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Exposure to Shampoo Made from Bangle Rhizome Extract (*Zingiber montanum*) to Histopathological Changes in the Kidney of Mice (*Mus musculus*)

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Abstract

Pyrethroid pesticide poisoning cases in developed and developing countries have a high incidence every year. One of the pyrethroid active substances that is widely used as an ingredient in anti-human lice shampoo (Pediculus humanus capitis) is permethrin 1%. The poisoning effect of permethrin-based shampoo as an anti-lice shampoo has been little studied, and there is a need to find alternative active ingredients from plants as a substitute for 1% permethrin, namely bangle (Zingiber montanum). This study aimed to determine the toxicity of shampoo made from 1% permethrin and bangle rhizome extract with graded doses through changes in kidney histopathology. The type of research was post test only control complete randomized design using 25 male mice, which were randomly divided into 5 groups, namely negative control (baby shampoo), positive control (1% permethrin shampoo), shampoo treatment made from bangle rhizome extract with graded doses (0.5%, 1%, and 2%). The shampoo was applied topically on the head for 14 days. After the mice were sacrificed, the kidneys were processed into tissues by paraffin method and stained with HE. The renal tubular injury was examined using a 400x magnification light microscope. The results showed the average changes in kidney histopathology in mice treated with 1% permethrin shampoo and 2% bangle rhizome extract shampoo, although not significantly different (p>0.05). Histopathological changes in the kidneys of mice that were seen were fatty degeneration, which caused swelling of the proximal tubules and necrosis of kidney cells

Keywords: *Bangle; Histopathologic; Kidney; Permethrin; Shampoo*

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INTRODUCTION

Human head lice (*Pediculus humanus* capitis) belong to the *Pediculidae* family. Head lice that infect the human head generally suck blood 2 to 6 times per day, and favor the head and behind the ears. The transmission of head lice can be done through shared items such as towels, blankets, hats, combs, and other personal items (Agustin, 2016). Head lice can affect anyone, especially children with an age range from 6 to 12 years, who have head to head contact (Massie et al., 2020; Saghafipour et al., 2017).

Each year in the United States, the incidence of pediculosis capitis is estimated to affect 6-12 million people (Dagrosa & Elston, 2017). The general picture is that the prevalence of *Pediculus humanus capitis* in developed countries is lower than in developing countries, including Indonesia (Wungouw et al., 2020). However, the exact incidence of pediculosis capitis is unknown because cases are not widely reported. In Indonesia, data on the prevalence of pediculosis capitis affecting all school-aged children is not yet available, but based on the results of a prevalence survey conducted in primary schools in Sabang City, Aceh Province, the result was 21.7% with the highest rate in Sukakarya District primary schools at 31.4% (Ramadhaniah et al., 2023).

Head lice can be transmitted directly and indirectly. Most commonly, it occurs through direct contact with the hair of a person with head lice. The most common mode of transmission is head-to-head contact. Head lice can spread quickly in children living in crowded neighborhoods, schools, dormitories, and others. Indirect spread can be through objects such as combs, hats, pillows, clothes, and others (Agustin, 2016; Devore et al., 2015).

The treatment of head lice involves eradicating the lice and their eggs and treating secondary infections. There are a variety of treatments used, such as pyrethrins in the form of lotions, shampoos, and creams, as well as other options such as pyrethroids, malathion 0.5% or 1%, lindane 1%, benzyl alcohol 5%, topical and oral ivermectin, and spinosad. However, using chemical insecticides to eradicate ticks can negatively impact health and the environment if not used appropriately. Overuse can also lead to resistance to insecticides. Therefore, other alternatives are needed, such as the use of natural insecticides like pyrethrins extracted from chrysanthemum flowers. Pyrethrins, the active compounds in *Chrysanthemum*, work by poisoning the nervous system of insects, causing paralysis and death in ticks (Abbasi et al., 2023; Verma & Namdeo, 2015).

Indonesia has many plants that can be used as natural insecticides. This is because plants in Indonesia contain a lot of content or secondary metabolites that have insecticidal functions, namely cyanide, steroids, tannins, flavonoids, saponins, alkaloids, and essential oils (Wahyuni et al., 2019) Bangle rhizome (*Zingiber montanum* is believed to be able to eradicate head lice (Soonwera, 2015). Based on phytochemical analysis of bangle rhizomes revealed the presence of phenylbutanoid compounds, cyclohexene derivatives, naphthoquinone, vanillin, vanillic acid, verric acid, terpenoids, β-sitosterol, and curcuminoids (Dechatiwongse & Yoshihira, 1973; Jitoe et al., 1994; Masuda et al., 1995) However, it is not possible to eradicate head lice if bangle rhizome extract is directly applied to the head, and it is necessary to test its toxicity in mammals. Therefore, bangle rhizome extract must first be processed into a shampoo preparation and then tested on male mice, and its toxicity is seen through changes in kidney morphology and histology.

