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ORIGINAL ARTICLE

The effect of telang flower extract on kidney function and histopathological features of obese rat kidneys

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Abstract

This study aims to determine the effect of oral administration of *telang* flower extract in several doses on histopathological changes in kidney organs. This study used a post test only control group design. Researchers used 6 Wistar rats for each experimental group, so that the total number of test animals in this study was 24. Grouping of test animals was done randomly into 4 test groups. Histopathological observations of kidney tissue were made by comparing the control and treatment groups. Changes observed will be the presence of fatty degeneration (vacuolization), necrosis and hydrophic degeneration. To obtain quantitative data, scoring was performed on each change found. The data from the study were then analyzed with the help of SPSS. Data normality test was analyzed by Kolmogorov-Smirnov test ($p > 0.05$). To test the significance between test groups, one-way analysis of variance or One Way ANOVA technique was conducted at 95% confidence level ($p < 0.05$). Further analysis or test was conducted using Post Hoc Test with LSD technique. The results showed that the administration of *telang* flower extract at a dose of 600mg/KgBB was effective in improving kidney function in obese Wistar white rats. This improvement is seen through increased levels of ureum, creatinine, and improved renal histological structure. Histopathological observation of kidney tissue showed that treatment group 3, which received *telang* flower extract at a dose of 600mg/KgBB, experienced significant improvement and was closer to the control group than the other groups.

Keywords: histopathology, kidney, obese rats, telang flower extract

Introduction

Obesity is a disease caused by excessive fat accumulation in the body. This condition leads to health issues across all age groups, including adults, teenagers, and children, in both developed and developing countries. The prevalence of obesity has increased alarmingly worldwide and has become a major health issue in today's society.[1] Obesity is associated with a higher risk of mortality and is known as a cause of chronic kidney disease. Kidney damage from obesity originates from renal hemodynamic changes, leading to hyperfiltration, albuminuria, and eventually, a decrease in glomerular filtration rate due to glomerulosclerosis. Additionally, hypertension and type 2 diabetes mellitus, conditions commonly associated with obesity, are both triggers for obesity-induced injury to the renal parenchyma and complications of being overweight. Obesity is also linked to poorer kidney transplant outcomes.[2]

Controlling diet and regular exercise have proven to be effective strategies for preventing obesity. Public interest in dietary cholesterol has increased significantly due to the relationship between plasma cholesterol levels and the risk of heart disease. For dieting, individuals need nutritional information provided by laboratories to producers because labels must inform customers about healthy foods and proper food choices to reduce nutrition-related diseases. Therefore, accurate evaluation of cholesterol content in foods is essential and encourages the development of technology for cholesterol quantification.[3]

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Another strategy that can be applied is exercise. Many studies report that regular exercise is also effective in maintaining cognitive function. However, many people cannot participate in regular exercise regimens due to various reasons, including time constraints and physical limitations. Observing this phenomenon, herbal medicine producers have started to manufacture anti-obesity herbal medicines.

The use of plant-based medicines, often referred to as herbal medicines, is an ancient practice that still persists today. The use of medicinal plants tends to be underreported scientifically, leading to chronic ambiguity about their therapeutic efficacy and acute and chronic side effects. Some may achieve the intended purpose of curing diseases, while others may not.[4,5] According to WHO, 88% of the world's population uses traditional medicine, and 90% of developing countries use traditional herbal medicines as primary health care.[6] The Indonesian community commonly consumes traditional herbal medicine known as *jamu*, an alternative medicine passed down through generations. *Jamu* remains popular for maintaining health and treating diseases. Indonesia's tropical climate also supports this, making Indonesia rich in natural resources..[7]

Traditional medicinal plants are crucial as curative and protective medicinal preparations. The use of medicinal plants growing on community land as part of traditional medicine procurement has been practiced by the community since ancient times. In Indonesian society, various traditional medicines made from natural spices have been used by ancestors, prepared alongside Borobudur Temple related to the processing/making of *jamu*. Various spices and *empon* as forms of Javanese local wisdom commonly referred to as *jamu* include turmeric-tamarind *jamu*, rice kencur *jamu*, paitan *jamu*, papaya leaf *jamu*, *chili puyang jamu*, and temulawak *jamu*. These *jamu* names are the main components in *jamu* ingredients, such as turmeric and tamarind in turmeric-tamarind *jamu*. In rice kencur *jamu*, the supporting components are rice and *kaempferia galanga*. Besides the main components, some herbalists can add various ingredients to complement the desired benefits. For example, in rice kencur *jamu*, besides the two main ingredients, rice and *kaempferia galang*a, kawak acid, kedawung seeds, ginger rhizomes, cardamom seeds, *temukunci*, cinnamon, turmeric, lime, and nutmeg are added.[8] More than 30,000 plant species grow in Indonesia, and about 9,600 species are known to have pharmacological activities[7], one of which is the *telang* flower.

