



THE DIFFERENCE OF HISTOPATHOLOGICAL IMAGE OF THE WISTAR RAT'S KIDNEY ADMINISTERED WITH GRADUAL DOSAGE OF PYRETHROID

Diaz Almayang¹, Saebani^{2*}, Tuntas Dhanardhono², Endang Ambarwati³, Ika Pawitra Miranti⁴

¹Undergraduate Program, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

² Department of Forensic and Medicolegal, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

³Department of Physiology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

⁴ Department of Anatomical Pathology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

* Corresponding author: Email: donsaebavo@gmail.com

ABSTRACT

Background: Pyrethroid pesticide poisoning cases in developed and developing countries have a high incidence every year. One of the active substances in pyrethroid widely used is cypermethrin. In the 2006-2016 period, 30.789 cases of pesticide poisoning caused by cypermethrin were reported in Jiangsu Province, China. Among these cases, 12.867 involved men and 17.922 involved women. Overall, 1.705 cases ended in death. The effects of cypermethrin intoxication on the kidneys, especially in humans, are little to be studied. This study aims to determine the difference in the histopathological image of the *Wistar* rat's kidney. **Method:** The experimental research of the *post-test only control group design* involved 24 male *Wistar* rats divided into 4 groups randomly, the control (not given cypermethrin), treatments of 125 mg/kgBW, 250 mg/kgBW, and 500 mg/kgBW. Cypermethrin are given orally for 14 days. After the rats were terminated, the kidney were processed to be paraffin-embedded tissues and stained with HE. Tubular injuries were examined using 400x magnification of light microscope and focused on closure of tubular lumen as well as proteinaceous cast inside the lumens. **Results:** The results of this study showed that the means of histopathological damage to the kidneys increased from control group to 500 mg/kgBW treatment group. Statistical analysis with *One way ANOVA* showed significant differences ($p < 0.001$), continued with *Post hoc games Howell* test, there was a significant differences between control group with 250 mg/kgBW treatment group and 500 mg/kgBW treatment group, and between 125 mg/kgBW treatment group with 250mg/kgBW treatment group and 500 mg/kgBW treatment group. There was no significant difference between the control group with 125 mg/kgBW group and 250 mg/kgBW treatment group with 500 mg/kgBW treatment group. **Conclusion:** There is a significant difference in renal histopathological image due to exposure to pyrethroid (cypermethrin) in gradual doses. The image of kidney damages can result in tubules injury which include: albuminous degeneration with narrowing of the tubular lumen and hyaline cast. The means of tubular injury rate will increase with increasing dose of pyrethroid.

Keywords: cypermethrin; kidney damage; pyrethroid

INTRODUCTION

Pesticides are chemical compounds that eradicate pests and it is widely used in various fields with the aim of reducing disturbance due to disturbing organisms.¹ In agriculture, farmers generally anticipate plant pests since early stage, from the beginning of planting. The presence of pests in the land will encourage farmers to use excess pesticides by increasing the dose, frequency of spraying and composition of the used pesticide mixture type. In fact, not a few farmers adhere to cover blanket system with or without the presence of pests, pesticides are still applied.²

To date, there are no pesticides that are truly safe.³ Pesticide toxicity is still quite high in humans and animals, so that poisoning by pesticides still often occurs either due to negligence, accidental contact, and some even

being misused on purpose. Another danger of pesticides is the suspicion that some of them are carcinogenic and can damage various organs in the body if poisoning occurs.⁴

There are five major groups of pesticides, such as organophosphates, carbamates, organochlorines, and pyrethroid. Pyrethroid is one of the synthetic insecticides currently being developed. Pyrethroid is synthetic insecticides that are similar to pyrethrin. Based on the time of its discovery, pyrethroid is divided into four generations, the first generation (Alletrin), the second generation (Rasmethrin), the third generation (Fanvarelan and Parmethrin) and the fourth generation (Cypermethrin). Cypermethrin is a class of insecticides that have specific properties for insect control, including: high effectiveness (as a contact and stomach poison), less toxic to mammals, and a relatively rapid loss of effectiveness.¹



In the 2006-2016 period, 30.789 cases of pesticide poisoning caused by cypermethrin were reported in Jiangsu Province, China. Among these cases, 12.867 involved men and 17.922 involved women. Overall, 1.705 cases ended in death, resulting in a mortality rate of 5.5%, comprising 765 men and 940 women. Of these cases a total of 23.557 were non-occupational intoxication primarily triggered by suicide or accidental consumption. This case mainly occurs in individuals aged 36-60 years.⁵

Based on data from the World Health Organization (WHO) and the United Nations Environment Program (UNEP) on workers in the agricultural sector, it is estimated that there are 1.5 million cases of pesticide poisoning, mostly in developing countries, 20.000 of which are fatal cases. Meanwhile, according to data from the National Poisoning Information Center in 2016 there were 771 cases of pesticide poisoning in various regions in Indonesia and cases of pesticide poisoning in Kulon Progo, there were 210 cases of poisoning by physical and clinical examinations, 50 of whom were examined in the laboratory with 15 results. people (30%) positive for poisoning. Sleman Regency area reported that of 30 pest control officers 14 people (46.66%) experienced symptoms of poisoning.⁶

