The Effect of Pagoda Flower Extract (*Clerodendrum Paniculatum*) on Liver Function and Histopathological Features of the Liver of White Rat Strains Male Wistar Infected with Leptospira Interrogans Bacteria

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ABSTRACT

This study was conducted to test whether the effect of pagoda flower extract (*Clerodendrum Paniculatum L.*) on liver function and histopathological features of the liver of male Wistar white rats infected with *leptospira interrogans* bacteria. This study used 24 experimental animal samples divided into 4 groups, namely the control group, treatment (P1, P2, P3) with different treatments. The results of the phytochemical test of pagoda flower extract (*Clerodendrum paniculatum L.*) has secondary metabolite content such as alkaloids, flavonoids, saponins, and tannins. On the 14th day of observation, the treatment obtained significant results to restore normal SGPT liver function in treatment group 1 with a value 30.17 ± 2.9, treatment 2 with value25.17 ± 2.92, pass 3 with a value of 20 ± 2. SGOT levels became normal in treatment group 2 with values77.5±13.3 and treatment group 3 with a value of62.3 ± 11.4. The histopathology results with a score of 1 which states normal liver function is in treatment group 3 (P3) with a dose of 200mg/kgBB pagoda flower extract. The results of the data analysis have been normally distributed with p>0.05.

Keywords: *Pagoda flower, Liver, Leptospira, SGOT, SGPT*

INTRODUCTION

The liver is the largest and most complex gland in the body, reddish brown in color, does many things to help digest food and metabolize nutrients in the digestive system. For some people, the function of the liver is only related to the digestive process, however, its function is very important for the body, from destroying toxins in the blood to helping the digestive process, it is very important to always maintain liver function. In excessive amounts, toxic substances can cause various types of liver damage, such as liver degeneration and necrosis.Liver damage can also be caused by bacteria through leptospirosis. Leptospirosis is

a zoonotic disease that can be transmitted and also cause an outbreak if prevention efforts are not carried out as early as possible. Leptospirosis is a disease caused by the bacteria Leptospira interrogans. This bacteria can spread through the urine or blood of infected animals. The worst possibility of liver damage through this bacteria can cause liver failure.Mild types of leptospirosis usually do not require special treatment. In severe cases, treatment is aimed at relieving symptoms and preventing complications. Histopathology examination is one way to see liver damage. This is because it allows to see the morphology and histological structure that changes and the level of damage to the liver organ. Some drugs that can be given include antibiotics, such as penicillin and amoxicillin, and others. Several types of herbal plants that contain antioxidants and antibacterials, one of which is pagoda leaf extract (*Clerodendrum Paniculatum L*). Antibacterial is a substance that has the ability to stop or even kill bacteria by stopping the metabolism of harmful microbes. Pagoda Flower Plant (*Clerodendrum paniculatum L.*) functions as an anti-aging, antioxidant, anti-inflammatory, anticancer, and antibacterial

LITERATURE REVIEW

The liver is an important organ of the body that is responsible for the metabolism of toxic substances. This can cause the liver to be an organ that is often targeted by toxicants, because toxic substances that enter the body through the gastrointestinal system are then absorbed and carried by the portal vein to the liver.(Huda MN, 2017). The liver is also the center of lipid metabolism, it extracts and processes lipids from food and is the main site of formation of lipoproteins, triglycerides, and cholesterol. The liver is also the main site of gluconeogenesis, which is important for maintaining blood glucose levels in the fasting state.(Yip, 2021). Imbalance in liver metabolism causes oxidative stress mainly caused by free radicals or called reactive oxygen species (ROS). Prolonged exposure to ROS without proper treatment causes severe liver damage and serious liver diseases including cirrhosis.(INE Lister, 2019). Another major function of the liver is the synthesis of bile acids from cholesterol, with secretion of these compounds into the bile, which facilitates the absorption of dietary fats and fat-soluble vitamins.(Moriles KE, 2022). Liver damage can also be caused by bacteria through leptospirosis disease. Leptospirosis is a zoonotic disease that can be transmitted and also cause an outbreak if prevention efforts are not carried out as early as possible.(Ministry of Health Regulation, 2010).

