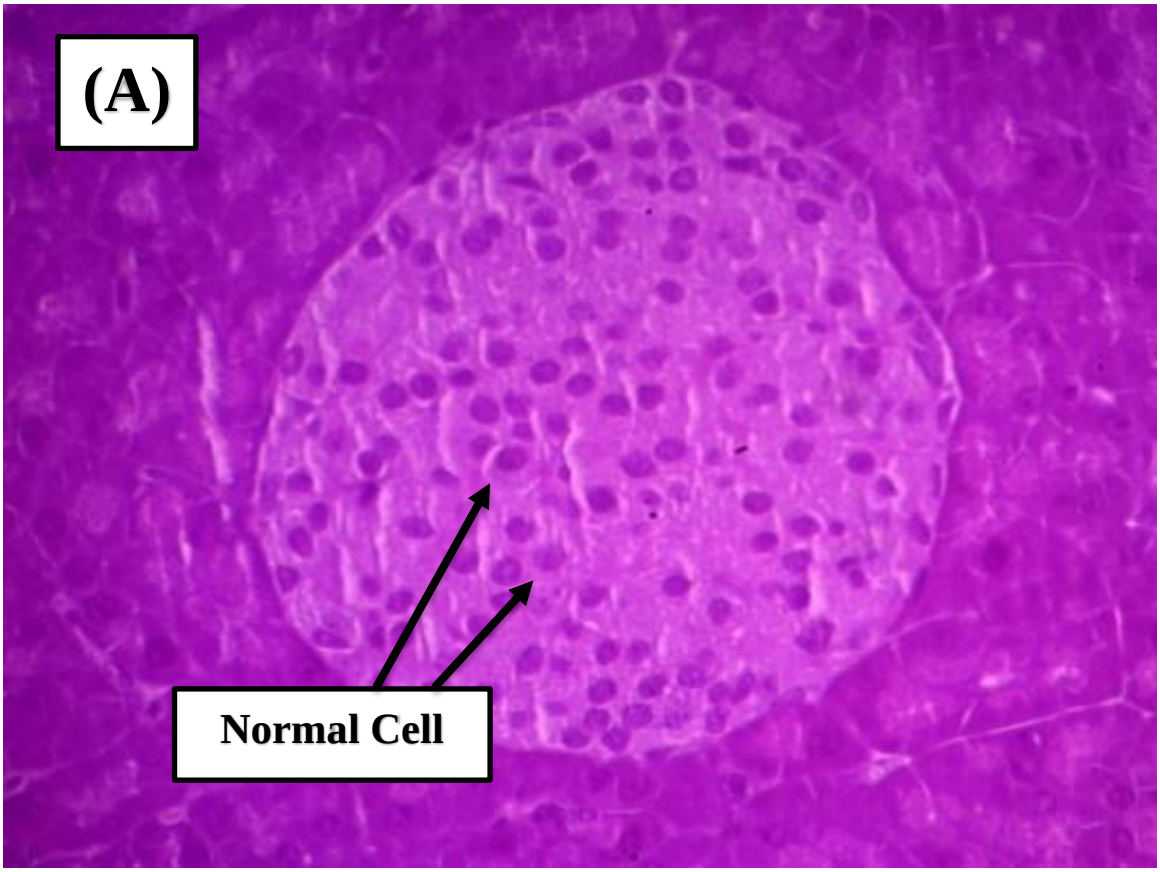
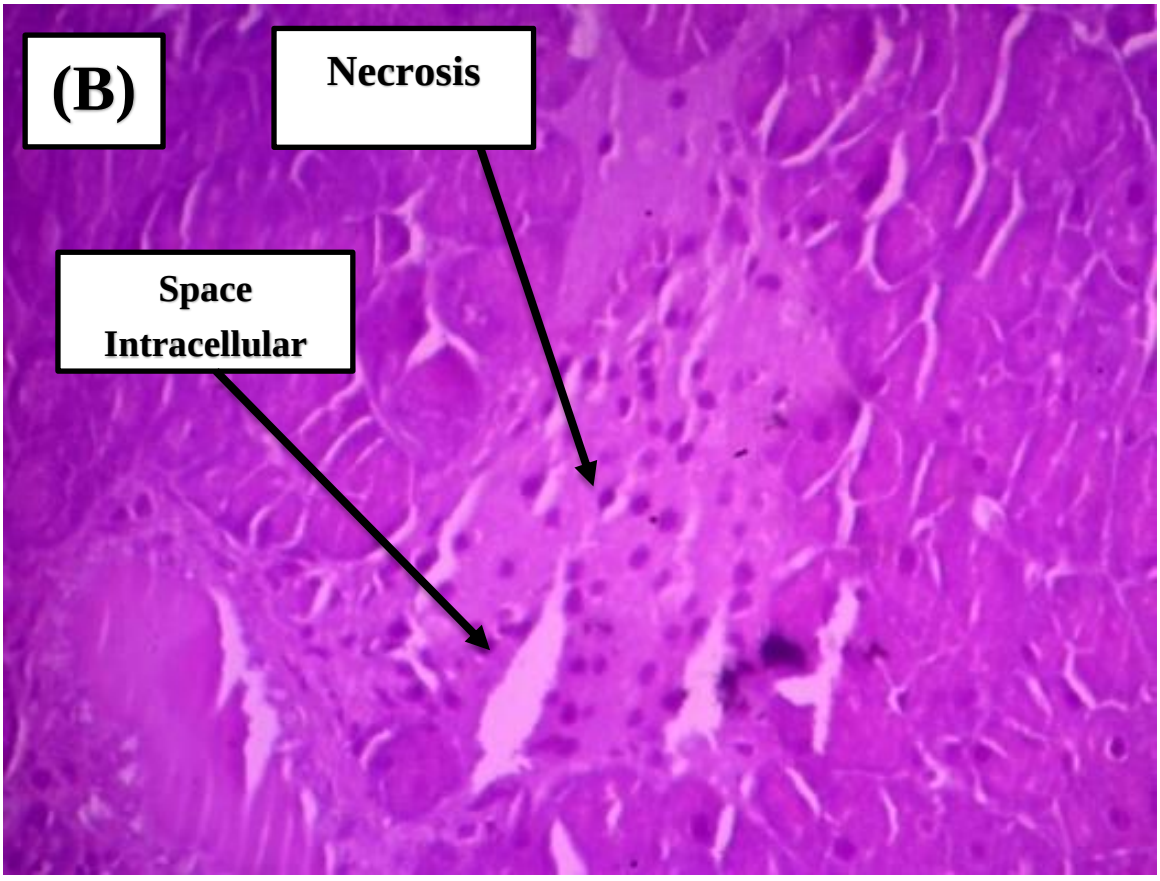