METHOD

Chemicals

The materials used were baby shampoo, store-bought 1% permethrin-based anti-lice shampoo, and shampoo made from bangle rhizome extract. Baby shampoo was used as the negative control group because its chemical composition is safe to use as a hair cleanser. Other chemicals were to make bangle rhizome extract and to make histopathology preparations.

Bangle Shampoo Making

The shampoo formula will be made using bangle rhizome extract, HPMC, sodium lauryl sulfate, methyl paraben, and distilled water. Making the shampoo begins with weighing HPMC, and heating distilled water as much as 20 mL, then HPMC is gradually put into the heated distilled water while stirring slowly until it forms a mixture. In a different container, methyl paraben was dissolved using ethanol until dissolved. Then, some of the distilled water was heated at 60°C and sodium lauryl sulfate was added, then stirred until homogeneous. After the mixture of sodium lauryl sulfate and distilled water is homogeneous, a mixture of HPMC and methyl paraben solution is added, then stirred until it thickens. After that, bangle rhizome extract was added according to the concentration (0.5%, 1%, and 2%). Then add distilled water until the total mixture is 50 mL and ready for use (Fauziah & Yamaesa, 2019).

Experimental Design and Animal Experiments

25 male Balb C mice aged 60 days with body weight ranging from 20-30 g were placed in separate cages in an air-conditioned room with a temperature of 25°C. The test animals were divided into five groups of 5 animals each. The test materials used were 1% permethrin-based anti-lice shampoo available in the market and those made from bangle rhizome extract at graded doses. So the division of the research group is a negative control group (baby shampoo), positive control group (1% permethrin shampoo), shampoo treatment group made from bangle rhizome extract with graded doses ranging from 0.5%, 1% and 2% (5 each). Baby shampoo is used in this study because it has been widely used in general due to its safe chemical composition. All rats had their body weight recorded daily and food and water intake changed daily for 14 days of treatment. On the 15th day, all rats were fasted at night, and sacrificed the next morning using cervical displacement. The kidneys were surgically removed, weighed and then the kidneys were prepared using routine HE staining. Observations made are changes in renal histopathology in the form of fatty degeneration and renal cell necrosis.

Kidney Histology Preparation

For histology analysis, kidney tissues were obtained from mice that had been exposed to shampoo for 14 days, and dissected on day 15. Specimens were fixed in 10% neutral buffered formalin solution. After fixation, the specimens were dehydrated with 70, 90 and 100% graded alcohol, then immersed and embedded in paraffin (Xu et al., 2018). After that, the paraffin blocks were cut into sections 4 μm thick using a microtome, then stained with hematoxylin and eosin. Histopathological changes in cross sections of kidney tissue were observed using a light microscope with an Olympus brand camera at 400x magnification.

Analysis Statistic

All data obtained, namely body weight, kidney weight and renal histopathological changes were processed using the one-way Anova test (SPPS for windows series 25.0).

RESULTS AND DISCUSSION

In this study, Balb C mice were used to study the organ toxicity of using 1% permethrin pyrethroid-based shampoo and bangle rhizome extract-based shampoo at graded doses up to 2%. This is because mice have been widely used as test animals for chemical nephrotoxicity studies, since mice are used to determine toxicological symptoms and tissue changes similar to humans. The study determined changes in body weight, organ weight, serum and urine biochemical parameters, and histopathological changes in the kidneys after 14 days of shampoo exposure.

Body Weight

In the chronic toxicity study, shampoo with 1% permethrin and graded doses of bangle rhizome extract up to 2% for 14 days did not cause clinical toxicity symptoms and death of mice. Compared to the control group with baby shampoo, the results were not significantly different ($p>0.05$). The effect of 1% permethrin-based shampoo and graded doses of bangle rhizome extract on body weight is presented in Figure 1. Body weight measurements for 14 days found no difference between the groups of mice as shown in Table 1 ($p>0.05$).