Leptospira bacteria are commensals in the kidneys and liver of mammals, including mice. (Wening Widjajanti, 2020). The worst possibility of liver damage through this bacteria can cause liver failure.There are no symptoms shown by Leptospira infection, as occurs in the manhole rat *Rattus norvegicus*.(Arief Mulyono, 2020). Based on the description, *Leptospira bacteria*

that are easily transmitted to mice are something that can be studied further in the function of organs in mice such as the liver.

If you are infected with this bacteria, one way to cure it is to take medications to relieve the symptoms and stop the bacterial infection.(Sri Wahyuni Nasution et al, 2021). Some drugs that can be given include antibiotics, such as penicillin and amoxicillin, and others.Some types of herbal plants that contain antioxidants and antibacterials, one of which is pagoda leaf extract (*Clerodendrum Paniculatum L*). In the study(Ihsanul Hafiz, 2019)Ethanol extract of pagoda flowers has anti-inflammatory properties at a dose of 100 mg/kg BW given to the sample.The pagoda flower plant (*Clerodendrum paniculatum L.*) is a species of the genus Clerodendrum and belongs to the verbenacea family which has many types of secondary metabolites, including alkaloids, flavonoids, steroids, terpenoids, saponins, and other phenolic derivatives. (KF Barus, 2013).Antibacterials are substances that have the ability to stop or even kill bacteria by stopping the metabolism of harmful microbes.(Nurhamidin SJ, 2022). Pagoda Flower Plant (*Clerodendrum paniculatum L.*) functions as an anti-aging, antioxidant, anti-inflammatory, anti-cancer, and antibacterial.(Banne et al., 2023).

METHODS

Laboratory experimental research is a type of research used in examining the effectiveness of pagoda flower extract on the liver function of male white rats (*Rattus norvegicus*) that have been infected with Leptospira Interrogans. The research design uses a post-test with control group design or controls the sample based on the treatment group. The research site was the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara in May - July 2024. Ethical Clearance was Aproved to the Health Research Ethics Commission Universitas Prima Indonesia (Approval No. [060/(KPEK-FKKGIK/2024). The sample in this study was 24 male rats which will be divided into 4 groups. Control group (K) treatment 1,2,3 (P1,P2,P3) which will each be given pagoda flower extract with a dose of 100gr, 150gr and 200gr. mice will be infected with bacteria through injection, then wait 24 hours after the induction of Leptospira Interrogans bacterial infection. Blood serum is tested positive for *Leptospira bacteria* if particle agglutination occurs on Leptospira antigens. Then the treatment was carried out in week 2 after the acclimatization process and preparation of test animals. Toxic symptoms observed were SGOT-SGPT examinations performed on day 7, day 14 and day 21, weighing of liver organ weights, macroscopic examination, histopathology (microscopy) of liver tissue performed on day 21. Then the data will be presented descriptively with the data normality test analyzed using the Kolmogorov smirnov approach ($p > 0.05$). Testing of significance between test groups was

carried out using the one-way analysis of variance technique or One Way ANOVA at a 95% confidence level ($p < 0.05$) (Ghozali, 2018).

RESULTS

This study used 24 male rats (*Rattus norvegicus*) of the Wistar strain that had been infected with *Leptospira bacteria* by injection once and the certainty of the rats being infected was checked using serological examination of rat blood serum. After that, the rats' body weight was examined before and after infection. It was seen that the average body weight of the rats decreased after 14 days of being induced by Leptospira interrogans bacterial infection. It was seen that the group that experienced the most drastic decrease was the Control group (P0) with an average difference of 23.16 grams from the average weight of the rats before being infected with the bacteria. This indicates a reaction to the increase in body weight of rats that had been infected with the bacteria. After ensuring that all mouse samples have been infected with bacteria, the next step in this study is to observe liver function through the levels of SGOT-SGPT in mice and provide treatment through pagoda flower extraction to mice infected with Leptospira interrogans bacteria and re-examine liver function through histopathological observations of mouse livers.