Cypermethrin intoxication doses 100 mg/kgBW may result from inhalation, oral and dermal exposure. Oral intoxication causes a wider range of symptoms and effects than inhalation and dermal. The effects of cypermethrin into the kidneys have been very little studied. The kidneys are one of the vital organs in the body and function to remove the waste products of the body's metabolism in the form of urine, including toxic substances that accidentally enter the body. The excretion of metabolite wastes in the kidneys can cause tissue damage due to contact with these toxic substances. Damage to the kidney can be seen by observing changes in the histopathological structure of the kidney, including tubulointerstitial damage in the form of tubular dilation, albuminous degeneration, and tubular cell necrosis. If left untreated, kidney tissue damage can lead to kidney failure which can lead to death.

METHOD

This study was an experimental study with a *post-test only control group design* involved 24 rats which are divided into 4 groups, that were control, exposure to 125 mg/kg, 250 mg/kg, and 500 mg/kg of body weight. Cypermethrin given orally for 14 days. After 14 days, the rats were terminated and the kidneys were examined by histopathological examination. The preparations were observed under a light microscope at 400 times magnification. Each preparation was observed in 5 different viewpoints which are considered to represent the whole description. Observations were made 2 times. The number of injured tubules was calculated including albuminous degeneration, tubular lumen narrowing, and *Hyalin cast*. The data obtained will be processed with a computer program. The data distribution was tested for normality with the *Saphiro-Wilk* test. The data were normally distributed with homogeneous variations, the difference test was carried out by *One-Way-ANOVA* followed by *Post Hoc Bonferroni*. The data had non-homogeneous variations, the difference test was carried out by *One-Way-ANOVA* followed by *Post hoc Games-Howell*. If the data distribution is not normal, then the *Kruskal-Wallis* test is carried out, if the p value is <0.05 then it is followed by the *Mann-Whitney test*.

RESULT

The renal histopathological preparations were made using Hematoxylin Eosin (HE) staining, then the tubules that were injured were read, including: albuminous degeneration, narrowing of the lumen, and the discovery of a *hyaline cast* with 400x magnification. Readings were carried out in 5 fields of view on each preparation.

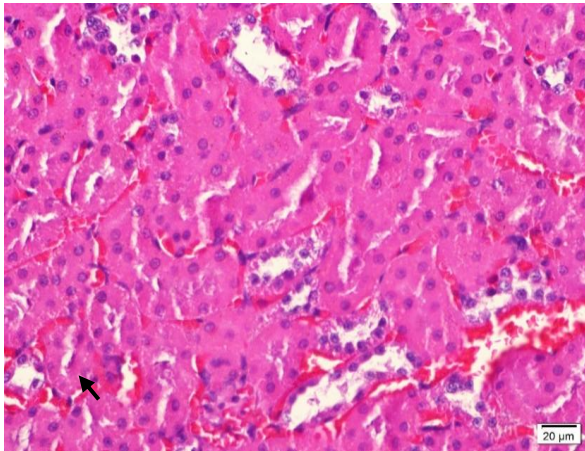


Figure 1. Closure of the lumen of the injured renal tubule (HE 400x)

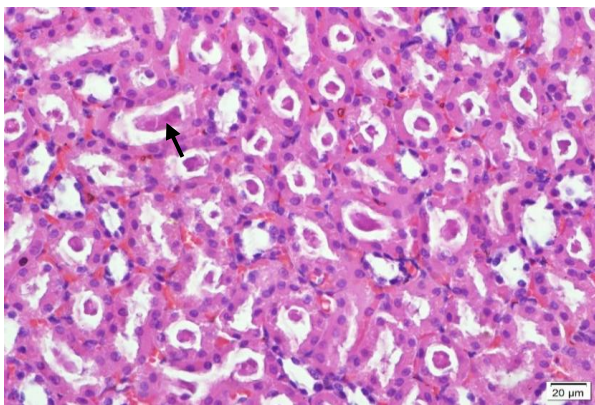


Figure 2. Hyaline cast in the tubular lumen of the injured kidney (HE 400x)

Table 1. Descriptive analysis results of the degree of renal histopathological damage for each group.