Kidney Weight

The effect of 1% permethrin-based shampoo and graded doses of bangle rhizome extract on kidney weight is presented in Figure 2. Measurement of kidney organ weight for 14 days found no difference between the groups of mice as shown in Table 2 ($p>0.05$). In general, weight loss and renal organ weight are simple and sensitive indices of toxicity after exposure to toxic substances (Amresh et al., 2008; Teo et al., 2002). Decreased body weight and internal organ weight will be indicators of adverse effects. Based on the one-way Anova test in Table 1 and Table 2, there was a decrease in body weight between the unexposed control group and the 1% permethrin shampoo group, and the shampoo treatment made from graded doses of bangle rhizome extract. Although the test results stated that there was no significant difference ($p>0.05$) in kidney weight, it did not mean that there were no changes in kidney histopathology of mice exposed to shampoo for 14 days.

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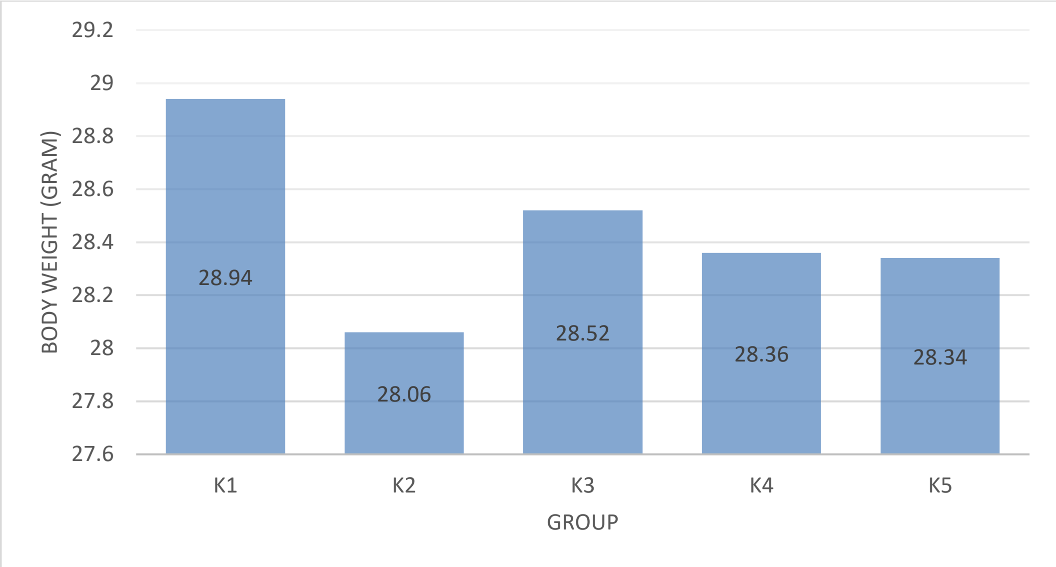

Figure 1. Graph of Mean Body Weight of Mice After 14 Days of Shampoo Exposure. (K1) Negative Control, (K2) Positive Control (1% Permethrin Shampoo), (K3) Shampoo Treatment of 0.5% Bangle Rhizome Extract, (K4) Shampoo Treatment of 1% Bangle Rhizome Extract, (K5) Shampoo Treatment of 2% Bangle Rhizome Extract.

Table 1. One-Way Anova Test of Body Weight of Mice After 14 Days of Shampoo Exposure

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		2.086	4	0.521	2.079	0.122
	Linear Term	Contrast	.405	1	0.405	1.615	0.218
		Deviation	1.681	3	0.560	2.234	0.116
Within Groups			5.016	20	0.251		
	Total		7.102	24			

Histopathologic Changes of the Kidney

The results showed that the use of 1% permethrin-based shampoo, which is a low dose, can still affect organ function, although statistical tests using one way Anova showed no significant differences in body weight, organ weight, changes in biochemical parameters, and changes in kidney histopathology (p>0.05). The kidney is a sensitive organ, and its function is affected by a number of factors including drugs and phytochemicals of plant origin (Chang et al., 2012; Mansour et al., 2003).

Changes in kidney histopathology in 1% permethrin-based shampoos and in graded doses of bangle rhizome extract up to 2% were proximal tubular swelling and ginal cell necrosis. This means that after exposure to shampoo for 14 days, no toxic symptoms or changes in biochemical parameters or changes in kidney histopathology were found in mice. Because the results of statistical tests using one-way Anova said there were no significant differences (p>0.05), the use of shampoo made from bangle rhizome extract at doses up to 2% is safe to use.