Before giving treatment, secondary metabolite testing was carried out using a phytochemical test with the results of pagoda flower extract (*Clerodendrum paniculatum L.*) positive for containing active compounds such as alkaloids, flavonoids, saponins, and tannins. The extract treatment was given for 14 days with a predetermined dose. Group K without treatment, p1 with 100 gr, P2 with 150 gr, P3 200 gr pagoda flower extract. Then, after the mice were given treatment in each group, then the mice were taken blood samples to see their liver function with the results of the SGOT-SGPT level test with the results of the SGOT value that had shown normal results were in treatment group 2 (P2) with a value of 77.5 ± 13.3 then in treatment group 3 (P3) with a value of 62.3 ± 11.4. For group P1, the SGOT value had exceeded the normal limit and received an average value and standard deviation of 90.5 ± 22.3. while the SGPT value with the average results and standard deviations that obtained normal values were in group P1 30.17 ± 2.9 then group P2 with a value of 25.17 ± 2.92 and P3 20 ± 2. Meanwhile, the control group had not obtained normal liver function results with SGOT values of 115.17 ± 17.05 and SGPT 46.83 ± 2.63.

Reporting Research Results

The following is the conceptual framework in this study:

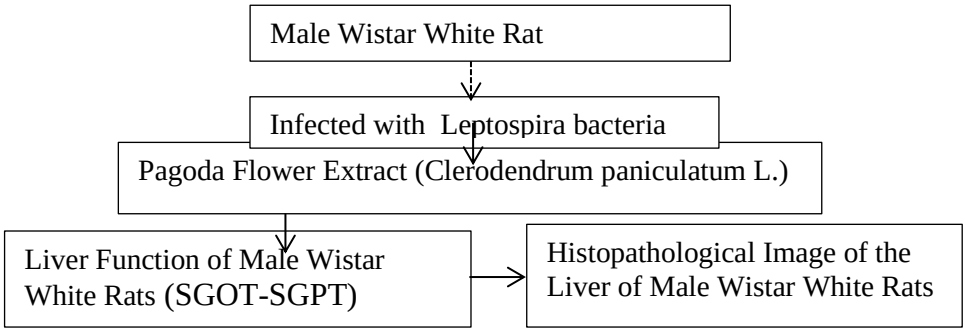


Figure 1 Conceptual Framework

Referring to the conceptual framework, the hypothesis in this study is that there is an effect of administering pagoda flower extract (*Clerodendrum Paniculatum* L) on liver function and histopathological features of the liver of male Wistar white rats infected with Leptospira interrogans bacteria.

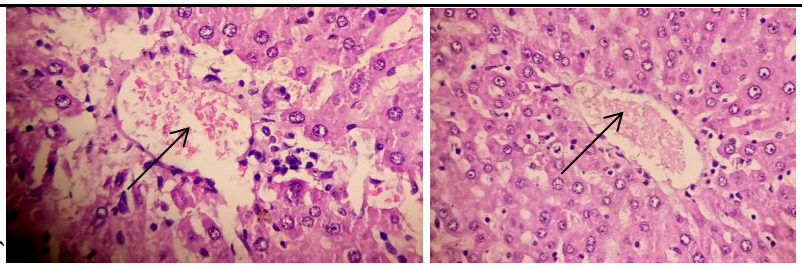
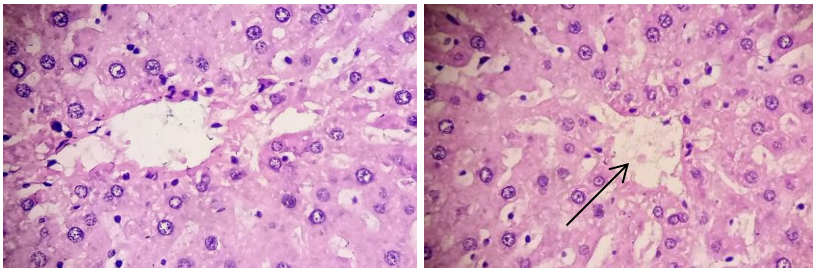
Table 1Average SGOT and SGPT