Telang flower or *Clitoria ternatea* is one of Indonesia's native local plants that has potential for further development. The wide genetic diversity of the *telang* flower based on morphological characteristics offers opportunities for research and development. The *telang* flower has many uses, including as a natural dye, food coloring, and cancer prevention due to its high antioxidant content, and also as an ornamental plant.[9] The *telang* flower thrives in Indonesian soil. Farmers can easily plant and cultivate the *telang* flower. This is related to the morphology of the *telang* flower, which supports its survival in the dry season. The community uses the *telang* flower as traditional medicine for tumor prevention, ear infections, skin conditions, and throat problems. Other secondary metabolites found in the *telang* flower include anthocyanins, triterpenoids, and phytosterols.[10]

The use of herbal medicines at certain doses is suspected to have different effects or indications on body organs, including the kidneys. Mammalian kidneys are target organs for various toxic agents due to their primary function as blood filters during the excretion process. Drug toxicity can result in damage to the functions of various organs, with nephrotoxicity (kidney poisoning) being common. The kidneys are very susceptible to damage from drugs and toxins. The mechanisms of kidney injury are diverse and not fully understood. However, the most common pathological findings in the setting of nephrotoxin exposure are acute tubular necrosis (ATN) and acute interstitial nephritis (AIN). Over the past two decades, publications on nephrotoxicity have increased substantially. They mainly concern drugs including nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, diuretics, paracetamol, contrast media, and chemotherapy.[11] Based on this phenomenon, this study is designed to investigate how the effects of oral administration of *telang* flower extract at various doses impact the histopathological changes in kidney organs.

Method

This study uses a post-test only control group design. This research type observes the control and treatment groups only after an intervention. The study took place in the Pharmacology and Pathology

Anatomy Laboratories at the Faculty of Medicine, University of North Sumatra, from October to December 2023. Sample calculation was conducted using the Federer formula, dividing the samples into four groups. Based on the calculations, the minimum number of test animals per group is 6 rats. Therefore, the researcher used 6 Wistar strain rats for each experimental group, totaling 24 test animals. The test animals were randomly assigned into 4 test groups.

Equipment used for making ethanol extract from *telang* flowers included maceration equipment, filters, a rotary evaporator, evaporation dishes, and a water bath. For in vitro testing, equipment included various sizes of volumetric flasks, test tubes, test tube racks, BioHit micropipettes (1000µL), measuring pipettes, spatulas, vials, incubators, pH meters, cuvettes, centrifuges, centrifuge tubes, and a UV-Vis spectrophotometer. Materials used were ethanol extract of *telang* flower (*Clitoria ternatea*) and chemicals like trichloroacetic acid (TCA), ethanol p.a (*Brataco*), and distilled water.

The *telang* flower extract preparation was modified from Rahayu's (2020) research. Raw materials were *telang* flowers sourced from farmers in Medan. These underwent wet sorting to separate rotten flowers, washing, and drying at 50°C in an oven. The simplicia powder was then ground with a blender and sieved with a 40-mesh sieve. Simplicia extraction was conducted using maceration. 100 grams of *telang* flower powder (*Clitoria ternatea*) was weighed and macerated with 96% ethanol (1:10 ratio) for 4 days. The extract was filtered to separate the residue and filtrate. The filtered extract was then evaporated using a rotary evaporator to obtain a thick extract. The doses of *telang* flower extract administered were 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW.