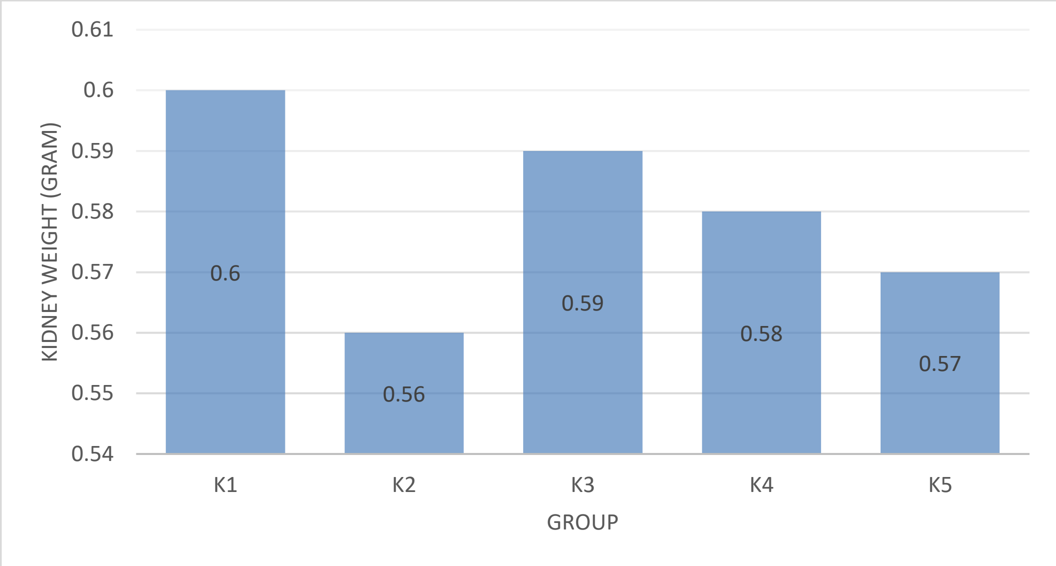

Figure 2. Graph of Average Kidney Weight of Mice After 14 Days of Shampoo Exposure. (K1) Negative Control, (K2) Positive Control (1% Permethrin Shampoo), (K3) 0.5% Bangle Rhizome Extract Shampoo Treatment, (K4) 1% Bangle Rhizome Extract Shampoo Treatment, (K5) 2% Bangle Rhizome Extract Shampoo Treatment

Table 2. One-Way Anova Test of Kidney Weight of Mice after 14 Days of Shampoo Exposure

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	(Combined)		0.004	4	0.01	0.971	0.445
	Linear Term	Contrast	0.001	1	0.001	0.532	0.474
		Deviation	0.003	3	0.001	1.117	0.366
Within Group			0.019	20	0.001		
	Total		0.023	24			

The results of this study also refer to the study of Koontongkaew et al., (2014) who observed the incidence of acute and chronic oral toxicity of Z. cassumunar extract granules in Sprague-Dawle rats, with a single dose of 5000 mg/kg body weight producing no signs of toxicity to the renal organs or treatment-related mortality over a 14-day observation period.

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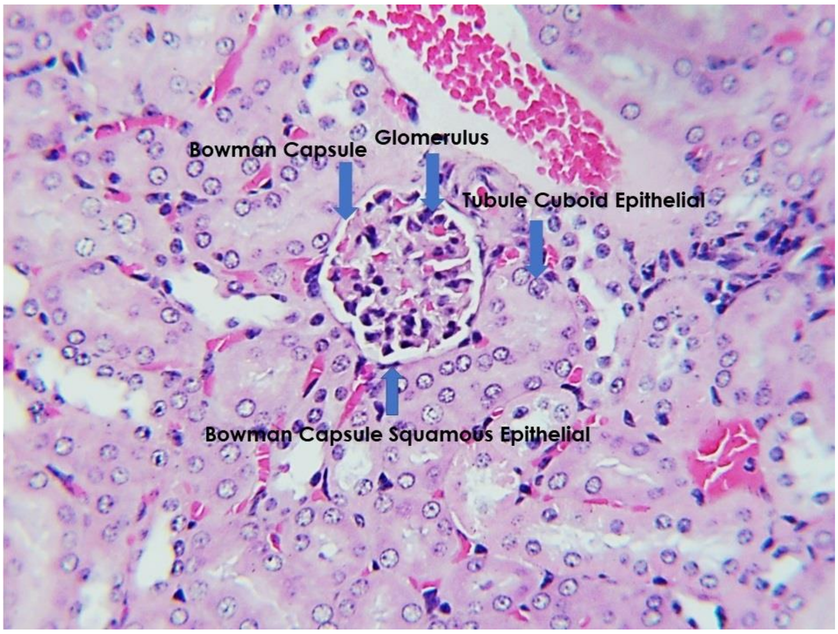