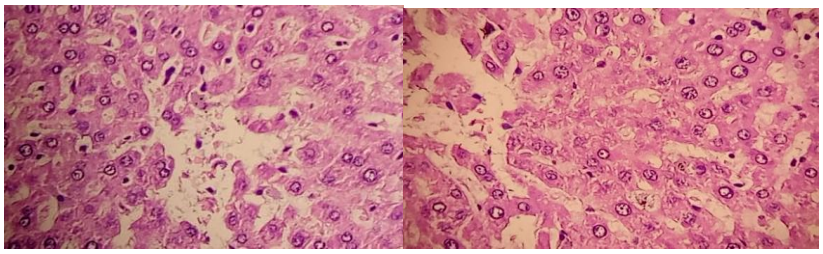
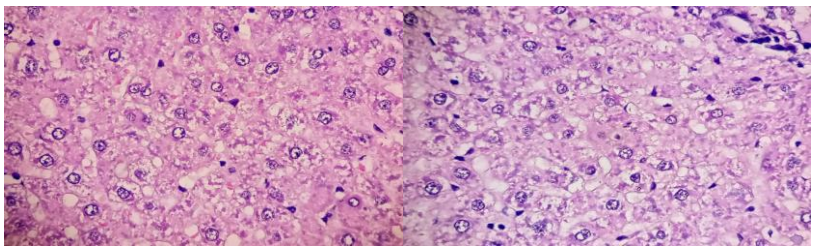
	Mean ±SD			
	H0		H14	
Group	**SGOT (U/L)**	**SGPT (U/L)**	**SGOT (U/L)**	**SGPT (U/L)**
P0	231.67±50.63	107±11.5	115.17±17.05	46.83±2.63
P1	231.5±45.01	111.83±13.73	90.5 ±22.3	30.17 ± 2.9
P2	214.83±16.9	110.6±9.35	77.5±13.3	25.17 ± 2.92
P3	213±25.7	108.6±11.6	62.3 ± 11.4	20 ± 2

The normal range of SGPT values in mice is 17.5-30.2 (IU/L), while the normal value of SGOT in mice is 45.7-80.8 (IU/L). From the table above, it can be seen that all groups of mice experienced abnormal liver function because the SGPT value was above 30.2 (IU/L) and also the SGOT value was 80.8 (IU/L). So from the data above, it can be seen that the increase in values far above normal for SGOT and SGPT was found in all groups on day 0, which was exactly 14 days after the acclimatization period and was infected with bacteria.*Leptospira interrogans*given to test animals. The highest average SGOT value before pagoda flower extract treatment (*Clerodendrum paniculatum L.*) is in the P0 group with a value of231.67±50.63 and the highest SGPT value in group P1 with an average value of 111.83±13.73.

From the table above, it can also be seen after 14 days and the treatment given, namelyIn the control group, the mice were only given distilled water. While in the treatment group, the mice were given pagoda flower extract liquid (*Clerodendrum paniculatum L.*) with different doses with SGOT value results that have shown normal results are in treatment group 2 (P2) with values77.5 ± 13.3 then in treatment group 3 (P3) with a value of 62.3 ± 11.4. For group P1, the SGOT value has exceeded the normal limit and gets an average value and standard deviation of 90.5 ± 22.3. while the SGPT value with an average result and standard deviation that gets a normal value is in group P1 30.17 ± 2.9 then group P2 with a value of 25.17 ± 2.92 and P3 20 ± 2. While the control group has not obtained normal liver function results with SGOT values of 115.17 ± 17.05 and SGPT 46.83 ± 2.63. thus the extractpagoda flower (*Clerodendrum paniculatum L.*) which is given toMale Wistar strain rats (*Rattus norvegicus*) were given significant results for 14 days of treatment to restore liver function to normal after the test animals underwent an acclimatization period to bacterial infection *Leptospira interrogans* for 14 days. Then conduct histopathological observations of the rat liver.