The parameters used to confirm obesity in rats were body weight and abdominal circumference. Rats were considered obese if the Lee index was > 0.300. The Lee index is a parameter to assess whether rats are obese. Obese male Wistar rats that had undergone an acclimatization period and received a high-fat diet were randomly divided into four groups, each consisting of 6 rats, and treated for 14 days. The treatments were as follows: a) Control Group (K-0): Rats were given standard pellet feed and distilled water at 200mg/kgBW per day per rat for 14 days; b) Treatment Group-1 (K-1): Rats were given a high-fat diet and *telang* flower extract at 200mg/kgBW per day per rat orally using a gavage for 14 days; c) Treatment Group-2 (K-2): Rats were given standard pellet feed and *telang* flower extract at 400mg/kgBW per day per rat orally using a gavage for 14 days; and d) Treatment Group-3 (K-3): Rats were given standard pellet feed and *telang* flower extract at 600mg/kgBW per day per rat orally using a gavage for 14 days.

Each treatment group was given *telang* flower extract orally every day. On day 14, the *telang* flower extract administration was stopped. The rats were then euthanized using ketamine, followed by necropsy. After opening the abdominal cavity, the kidneys were removed and placed in a pot containing 10% Neutral Buffered Formalin. On day 15, the rats were anesthetized, and blood samples were collected from the orbital vein using a capillary pipette, totaling 3 cc, and stored in an EDTA (Ethylenediamine Tetraacetic Acid) tube in a cool box. Blood samples were analyzed at the University of North Sumatra Laboratory for amylase and lipase levels. Using a pipette, the blood samples were processed and then applied to the Creatinine (CREA) and Blood Urea Nitrogen (BUN) test kits to determine creatinine and urea levels in the blood. After placing the blood samples into the CREA and BUN test kits, both were placed in the measurement chamber of the Reflovet Plus device and the chamber lid was closed. After a few minutes (about 2-3 minutes), Reflovet Plus displayed and printed the analysis results. These results were used to evaluate kidney function by analyzing blood creatinine and urea levels.

Histopathological preparations were made by fixing kidney organs using 10% Neutral Buffered Formalin solution, then cutting and placing them in plastic specimen containers. The tissues underwent dehydration using graded alcohol concentrations (70%, 80%, 90%, absolute alcohol I, absolute II) for 2 hours each. Clearing was done with xylol, followed by embedding in paraffin to create paraffin blocks, which were stored in a refrigerator. The paraffin blocks were then thinly sectioned (5-6 µm) using a microtome. The sections were floated in warm water (60°C) to stretch the tissues and prevent folding. The sections were then placed on glass slides and stained with Hematoxylin and Eosin (HE) for microscopic examination.

Histopathological examination of kidney tissues was done by comparing control and treatment groups. Observed changes included fatty degeneration (vacuolization), necrosis, and hydropic degeneration. To obtain quantitative data, scoring was conducted for each observed change using a light

microscope at 400x magnification. The scoring system used was based on kidney damage: Score 0 = no histopathological damage; Score 1 = mild focal damage; Score 2 = moderate multifocal damage; Score 3 = severe diffuse damage.

Data normality was tested with the Kolmogorov-Smirnov test ($p > 0.05$). Significance testing between groups was performed using One-Way ANOVA at a 95% confidence level ($p < 0.05$). Further analysis or post-hoc tests were conducted using the LSD method.

Table 1. Rat weight

Parameter	Group	Average	
		Before a high-fat diet	After a high-fat diet
Weight	Control	235 gr	237 gr
	P1	239 gr	339 gr
	P2	241 gr	346 gr
	P3	243 gr	345 gr
Naso-anal length	Control	214 mm	216 mm
	P1	217 mm	220 mm
	P2	213 mm	216 mm
	P3	216 mm	218 mm
Lee Index	Control	0.28	0.28
	P1	0.28	0.31
	P2	0.29	0.32
	P3	0.28	0.32

Results

This study utilized test animals that were given a high-fat diet, primarily quail egg yolk, for 14 days to induce obesity. The cholesterol in this diet increased cholesterol levels in the rats, leading to obesity. The body weight of the test animals was measured before and after the 14-day high-fat diet to confirm the diet's effectiveness in inducing obesity.