Figure 3. Photomicroscopic Cross Section of Kidney of Control Group Mice with Baby Shampoo (HE, 400x). No Visible Damage or Histopathological Changes in the Tubule Epithelium or Other Constituent Cells

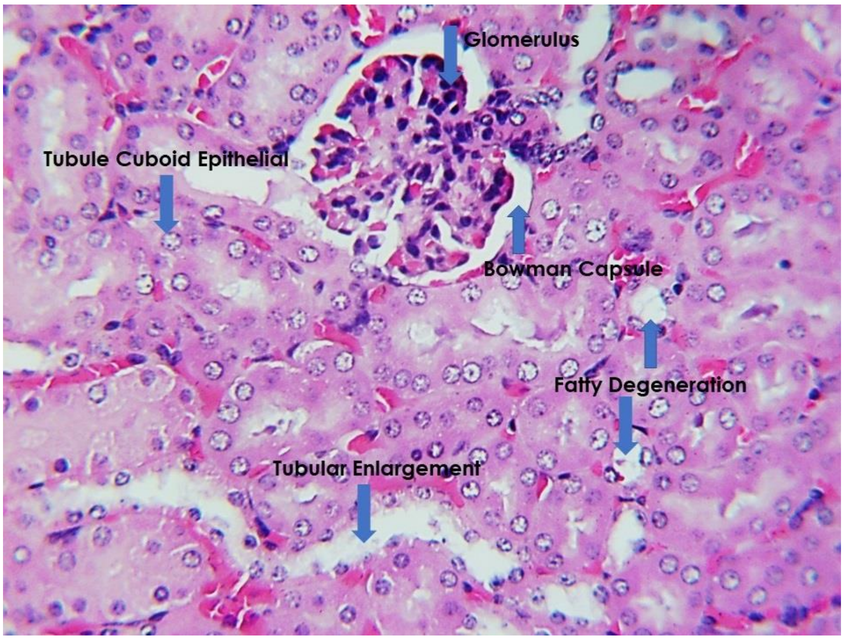

Figure 4. Photomicroscopic Cross Section of Kidney of Control Group Mice with 1% Permethrin Shampoo (HE, 400x). Histopathological Changes are Visible, Namely Fatty Degeneration in the Kidney Medulla and Cause Tubular Enlargement

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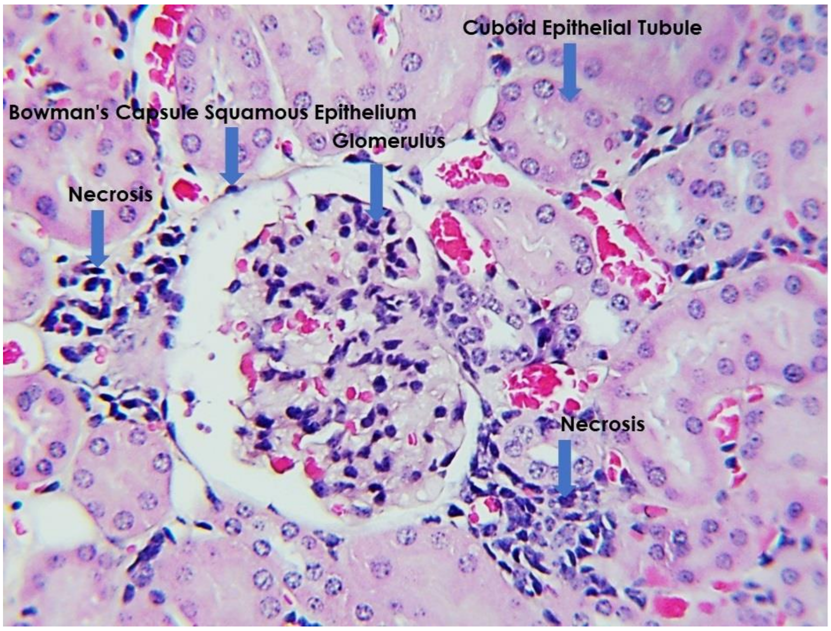

