The Effect of Pagoda Flower Extract (*Clerodendrum paniculatum*) on Kidney Function and Histopathology of the Kidneys of Male Wistar White Rats Infected with *Leptospira interrogans* Bacteria

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ABSTRACT

This study was conducted to test the effect of pagoda flower extract (Clerodendrum paniculatum. L) on reducing cholesterol levels and histopathological features of the liver of obese white Wistar rats. This study used 24 white Wistar rats divided into 4 groups, namely K, P1, P2, and P3, with different dosage treatments. The results of the phytochemical test of pagoda flower extract contained alkaloids, flavonoids, saponins, and tannins. This study was conducted with an initial stage of testing a high-fat diet for 14 days. The results showed that the rats obtained a Lee index value of 0.31 in treatment groups 1, 2, and 3. Based on the results obtained, there was a decrease in total cholesterol levels in rats. The group was given pagoda flower extract with a dose of 150 mg/kgbband 200 mg/kg bb no longer experienced high cholesterol levels because their cholesterol levels were <54mg/dl. Microscopic images of the liver of rats in group P3 with rats given a dose of 200 mg/kgbbpagoda flower extract looked normal, with no inflammation, necrosis, or fatty deposits. Data analysis using the Kolmogorov-Smirnov test The data results for each group were usually distributed.

Keywords : Pagoda flower, Cholesterol, Liver, Obesity

INTRODUCTION

The kidneys are one of the most important organs in the body. They are also responsible for filtering various wastes in the blood before being excreted through urine. The kidneys are two bean-shaped organs in the urinary system. This organ also filters blood before returning it to the heart. Some of its functions include Maintaining fluid balance in the body, regulating and

filtering minerals from the blood, filtering waste materials from food, drugs, and toxic substances, creating hormones that help produce red blood cells and improve bone health, and regulating blood pressure. Several factors cause decreased kidney function and kidney damage, namely, if the kidney organs are infected with bacteria. The bacteria often infected in animals, one of which is the rat, is the bacterium Leptospira interrogans. The kidney is Leptospira's main target during acute and chronic infections.

Leptospira will colonize in the proximal tubules of the host kidney because the conditions in the proximal tubules of the kidney support Leptospira in growing and reproducing itself. One of the animals that can contract leptospirosis is the rat. One of the treatments if infected with this bacteria is drugs to relieve symptoms and treat infections caused by bacteria. Some drugs usually given are antibiotics, such as penicillin, amoxicillin, and so on. The Pagoda flower plant (Clerodendrum paniculatum. L.) is a plant and one of the species of the genus Clerodendrum. This plant is one of the verbenacea tribe, which is rich in its secondary metabolite content diversity. The benefits of the flowering plant (Clerodendrum paniculatum. L.) are that it functions as an anti-aging, antioxidant, anti-inflammatory, anti-cancer, and antibacterial.

LITERATURE REVIEW

The kidneys are one of the most important organs in the body. This organ plays several roles, including filtering various wastes in the blood to be excreted through urine. The kidneys regulate the body's fluid, electrolyte, and acid-base balance by filtering blood, selectively reabsorbing water, electrolytes, and nonelectrolytes, and excreting excess as urine. The kidneys also excrete metabolic waste products (e.g., urea, creatinine, and uric acid) and foreign chemicals (Oktaria, 2017). The kidneys maintain the body's homeostasis by controlling the concentration of plasma components, especially electrolytes, and water, and excreting metabolic waste (Sherwood L., 2014). Since nearly 25% of the total blood flow goes to the two kidneys, they have much to do to perform their excretory duties. Because of the large amount of blood flow to the kidneys, they are highly exposed to substances circulating in the circulatory system. Toxic substances can easily cause changes in the structure and function of kidney tissue (Moore, 2012).