Figure 3. Graphic of Mann-Withney Test Result on Liver Histopathology

Description: a: Significantly different with P0; b: Significantly different with P1; c: Significantly different with P2; d; significantly different with P3


(A)
Normal Cell


(B)
Necrosis
Space
Intracellular

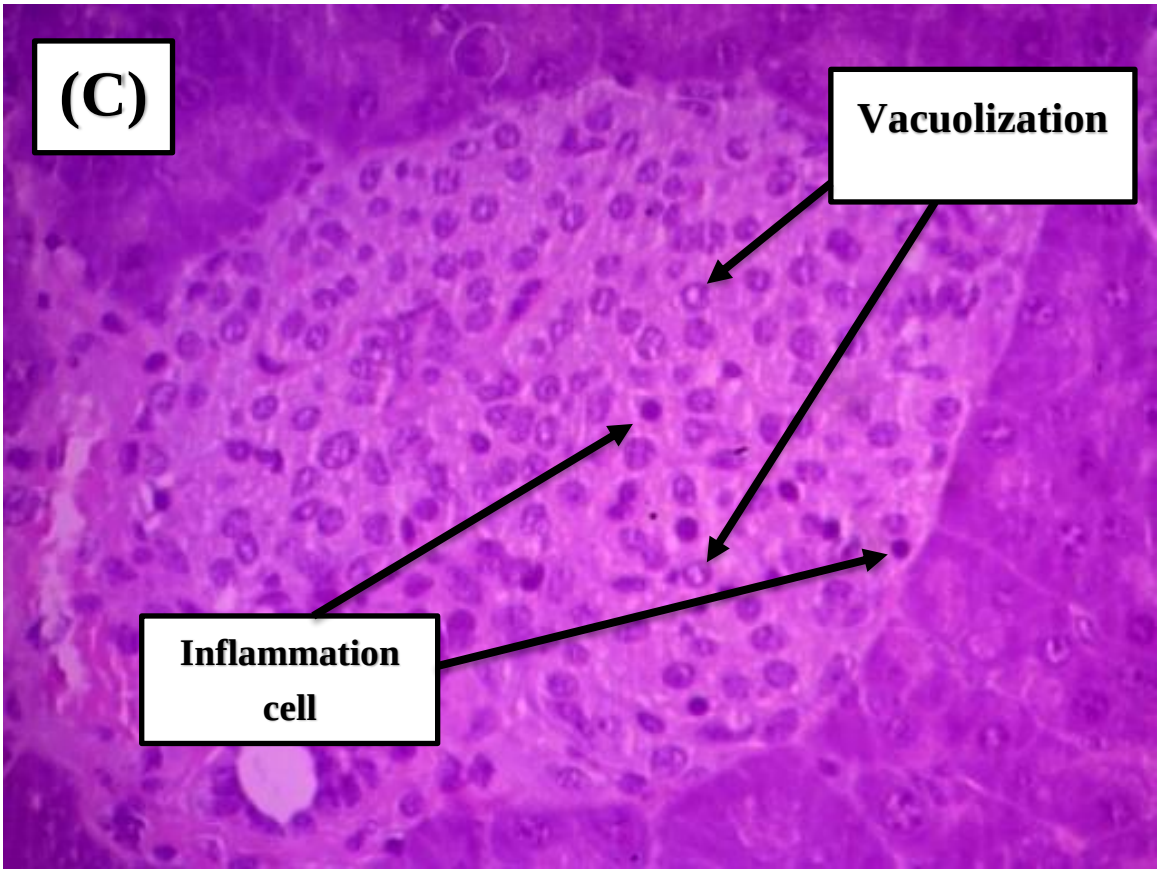


Figure 4. A: Normal Pancreatic Cell; B: Necrosis Pancreatic Cell; C: Vacuolization and Inflammation Cell