Measurement of body weight and nasoanal length was conducted to calculate changes in the Lee index in rats fed a high-fat diet. Before the diet, the Lee index values for treatment groups 1 and 3 were 0.28, while group 2 was 0.29, still below 0.3 indicating non-obesity. After 14 days of quail egg yolk diet, the Lee index values increased: group 1 to 0.31, and groups 2 and 3 to 0.32. From this data, it is concluded that the rats in the treatment groups were already obese before testing with butterfly pea flower extract for kidney function improvement (see Table 1).

Observations showed changes in urea levels in the treatment groups during this study (Table 2). The control group initially had a urea level of 17.1 mg/dl before treatment, and after 14 days, the urea level increased to 18.03 mg/dl, but still within the normal range. Meanwhile, treatment group 1, given a high-fat diet, initially had a urea level of 33.16 mg/dl, but after being given butterfly pea flower extract at a dose of 200 mg/kg BW, the urea level decreased to 23.96 mg/dl. Treatment group 2 with the same diet had an initial urea level of 22.93 mg/dl, and after administration of butterfly pea flower extract at a dose of 400 mg/kg BW, the urea level dropped to 21.08 mg/dl. Treatment group 3, with a high-fat diet, initially had a urea level of 33.6 mg/dl, but after administration of butterfly pea flower extract at a dose of 600 mg/kg BW, the urea level drastically decreased to 18.9 mg/dl. From these results, the researchers concluded that treatment group 3 experienced the most significant decrease in urea levels, approaching the urea levels of the control group. Meanwhile, treatment group 1 had the lowest decrease in urea levels compared to treatment groups 2 and 3. This indicates that higher doses of butterfly pea flower extract can provide better effects in reducing urea levels in the treatment groups.

Table 2. Creatinine from ureum up

Group	Repetition	After a high-fat diet (mg/dl)		After treatment (mg/dl)	
		Urea	Creatinine	Urea	Creatinine
Control	1	17.1	4.2	17.9	4.7
	2	16.2	3.8	17.3	4.1
	3	18.1	3.1	19.1	3.9
	4	17.5	3.9	18.2	4.4
	5	16.8	4.1	17.1	4.8
	6	16.9	4.3	18.6	5.1
	Average	17.1	3.9	18.03	4.5
Treatment I	1	37.4	10.3	24.2	8.3
	2	30.3	11.8	23.3	7.9
	3	31.5	10.8	22.9	8.1
	4	33.2	10.5	24.5	9.1
	5	34.9	8.7	25.2	7.2
	6	31.7	9.7	23.7	8.6
	Average	33.16	10.3	23.96	8.2
Treatment II	1	32.5	11.2	20.1	7.1
	2	34.1	11.4	22.3	6.9
	3	38.1	9.7	19.8	5.4
	4	30.2	8.9	20.8	7.4
	5	30.5	9.9	21.6	6.3
	6	32.2	10.1	21.9	7.8
	Average	32.93	10.2	21.08	6.81
Treatment III	1	33.8	10.8	19.9	6.1
	2	32.6	11.6	18.2	5.4
	3	36.1	11.1	19.2	3.7
	4	37.2	8.5	18.7	4.2
	5	30.3	9.2	19.6	4.8
	6	31.6	10.2	17.8	5.7
	Average	33.6	10.23	18.9	4.98

Comparison of creatinine levels among the treatment groups in the study showed the most significant reduction in the group receiving

Clitoria ternatea flower extract at a dose of 600 mg/kg BW (group 3), which was close to the control group. The decrease in creatinine levels in group 1 (dose 200 mg/kg BW) was the smallest.

Based on the results of the phytochemical test, it can be concluded that the extract of moringa flower contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and steroids. Subsequently, histopathological observations were made using a light microscope with 400x magnification. The aim of this observation was to examine the structure and morphology of the cells in each liver tissue specimen in the control and treatment groups. The administration of moringa flower extract was carried out daily in the morning.