Table 2 Histopathological Description of Rat Liver Function

Group	Histopathological Image Results of Rat Liver Function
Control (PO)	 Microscopic image of the liver of Wistar rats in the Control group (P0). Fatty degeneration (white arrow), necrotic congestion, inflammatory cell infiltration are visible. The scoring is 4, which means necrosis is seen in the liver cells.
Treatment P1	 Microscopic picture of the liver of mice in treatment group 1 P1. There is a picture of fatty degeneration liver which is quite extensive and widespread, parenchymatous degeneration or bleeding in liver cells,

	inflammatory cell infiltration, the scoring in this group is 3, namely there are changes in the form of hydropic degeneration.
P2 Treatment	 Microscopic image of the liver of mice in Treatment Group 2 (P2). The liver shows fatty degeneration, congestion, and inflammatory cell infiltration, scoring for this group is 2.
P3 Treatment	 Microscopic image of the liver of mice in treatment group 3 (P3). It looks normal, no inflammation is seen, cells are starting to improve, no necrosis and fatty deposits are seen. And the scoring for this image is 1, which is normal.

After conducting histopathological descriptions, the researchers then analyzed the results of liver function data by testing the data on the SPSS for Windows application for data analysis.

Table 3 Normality Test

One-Sample Kolmogorov-Smirnov Test		
	SGOT	**SGPT**
N	24	24
Kolmogorov-Smirnov Z	.797	.775
Asymp. Sig. (2-tailed)	.549	.586

Normality testing using the One-Sample Kolmogorov Smirnov Test technique with the following criteria:Data is said to be normal if the significance value is**greater than 0.05**and the data is said to be abnormal if the significance value is less than 0.05. So from the table above the 2-tailed significance results in the SGOT examination 0.549> 0.05 and SGPT 0.586> 0.05 so the data is normally distributed.

Table 4 ANOVA Test Results

		df	Mean Square	F	Sig.
SGOT	Inter Group	3	3005.486	10,935	.000
	In Group	20	274,858		
	Total	23			
SGPT	Inter Group	3	811,153	115,467	.000
	In Group	20	7.025		
	Total	23			

From the test results above, the significance value at a 95% confidence level (p <0.05) is 0.00 for SGOT liver function and a significant value of 0.00 for SGPT liver function. so there is a significant difference in each average value between sample groups. means Ho is rejected, so the conclusion obtained is that there is or there is a significant difference in the average (mean) percentage of liver function from the three groups. Next, see the results of the homogeneity test whether the group comes from a population that has the same variance

Table 10 Homogeneity Test

	Levene Statistics	df1	df2	Sig.
SGOT	1,851	3	20	.171
SGPT	.428	3	20	.735

The results showed that the variance of the research data for the variables of group P0, group P1, P2 and group P3 was homogeneous or came from a population that had the same variance, namely 0.171 (p>0.05) for the analysis data of SGOT liver function on the 14th day during the treatment of pagoda flower extract, and 0.735 (p>0.05) analysis of SGPT liver function on the 14th day during the treatment of pagoda flower extract (*Clerodendrum paniculatum L.*).

DISCUSSION

The liver is an organ that plays an important role as a chemical factory for the body because the liver carries out a series of very complex tasks to keep the body in a healthy condition.(Francis, 2021).If the liver does not function, a person cannot survive more than one day.(Virani et al., 2020).This process can also easily cause changes in the structure of liver cells, both structurally and functionally, to the liver.

This study used male wistar rats (*Rattus norvegicus*) weighing 160-250 grams and aged 2-3 months. With the use of the number of research samples, namely 24 male rats which will be

divided into 4 groups where each group consists of 6 rats. In this study, the rats will be infected with bacteria and wait 24 hours after the induction of Leptospira Interrogans bacterial infection. This study was conducted to analyze the effect of administering pagoda flower extract (*Clerodendrum Paniculatum*) on liver function and histopathological features of the liver of male white wistar rats infected with Leptospira Interrogans bacteria.

Before the extract is givenpagoda flower (*Clerodendrum Paniculatum*), the mice were first measured for weight and given standard feed for 14 days. And the mice were infected with Leptospira Interrogans bacteria. In measuring the weight after the mice were infected with bacteria, it was found that the average weight of the mice decreased.It can be seen that the group that experienced the most drastic decrease was the Control group (P0) with an average difference of 23.16 grams from the average original weight of the mice before being infected with the bacteria. This indicates a reaction to the increase in body weight of mice that have been infected with the bacteria.Then the mice were tested again for SGPT and SGOT levels. All groups of mice experienced abnormal liver function because the SGPT value was above 30.2 (IU/L) and also the SGOT value was 80.8 (IU/L).