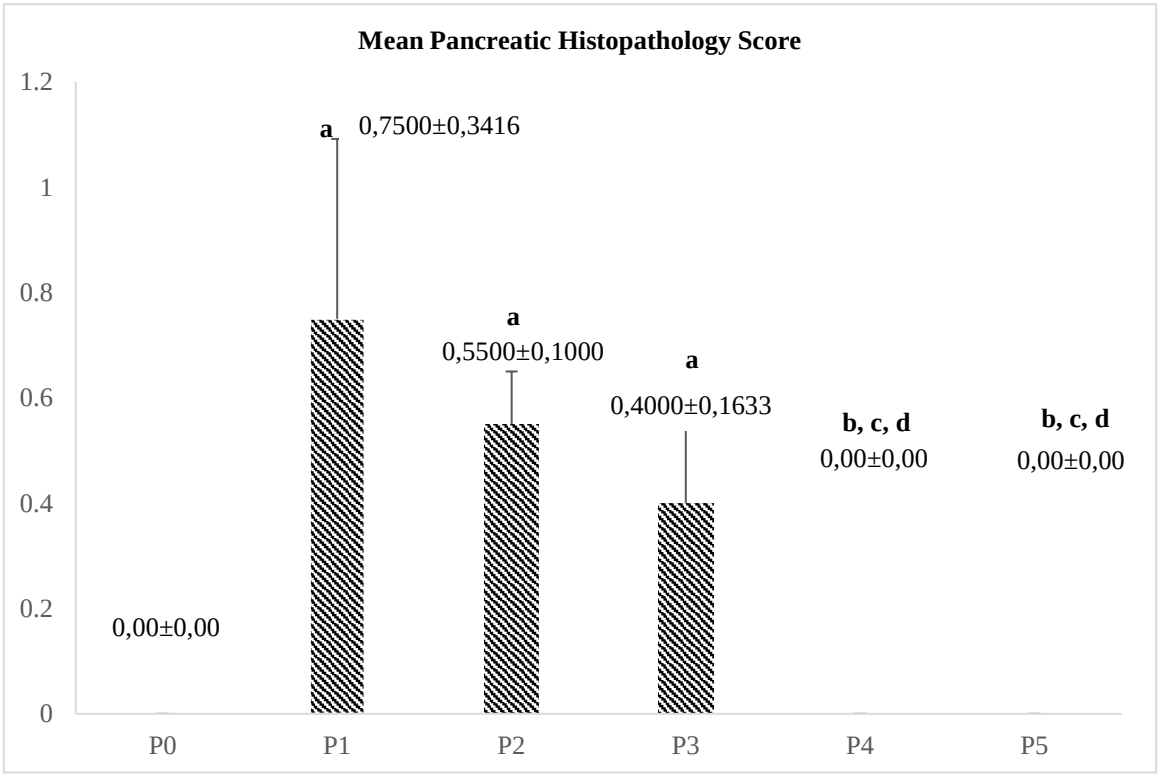


Figure 5. Graphic of Mann-Withney Test on Pancreatic Histopathology
Description: a: Significantly different with P0; b: Significantly different with P1; c: Significantly different with P2; d; significantly different with P3

Based on the Table 1, there is an increase in total cholesterol levels in groups P1, P2, P3, P4, and P5 caused by feeding consistently high cholesterol every day for 14 days to affect cholesterol levels. After checking cholesterol in the P1 group on Day 15, then the P1 group was immediately terminated because of the risk of cell regeneration in the liver. Then followed by administration of Macassar fruit extract for 14 days in Group P2 (dose 10mg/kgBB), P3 (dose 20mg/kgBB), P4 (dose 30mg/kgBB), and administration of simvastatin drugs in Group P5, and on the 28th day of checking cholesterol levels again. In checking the obtained reduction in cholesterol levels in groups P2 (giving fruit extract Macassar 10mg/kgBB), P3 (giving fruit extract Macassar 20mg/kgBB), P4 (giving fruit extract Macassar 30mg/kgBB), and P5 (giving simvastatin 0.08 mg/30gBB), where the highest reduction in cholesterol levels is in Group P4 by giving fruit Macassar 30mg/kgBB which lowers cholesterol levels of 146.8 to 105.2 mg/dL. The results of the Mann-Whitney statistical test (Figure 1) proved that there was a significant difference in total cholesterol values between the P0 group (normal control) and the P1 Group (positive control) with a p value<0.05. It can be concluded that feeding high cholesterol for 14 days can increase the value of total cholesterol in the blood until it reaches the stage of hypercholesterolemia. Then there is a significant difference between the group P0 (normal control) with P2, P3, P4, and P5 with P>0.05 and there is a significant difference between the group P1 (positive control) with P2, P3, P4, and P5 with p<0.05. Therefore, it can be concluded that the administration of Macassar fruit extract (*Brucea javanica*) with a dose of 10mg/kgBB, 20mg/kgbb, and 30mg/kgbb and simvastatin Drug Administration can reduce total cholesterol values from hypercholesterolemia to normal cholesterol values resembling normal control group (P0).

3.2. Liver Histopathology