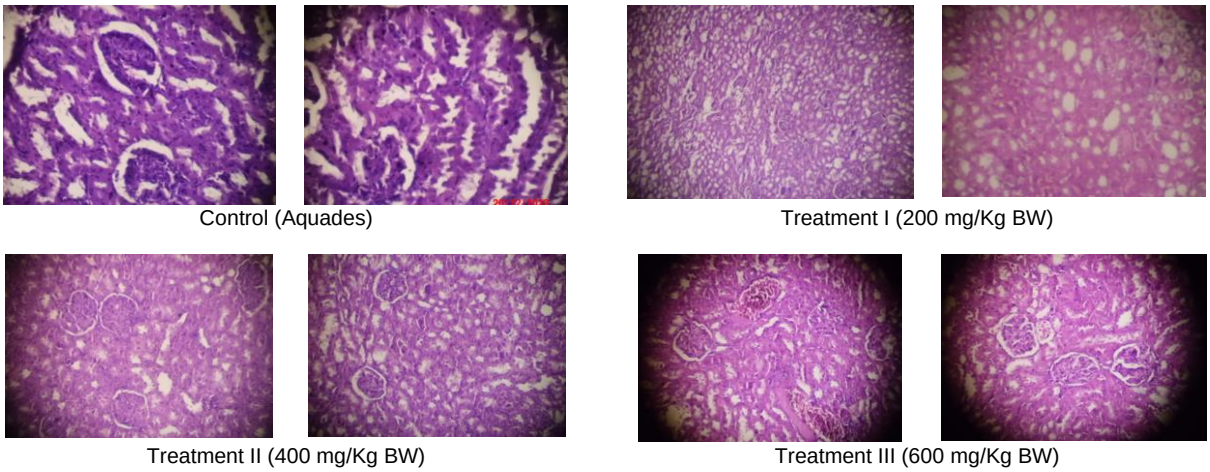

Figure 1. Histopathological Images of Kidney Tissue

Histopathological observations showed significant differences in kidney cell appearance between the control and treatment groups. The control group, which received regular pellet feed and distilled water, showed normal kidney histology with a score of 0, indicating no damage to the kidney tissue. This normal condition was due to the absence of a high-fat diet, serving as a reference for comparison with the treatment groups. Treatment group 1, given a high-fat diet and butterfly pea flower extract at a dose of 200 mg/kg BW, showed changes in kidney structure due to exposure to the high-fat diet and obesity. Histology of this group revealed significant kidney cell damage, indicated by a score of 4 (severe damage). Meanwhile, treatment group 2, with butterfly pea flower extract at a dose of 400 mg/kg BW, showed improvement in kidney histology structure despite still having moderate multifocal damage, indicated by a score of 2. Treatment group 3, which received a high-fat diet and butterfly pea flower extract at a dose of 600 mg/kg BW, showed kidney histological structure close to the control group with a score of 0. This indicates the positive potential of the combination of a high-fat diet and butterfly pea flower extract in improving histopathological damage in kidney tissue.

The results of the normality test indicate that all data are normally distributed ($p > 0.05$). Therefore, the homogeneity test was continued using the Levene test to determine if each group's population variance is equal or homogeneous.

Table 3. Normality Test Results

Group	df	Sig
Control	6	.200
P1	6	.200
P2	6	.200
P3	6	.200

The homogeneity test using the Levene test showed a significance probability value of 0.715, which is greater than 0.05. Thus, it can be concluded that the control group, treatment group 1, treatment group 2, and treatment group 3 come from populations with equal variances, or are homogeneous. Next, a One-Way ANOVA test was conducted to test the significant effectiveness among the trial groups.

Tabel 4. Homogeneity Test Results

Levene static	df1	df2	Sig
2.322	3	20	.106

Urea level observation results

The One-Way ANOVA test results showed a significance value of 0.000, or < 0.05, indicating a significant difference between the control group and the treatment groups based on the analyzed data.

Table 5. One *Way Anova test results* on urea levels

	Sum	df	Mean square	F	Sig
Intergroup	126.015	3	42.005	56.553	.000
In Groups	14.855	20	.743		
Total	140.870	23			

Table 6. *Post-Hoc* LSD test results on urea levels

Group		Mean difference	Sig
Control	Treatment 1	-5.93333*	.000
	Treatment 2	-3.05000*	.000
	Treatment 3	-.86667	.097
P1	Control	5.93333*	.000
	Treatment 2	2.88333*	.000
	Treatment 3	5.06667*	.000
P2	Control	3.05000*	.000
	Treatment 1	-2.88333*	.000
	Treatment 3	2.18333*	.000
P3	Control	.86667	.097
	Treatment 1	-5.06667*	.000
	Treatment 2	-2.18333*	.000