After 14 days and the treatment given isIn the control group, the mice were only given distilled water. While in the treatment group, the mice were given pagoda flower extract liquid.(*Clerodendrum paniculatum L.*)with different doses with SGOT value results that have shown normal results are in treatment group 2 (P2) with values77.5 ± 13.3 then in treatment group 3 (P3) with a value of 62.3 ± 11.4. For group P1, the SGOT value has exceeded the normal limit and gets an average value and standard deviation of 90.5 ± 22.3.

Meanwhile, the SGPT value with the average results and standard deviations that obtained normal values were in group P1 30.17 ± 2.9 then group P2 with a value of 25.17 ± 2.92 and P3 20 ± 2. Meanwhile, the control group had not obtained normal liver function results with SGOT values of 115.17 ± 17.05 and SGPT 46.83 ± 2.63. Thus, the extractpagoda flower(*Clerodendrum paniculatum L.*) which is given toMale Wistar strain rats (*Rattus norvegicus*) were given significant results for 14 days of treatment to restore liver function to normal after the test animals underwent an acclimatization period to bacterial infection.*Leptospira interrogans*for 14 days.

From the histopathological picture, the control group (P-0) which was only given rat pellet feed + distilled water/day/head for 14 days was the group with the worst liver function picture with a score of 4 due to the appearance of fatty degeneration, congestion, necrosis, and inflammatory cell infiltration. Treatment Group I (P-1) which was given rat pellet feed +extractpagoda flower(*Clerodendrum paniculatum L.*)with a dose of 100mg/BW 1ml and given distilled water/day/head for 14 days, the histopathological results were scored 3, namely there were changes in the form of hydropic degeneration or fatty liver cells with quite a lot of

degenerated liver and spread and also inflammatory cell infiltration. Treatment Group 2 (P-2) was given rat pellet feed +extractpagoda flower(*Clerodendrum paniculatum L.*)with a dose of 150mg/BW 1ml and given distilled water/day/head for 14 days, the histopathological results were scored 2 because there were changes in the form of parenchymatous degeneration or bleeding in liver cells. While Treatment Group 3 (P-3) was given rat pellet feed +extractpagoda flower(*Clerodendrum paniculatum L.*)with a dose of 200mg/BW and given distilled water/day/tail for 14 days had normal results (score 1), no inflammation was seen, cells began to improve, no necrosis and fatty deposits were seen. So it can be seen that the pagoda flower extract(*Clerodendrum paniculatum L.*)effective in healing decreased liver function due to infection with the bacteria Leptospira Interrogans.

This is in line with previous research that discussed the antibacterial content of pagoda flower extract, namely in previous research.(Ihsanul Hafiz, 2019)regarding the antioxidant content in pagoda flowers with a strong ppm value, which can be used as a defense for the body attacked by viruses and bacteria.

For the results of data analysis have been normally distributed with $p > 0.05$ results on SGOT and SGPT liver function values in the treatment group and control group. In the significance test with the one way anova method, the sig value <0.05 was obtained, so there was a significant difference in each average value between sample groups. Likewise, homogeneity has shown that the data has been homogeneous because the variables of group P0, group P1, P2 and group P3 come from populations that have the same variance.

CONCLUSION

1. In this study, it was found that there was an effect of administering pagoda flower extract (*Clerodendrum Paniculatum*) on the liver function of mice infected with *Leptospira bacteria* and its histopathological features.
2. Secondary metabolite content in pagoda flower extract (*Clerodendrum paniculatum L.*)positive for containing active compounds such as alkaloids, flavonoids, saponins, and tannins.
3. Extractpagoda flower(*Clerodendrum paniculatum L.*) which is given toMale Wistar strain rats (*Rattus norvegicus*) for 14 days of treatment obtained significant results to restore normal SGPT liver function in treatment groups 1, 2 and 3 and SGOT levels to normal in treatment groups 2 and 3.
4. The histopathological picture of the control group got a score of 4 with the worst results, and the best result with a score of 1 and stated normal liver function was in treatment group 3 (P3) with a dose of pagodA flower extract.200mg/kgBW

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