Histopathological picture in Group P0 shows the condition of normal liver cells. In Group P0 showed histopathological picture with the highest degree of damage in the presence of degenerated cells and necrosis. Kruskal-Wallis statistical test to see statistically significant differences in all groups. The results of the Kruskal-Wallis test obtained $p = 0.000$, which shows there are significant differences between the treatment groups, followed by Mann Whitney post hoc test to see significant differences between the two groups. In the Mann-Whitney test, it was found that there was a significant difference between the P0 Group (negative control) and the P1 Group (positive control) with a p value < 0.05. Therefore, it can be said that feeding high cholesterol for 14 days has an effect on liver damage Swiss Webster mice (Mus musculus), shown by the damage to hepatocyte cells around the central vein with a picture of parenchymatous degeneration, hydrophic degeneration, and necrosis in hepatocytes cells. Comparison in Group P1 (positive control) with P2 (administration of Macassar fruit dose of 10mg / kgBB) and P1 (positive control) with P3 (administration of Macassar fruit dose of 20mg / kgBB) has no significant difference ($p > 0.05$). This shows that the administration of Macassar fruit dose of 10mg / kgBB in Group P2 and administration of Macassar fruit dose of 20mg / kgBB in Group P3 cannot repair damage to hepatocyte cells in the area around the central vein. Comparison between group P1 (positive control) and P4 (administration of Macassar fruit dose 30mg/kgBB) has significant differences ($p < 0.05$), but in comparison with P4 group P0 (negative control) there are significant differences, it was concluded that the administration of Macassar fruit (*Brucea javanica*) with a dose of 30mg/kgBB can repair liver damage seen from the improvement of histological picture but not statistically. Comparison between group P1 (positive control) and P5 (simvastatin Drug Administration) has significant differences ($p < 0.05$), and the comparison between group P0 (negative control) with group P5 has no significant differences, so it can be concluded that the administration of simvastatin drug in Group P5 can improve liver damage statistically and histological picture. In this study proved that using a higher dose of Macassar fruit (*Brucea javanica*) could increase the rate of improvement in the liver. Therefore, it is necessary to do further research by using a higher dose of Macassar fruit to find an effective dose that can repair liver damage statistically to resemble normal (negative control). In a study conducted by [12] examined the toxic dose of Macassar fruit extract (*Brucea javanica*) with research results showing indications of safe use of doses up to 2000 mg/kgBB where there are no clinical signs of toxicity in the study.