Leptospira bacteria are commensals in the kidneys and liver of mammals, including rats (Wening Widjajanti, 2020). The kidney is Leptospira's main target during acute and chronic infections. Leptospira will colonize the proximal tubules of the host kidney because the

conditions in the proximal tubules of the kidney are very supportive for Leptospira to grow and reproduce (Faine S, 2009). Mild leptospirosis generally does not require special treatment and may even resolve within 7 days. Mammals infected with Leptospira show various symptoms. Leptospirosis has acute, moderate, mild, or chronic symptoms. Clinical signs are often associated with kidney, liver, and reproductive failure. Leptospira pathogenicity histopathologically damages the liver and kidney (dr. Devia Irine Putri, 2022). According to (Tucunduva MT, 2017) One of the animals that can be infected with leptospirosis is rats. Although rats are the primary source of the spread of leptospirosis to humans, there are not many reports discussing the histopathology of the kidneys of rats infected with Leptospira bacteria. Currently, herbal products are widely used to control or prevent the disease, and some plants continue to be tested for their pharmacological properties, such as antibiotic, anti-apoptotic, antioxidant, and anti-inflammatory properties (Nurhamidin S, 2022).

Pagoda flower plant (Clerodendrum paniculatum. L). can grow in highland areas with an altitude of 1,300 meters above sea level, and all species of this flower can grow in almost all tropical habitats, tropical rainforests, bushes, and places close to water sources (Princes, 2023). The secondary metabolites of this genus have various biological functions, such as preventing viruses from attacking plants and acting as protectors and insecticides (Ihsanul Hafiz, 2019). Traditional medicine often uses pagoda flowers to relieve various inflammatory conditions, such as joint pain and sore throat. Antioxidants are compounds that can prevent and repair damage to cells in the body (Regitha Claudia, 2024). The Pagoda Flower Plant is an anti-aging, antioxidant, anti-inflammatory, anti-cancer, and antibacterial (Banne et al., 2023).

METHODS

Laboratory experimental research is used to examine the effectiveness of pagoda flower extract administration in the kidney function of male rats (Rattus norvegicus) Wistar strain infected with leptospira bacteria along with their kidney histopathology. The place of research is in the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara in May - July 2024—ethical Clearance supervised by Health Research Ethics Commission (KPEK) Universitas Prima Indonesia. The sample in this study was 24 male rats, which were divided into 4 groups. Control group (K) treatment 1,2,3 (P1, P2, P3), which each would be given pagoda flower extract with a dose of 100 mg/kg bb, 150 mg/kg bb, and 200 mg/kg bb. Then, the phytochemical content of pagoda

flower extract will be tested, and kidney function tests (serum and keratin) will be conducted on rats that are declared positive for leptospira bacteria infection. After that, the process was observed for 14 days, after which the extract was given, the histopathology of the rat kidneys was observed, and scoring was performed. Then, the data will be presented descriptively, with the data normality test analyzed using the Shapiro-Wilk approach ($p > 0.05$). Significance testing 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 checked before and after infection. From the observation data, the rats' body weight decreased after 14 days of being induced by Leptospira interrogans bacterial infection. The group that experienced the most drastic decrease was group P2, with an average difference of 21.16 grams from the average original weight. This indicates a reaction to the increased body weight of rats infected with the bacteria. Before giving the pagoda flower extract treatment, the pagoda flower extract was first made using a maceration technique. The dried pagoda flower powder was extracted using 96% ethanol, then filtered, and the filtrate was collected; the residue was then macerated again with the same method, then filtered, and the filtrate was evaporated. After that, 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 mg/kg bb, P2 with 150 mg/kg bb, and P3 with 200 mg/kg bb pagoda flower extract. Then, after the rats were treated in each group, they were taken blood samples to see their kidney function, with the results of the keratin level test for the Control group (K) getting the largest average for keratin levels (mg / dL) in mouse samples. Group P2 also got the most minor average results from groups P1 and P3. Regarding urea levels, group P2 rats got the most minor average results, while the Control group (K) got the largest average for urea levels (mg / dL) in mouse samples.

This study hypothesizes that administering pagoda flower extract (Clerodendrum paniculatum. L) affects kidney function and histopathological features of male Wistar strain white rats infected with Leptospira interrogans bacteria.