Figure 5. Photomicroscopic Cross Section of Kidney of Control Group Mice with 1% Permethrin Shampoo (HE, 400x). Histopathologic Changes are Visible, namely Cell Necrosis in the Kidney Medulla

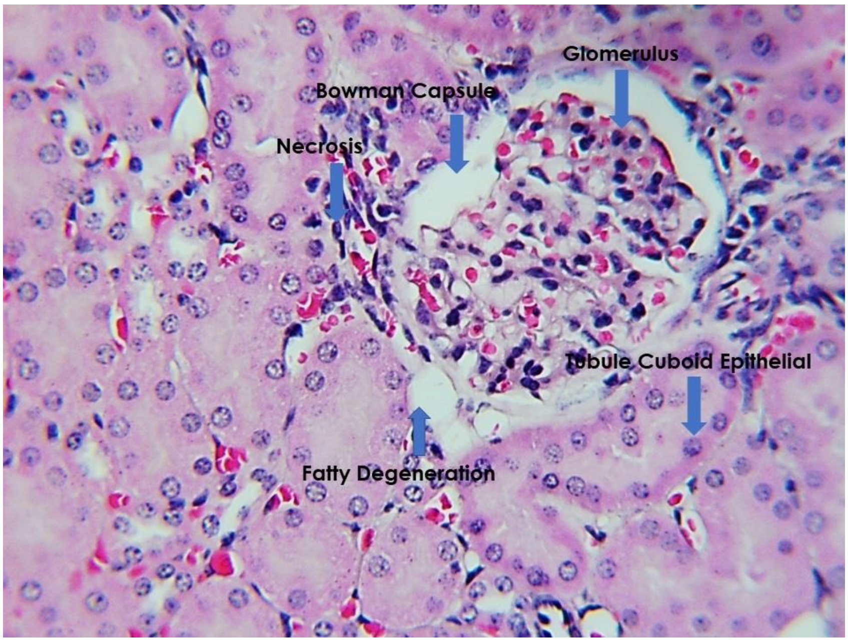

Figure 6. Photomicroscopic Cross Section of Kidney of Control Group Mice with 2% Bangle Rhizome Extract Shampoo (HE, 400x). Histopathologic Changes are Visible, namely Cell Necrosis in the Kidney Medulla and Fatty Degeneration

Permethrin is a synthetic neurotoxic pyrethroid used in the treatment of pediculosis (Nanda & Juergens, 2020). Permethrin has the ability of neurotoxicity by acting on the nervous system (Nanda & Juergens, 2020; Toynton et al., 2009). The mechanism is by interfering with sodium channels that cause depolarization, leading to respiratory paralysis in affected head lice. Permethrin also slows the closure of Na^+ channels, causing neuronal dysfunction. This causes respiratory paralysis in the louse, which can leado death (Gammon, 2014) .

The results of research conducted by Calvert (2016) stated that the administration of permethrin at a dose of 100 mg/kgBB orally or through skin exposure can cause poisoning. To determine the level of poisoning caused can be by observing the kidney organ, which functions to remove the body's metabolic waste products in the form of urine, including toxic substances. Residual metabolites that are toxic in the kidney can cause kidney tissue damage. Histopathological changes in the kidney due to exposure to toxic substances include interstitial tubule damage in the form of tubule dilation, fatty degeneration, and necrosis of tubule epithelial cells. Substances that are toxic in the kidney are also called nephrotoxic. The necrotoxic state can be seen in the proximal tubules, which are the part that is easily damaged by exposure to these toxic substances. Damage to the proximal tubule is seen in the form of damaged epithelium and causes leakage, so that the toxic material can easily flow through the proximal tubule and accumulate in it.

Grewal et al., (2010) have also previously observed exposure to cypermethrin, which is in the same class as permethrin. Toxicity begins with the excretion process in the kidney organ, which disrupts the reabsorption process and reduces the function of the proximal tubules, which in turn causes the epithelium that makes up the proximal tubules to undergo apoptosis. Proteins that are removed from the glomerulus through the filtration process cannot be completely absorbed by the proximal tubule epithelium, so the proteins accumulate in the tubule lumen.

CONCLUSION

Exposure to 1% permethrin-based shampoo and bangle rhizome extract-based shampoo at graded doses up to 2% caused changes in body weight, kidney weight, and histopathological changes in the kidneys of male mice, although not significantly different. Histopathological changes in the kidneys included proximal tubule swelling and renal cell necrosis. Shampoo made from bangle rhizome extract is safe to use as an anti-lice shampoo (*Pediculus humanus capitis*).

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