3.3. Pancreatic Histopathology

Histopathological picture in the negative control group (P0) with standard feeding had a normal microscopic picture of the uniform cell shape and clearly granulated cell nuclei. The positive control group (P1), which is the group given high cholesterol feed has a microscopic image of the islets of Langerhans with the highest degree of damage. The involvement of cholesterol in the destruction of the cells of the islets of Langerhans was proved in the study of [13] who stated a diet high in cholesterol causes beta cell apoptosis, degeneration of islets of Langerhans cells, adipocyte accumulation, and cell vacuolization. High levels of cholesterol in the body provoke an increase in fatty acids in the body. An increase in fatty acids leads to the accumulation of excess fat in various organs, one of which is the pancreas. The accumulation of pancreatic fat will cause the disease of Non Alcoholic Fatty Pancreas Disease (NAFPD) [14]. The Mann-Whitney test results obtained most of the significant differences in the two groups (Figure 5). Group P0 (negative control) with group P1 (positive control) Mann-Whitney test results $p = 0.018$ ($p < 0.05$) so that between the two groups showed a significant difference. Based on these results, Group P1 (positive control) showed a picture of damage to the islets of Langerhans when compared to group P0 (negative control). Comparison in Group P0 to treatment group P2 and P3 showed significant differences, but not with group P4 and P5 ($p > 0.05$). This showed that the treatment groups P2 and P3, each of which was given Macassar fruit extract *(Brucea javanica*) doses of 10mg/kg and 20mg/kg, did not provide statistically significant improvement. In contrast to the P4 treatment group, which showed that the administration of Macassar fruit extract (*Brucea javanica*) at a dose of 30 mg/kgBB was able to significantly repair damage to the islets of Langerhans due to a high cholesterol diet. Statistical tests in Group P5 to group P4 showed no significant difference. This explains that the administration of

simvastatin can provide a repair effect as good as that of the P4 group. The results of this study explain that the administration of Macassar fruit extract (*Brucea javanica*) can repair damage to the pancreatic islets of Langerhans due to high cholesterol diet with an effective dose obtained is 30mg/kgBB. In a study conducted by [13] who examined pancreatic damage due to high cholesterol diet explained that pancreatic damage due to dietary cholesterol is not only found in the islets of Langerhans but also involves cells outside the islets of Langerhans. Thus, further research is needed on the improvement effect that Macassar fruit extract can provide on the overall picture of pancreatic histopathology.

4. Conclusions

High cholesterol diet causes damage the liver and pancreas. In this study damage to the liver and pancreas due to high cholesterol diet will be treated using a multilevel dose of Macassar fruit extract. The results of this study concluded that the administration of Macassar fruit extract has not found an effective dose for improvement in liver damage. The result was different in histopathological observations and statistical tests of pancreatic organs, the administration of Macassar fruit extract at a dose of 30 mg / kgBB can have a significant improvement effect on pancreatic damage.

Acknowledgements

This research has been conducted in Research Laboratory of Faculty of Medicine and Health Sciences, Bengkulu University, Anatomical Pathology Laboratory of M. Yunus Hospital, and SBIH Ruyani.

Ethical Approvals

The ethical clearance of this study was permitted by the Bengkulu University Health Research Ethics Committee number 92/UN30.14.9/LT/2023.

Declaration of Interest Statement

The authors assert that they have no conflicts of interest.

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