Table 1. Rat Keratinin Level Test Result Data

Variables	**Group**				**Sig**
	K	**P1**	**P2**	**P3**	
	n=6Mean±SD	**n=6**	**n=6**	**n=6**	
		Mean±SD	**Mean±SD**	**Mean±SD**	
Keratin	1.315±0.015	0.768±0.022	0.615±0.176	0.855 ± 0.258	
Shapiro Wilk	0.389	0.801	0.505	0.272	
One Way ANOVA					0,000

The results of creatinine level and statistics mentioned in Table 1, in the control group (K) with the treatment of rats infected with Leptospira interrogans bacteria but only given standard feed without being given pagoda flower extract treatment obtained an average keratin content and standard deviation of 1.3150 ± 0.015 and obtained the lowest results compared to other groups. Treatment group 1 (P1), which had been infected with bacteria and received 100 gr of pagoda flower extract, obtained an average result and standard deviation of 0.768 ± 0.0229. Treatment group 2 (P2) in the group of rats that had been infected with bacteria and received 150 gr of pagoda flower extract obtained an average result and standard deviation of 0.615 ± 0.176, where these results show a decrease in the average keratin content of group P1. Then, in treatment group 3 (P3), the group of rats that had been infected with bacteria and were given 200 grams of pagoda flower extract obtained average results and standard deviations of 0.855 ± 0.258.

In the normality test, all the data has been customarily distributed; this can be seen from the results of the Shapiro Wilk normality test data, showing that the data for groups K, P1, P2, and P3 have a significance of >0.05, so the data is usually distributed. In this test, the One-

way ANOVA result at sig 0.00 means that pagoda flower extract affects kidney function (keratin) in rats infected with Leptospira interrogans bacteria.

Table 2. Data on Urea Levels in Rats

ariables	**Group**				**Sig**
	K	**P1**	**P2**	**P3**	
	n=6Mean±SD	**n=6**	**n=6**	**n=6**	
		Mean±SD	**Mean±SD**	**Mean±SD**	
urea	36.92±0.531	20.255±0.363	17,018±0,256	17,069 ± 0.459	
Shapiro Wilk	0.328	0.221	0.259	0.163	
One Way ANOVA					0,000

Urea levels in Table.2 as seen in the control group (K) with rats infected with bacteria Leptospira interrogans and only given standard feed without being given pagoda flower extract treatment, the average results and standard deviations were obtained.36.92±0.531 and got the highest results compared to other groups. In the treatment group 1 (P1), which had been infected with bacteria and received 100 gr of pagoda flower extracted, the average and standard deviation results were 20.255±0.363, and in the treatment group 2 (P2) in the group of rats that had been infected with bacteria and received 150 gr of pagoda flower extract. Get the average and standard deviation results in the smallest of the other groups, namely17.018±0.256. Then, in treatment group 3 (P3), a group of rats had been infected with bacteria and were given 200 gr of pagoda flower extract. Get the average and standard deviation results17.069 ± 0.459.

In the normality test, it can be seen that all the data has been customarily distributed; this can be seen from the results of the Shapiro Wilk normality test data showing that the data for groups K, P1, P2, and P3 have a significance of >0.05 so that the data is usually distributed. In this test, the One-way ANOVA result at sig 0.00 means that pagoda flower extract affects kidney function (serum) in rats infected with Leptospira interrogans bacteria. Next, see the results of the histopathology of the kidney function of rats after being given pagoda flower extract treatment for 14 days:

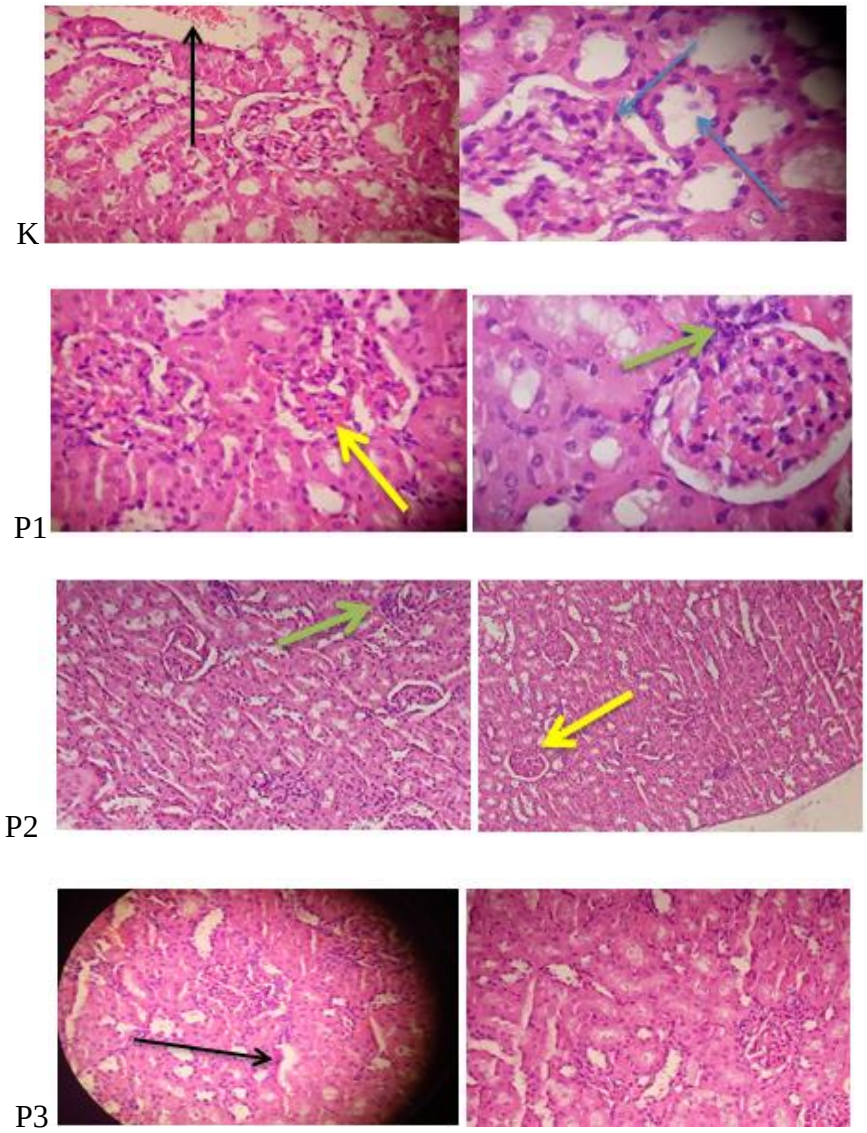

Figure 1. Histology results from rats kidney

Histology results in Figure 1 show that in the kidney organs in the control group, there is severe necrosis (blue arrow) and severe inflammation (black arrow), so this group gets a score of 3. There is moderate necrosis (yellow arrow) and bleeding (Bowman's Space Edema or bleeding), so this group gets a score of 2. There is moderate necrosis (yellow arrow) and bleeding (there is Bowman's Space Edema), so this group gets a score of 2. There is an inflammatory cell infection, so treatment group 3 gets a score of 1 because there is still an infection in the kidney organs of the rats.

In Table 3, the differences in kidney histology damage between groups K, P1, P2, and P3 are shown, and groups K and P1 experienced the most severe histopathological damage compared to the other treatment groups.

Table 3. Kidney morphology status of each rats control and treatment groups

Group	Hypertrophy Glomerulus	Tubular Hemorrhage	Pycnosis Tubule	Information
K	++	+	++	Much hypertrophy and pyknosis, little hemorrhage
P1	++	++	++	Lots of hypertrophy, hemorrhage and pyknosis
P2	+	++	+	Lots of hemorrhage, little hypertrophy and pyknosis
P3	++	+	+	Much hypertrophy, little hemorrhage and pyknosis

Description: The (+) sign indicates that there is little change, the (++) sign indicates that there are many changes in the histopathological picture of the kidneys.

DISCUSSION

The kidneys are one of the most important organs in the body because they regulate the balance of body fluids, electrolytes, and acids by filtering blood, selectively reabsorbing water, electrolytes, and nonelectrolytes, and excreting excess as urine. The kidneys also excrete metabolic waste products (e.g., urea, creatinine, and uric acid) and foreign chemicals (Oktaria, 2017). Kidney function tests usually examine urea, creatinine, and glomerular filtration rates. Typical results for both tests indicate that the kidneys are working well. Impaired kidney function can lead to kidney failure. Kidney failure is characterized by a glomerular filtration rate that functions only 10–20%, high urea and creatinine levels, anemia, azotemia, metabolic acidosis, polyuria, and nocturia (K, 2014). Urea and creatinine are compounds that can describe normal kidney function.