Table 7. One *Way Anova test results* on creatinine levels

	Sum	df	Mean square	F	Sig
Intergroup	54.041	3	18.014	28.529	.000
In Groups	12.628	20	.631		
Total	66.670	23			

Table 8. *Post-Hoc* LSD test results on urea levels

Group		Mean difference	Sig
Control	Treatment 1	-3.70000*	.000
	Treatment 2	-2.50000*	.000
	Treatment 3	-.48333	.305
P1	Control	3.70000*	.000
	Treatment 2	1.20000*	.017
	Treatment 3	3.21667*	.000
P2	Control	2.50000*	.000
	Treatment 1	-1.20000*	.017
	Treatment 3	2.01667*	.000
P3	Control	.48333	.305
	Treatment 1	-3.21667*	.000
	Treatment 2	-2.01667*	.000

The results of the analysis showed that there was a significant difference between the control group and the treatment group 1 (p=0.000) and the treatment group 2 (p=0.000), but there was no significant difference between the control group and the treatment group 3 (p=0.097) (see Table 6).

Creatinine level observation results

The One-Way ANOVA test results showed a significance value of 0.000, or < 0.05, indicating a significant difference between the control group and the treatment groups based on the analyzed data (see Table 7).

The results of the analysis showed that there was a significant difference between the control group and treatment groups 1 (p= 0.000) and 2 (p= 0.000) and there was no significant difference with treatment group 3 (p= 0.305) (see Table 8).

Discussion

The One-Way ANOVA results showed a significance of 0.000, which is less than 0.05, indicating a significant difference between the control group, treatment 1, treatment 2, and treatment 3. A Post-hoc LSD test was conducted to analyze the differences in mean urea and creatinine levels between groups. The Post-hoc LSD test results for urea levels showed significant differences between the control group and treatments 1 and 2, but not with treatment 3. Treatment group 3 (600 mg/kg BW of butterfly pea flower extract) had urea levels not different from the control group, while treatments 1 and 2 did differ. The creatinine level analysis showed significant differences between the control group and treatments 1 and 2, but not with treatment 3. The control group and treatment 3 did not differ significantly in creatinine levels.

The histological condition of the kidneys in rats that had undergone the trial process was then analyzed. The control group had a normal kidney histology. The histopathological observations of the kidneys in the control group served as a reference for describing other groups and as a comparator. In treatment group 1, given a high-fat diet and butterfly pea flower extract at a dose of 200 mg/kg BW, differences in kidney structure were observed due to exposure to a high-fat diet and obesity. The histology of treatment group 1 showed kidney cell damage, categorized with a score of 4 (severe/diffuse damage). Treatment group 2, given butterfly pea flower extract at a dose of 400 mg/kg BW, showed improvement in kidney histology structure, although there was still moderate/multifocal damage, categorized with a score of 2. Treatment group 3, given a high-fat diet and butterfly pea flower extract at a dose of 600 mg/kg BW, showed kidney histological structure close to the control group, categorized with a score of 0.

These observations indicate that administering butterfly pea flower extract at a dose of 600 mg/kg BW can improve kidney tissue structure in male Wistar rats with obesity. This improvement is evident from the histopathological examination of the kidneys in the control group and treatment group 3, showing minimal differences. The improvement in the kidney histology structure of white rats is due to the secondary metabolites contained in *Clitoria ternatea* flower extract, such as flavonoids, saponins, tannins, and triterpenoids.

A phytochemical study revealed that *Clitoria ternatea* flower extract contains flavonoids, saponins, and tannins, consistent with previous research by Azzahra et al. The flavonoids in the extract contribute to

improving kidney function impaired by obesity through their antioxidative properties, increasing levels of oxidative stress-reducing species, and acting as mediators in generating an antioxidant response in the body. Additionally, flavonoids modulate inflammatory markers, show anti-inflammatory effects, and protect cells from apoptosis in the kidneys.

Conclusion

The administration of *telang* flower extract at a dose of 600mg/Kg BW was effective in improving kidney function in white rats of the obese Wistar strain. This improvement can be seen through increased levels of urea, creatinine, and improvement of the histological structure of the kidneys. The results of histopathological observation of kidney tissue showed that treatment group 3, which received *telang* flower extract at a dose of 600mg/Kg BW, experienced significant improvement and was close to the control group compared to the other group.

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