Before further research, the pagoda flower (Clerodendrum paniculatum. L .) was tested for its content by phytochemical testing on its extract. Phytochemical testing was carried out to determine whether metabolite compounds were contained in the pagoda flower extract. The test results show that the extract pagoda flower (Clerodendrum paniculatum. L.) positively

contains active compounds such as alkaloids, flavonoids, saponins, and tannins. As is known, alkaloids are effective as antimicrobials, anti-diabetics, antibacterials, and anti-diarrhea.

Normal ketamine levels in rats range from 0.2 to 0.8 mg/dL. The research showed that the control group (K) was not given pagoda flower extract. Get the average and standard deviation results1.315±0.015; this indicates that the keratin levels are above normal, and it can be said that the rats are in a state of impaired kidney function through their keratin levels. Treatment group 1 (P1) received 100 grams of pagoda flower and extracted the average and standard deviation results0.768±0.0229. In treatment group 2 (P2), with the administration of 150 gr of pagoda flower extracted, the average and standard deviation results0.615±0.176, then in both groups (P1 and P2), the keratin levels reached normal levels. At the same time, for treatment group 3 (P3), which was given 200 grams of pagoda flower extract, the average and standard deviation results0.855 ± 0.258, where these results indicate the maximum standard limit for keratin levels.

In examining urea levels, normal urea levels in rats range from 15 to 21 mg/dL. The average results and standard deviations were obtained in the control group (K) without being given pagoda flower extract treatment.36.92±0.531 This indicates that the urea level is above normal, so there is a disruption of kidney function in the examination of the urea levels of rats. In the treatment group 1 (P1), which was given 100 gr of pagoda flower extracted, the average and standard deviation results20.255±0.363, and in the treatment group 2 (P2) received 150 gr of pagoda flower extracted, the average and standard deviation results17.018±0.256. Then, in the treatment, group 3 (P3) received 200 gr of pagoda flower extract, the average and standard deviation results17.069 ± 0.459. So groups P1, P2, and P3 showed normal urea levels, and group P2 got the minor average results, while the Control group (K) got the largest average for urea levels (mg/dL) in rat samples.

The histopathology results describe that the control group (K) in the kidney organ image showed severe necrosis and inflammation, so this group got a score of 3. In treatment groups 1 and 2 (P1 and P2), there was moderate kidney necrosis and bleeding (Bowman Spatium Edema), so this group scored 2. While in treatment group 3, the organ still showed inflammatory cell infection, so treatment group 3 got a score of 1. So the extractPagoda flowers (Clerodendrum paniculatum. L .) effectively heals the kidney function of rats that experience decreased function when viewed from the histopathological picture caused by injection of leptospira bacteria.

This is in line with previous research (Banne et al., 2023) related to studying the content of pagoda flower extract with pharmacological activities such as anti-aging, antioxidant, anticancer, anti-inflammatory, and antibacterial activities. Also, in research conducted by researchers related to bacterial infections of mouse samples, it can be proven that pagoda flower extract has good properties in curing bacteria in the body.

CONCLUSION

In this study, it was found that there was an effect of administering pagoda flower extract (Clerodendrum paniculatum. L.) on the kidney function of male Wistar strain white rats infected with Leptospira interrogans bacteria, as seen from the results of urea and keratin levels and histopathological examination of the rat kidneys which improved after being administered pagoda flower extract. The content of secondary metabolites in pagoda flower extract (Clerodendrum paniculatum. L.) through phytochemical tests shows that pagoda flowers positively contain active compounds such as alkaloids, flavonoids, saponins, and tannins. So, pagoda flower extract has antioxidant and antibacterial benefits. In this study, the control group of rats was not given pagoda flower extract, P1 with a dose of 100 gr, P2 150 gr, and P3 with a dose of 200 gr where the kidney function (urea and keratin levels of rats) that had been infected with Leptospira interrogans bacteria and received pagoda flower extract treatment (Clerodendrum paniculatum. L.) for the groupP1 and P2 creatinine levels have reached normal levels. In contrast, urea levels for groups P1, P2, and P3 have reached the expected results. Results histopathology describes that the control group (K) in the picture of the kidney organ experienced severe necrosis and inflammation. In treatment groups 1 and 2, moderate necrosis and bleeding (Bowman's Spatium Edema) were seen in the kidney organ. While in treatment group 3, inflammatory cell infection was still seen in the organ so that treatment group 3. So grou,p 3 is the best dose for treating kidney function in rats infected with Leptospira interrogans bacteria.

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