Variabel	Mean ± SD	Minimum	Maximum
Control	2,63 ± 0,56	1,8	3,2
125 mg	5,17 ± 2,92	0,8	8,2
250 mg	13,75 ± 3,48	10,6	18,2
500 mg	17,00 ± 4,30	11,2	21,2

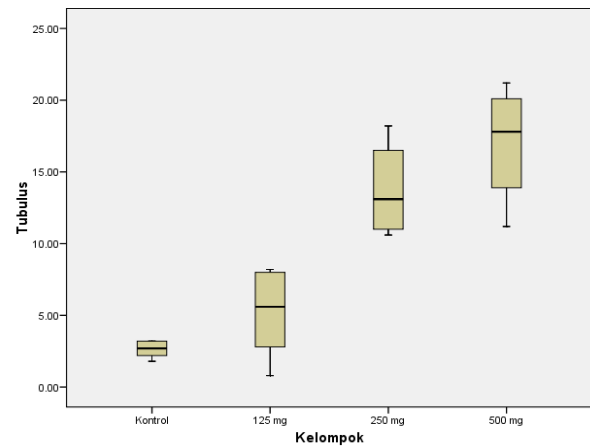


Figure 3. Boxplot of the kidney damage level for each treatment group.

Based on Table 1 and Figure 3, it can be seen that the mean kidney damage in rats will get heavier as the pyrethroid dose increases.

Table 2. One Way ANOVA Test Results.

Variabel	Mean ± SD	p	Homogeneity
Control	2,63 ± 0,56	<0,001*	0,048 (not homogen)
125 mg	5,17 ± 2,92		
250 mg	13,75 ± 3,48		
500 mg	17,00 ± 4,30		

Explanation: * Significant (p <0.05)

Based on table 2, it can be concluded that this study hypothesis was accepted, that was the administration of pyrethroid orally causes changes in the histopathological description of the kidneys in *Wistar* rats (p = <0.001).

Table 3. Hasil uji *post hoc* Games-Howell

Variabel	125 mg	250 mg	500 mg
Control	0,267	0,022*	0,020*
125 mg	–	0,028*	0,019*
250 mg	–	–	0,663

Explanation: * Significant (p <0.05)

Based on table 3, it can be concluded that there were significant differences between treatment groups (p <0.05).



CONCLUSION

The kidney is one of the organs that has the function of removing substances that are no longer needed by the body or toxic substances so that the kidneys are often exposed to nephrotoxic substances. In nephrotoxic cases, the proximal tubule is the part that is prone to damage. This is because the epithelium is weak and leaks easily so that the nephrotoxic material flows easily to the proximal tubule and accumulates in it.

Based on descriptive analysis, the mean number of rats' kidney tubule damage in the control group was 2.63 where this figure was the lowest average, the 125 mg/kgBW treatment group had a mean of 5.17, the 250 mg/kgBW treatment group had a mean of 13.75, and the treatment group 500 mg/kgBW has the highest average of 17. In the control group, damage was found, possibly because the rats in the control group had previous abnormalities or the bias from albuminous degeneration because this degeneration normally occurs in post-mortem tissue.

The histopathological changes found in the renal tubules are albuminous degeneration, lumen constriction, and *hyaline cast*. The results of microscopic observations of *Wistar* rats' kidneys were analyzed by *One way ANOVA* test, which showed significant differences in histopathological features between the control group and the treatment group. Based on further statistical tests, namely the *Post hoc Games-Howell* test, there was a significant difference in the histopathological description between the control group and the 250 and 500 mg/kgBW treatment group, and the 125 mg/kgBW treatment group with the 250 and 500 mg/kgBW dose group. This supports the study findings in descriptive analysis. However, between the control group with the 125 mg/kgBW dose group, and the 250 mg/kgBW dose group with the 500 mg/kgBW dose group showed insignificant differences in histopathological features. This insignificant difference in histopathological features is probably due to the small number of study samples and the difference in dosage that is not much adrift.

These results were in line with a study by Grewal K, et al in 2010 entitled "Toxic Impacts of Cypermethrin on Behavior and Histology of Certain Tissues of Albino Rats". The study used a much lower dose of cypermethrin than this study, namely

5 and 20 mg/kgBW with a much longer duration of 30 days. It is concluded that the greater the dose of cypermethrin given, the more severe the histopathological damage to the kidneys, the damage includes narrowing of the tubular lumen, albuminous degeneration, and necrosis.⁹ Similar results were obtained by a study at the Bogor Agricultural University, entitled "Organ Histopathological Overview Liver and Kidney in Acute Intoxication of Insecticides (Metoflutherin, D-Phenothrin, D-Allethrin) with Multilevel Doses". The experimental study which also used *Wistar* rat subjects also found that the administration of pyrethroid of the Metoflutherin, D-Phenothrin, and D-Allethrin groups orally would cause damage to the rats' kidneys characterized by *hyaline cast*, tubular lumen dilation, Bowman's space edema, albuminous degeneration, as well as congestion. The changes that occur tend to increase with increasing doses. The highest dose, 5000 mg/kgBW, is the dose that causes the most cell deaths and causes 60% of the rat population 24 hours after giving the insecticide formulation. The study used 25 rats divided into 5 groups, namely the pyrethroid dose of 5000 mg/kg, 2500 mg/kg, 1250 mg/kg and 625 mg/kg of body weight.¹⁰

Descriptively, tubular damage occurred mostly in the 500 mg/kgBW dose group. This is thought to be due to the effect of the insecticide formulation, cypermethrin, which is toxic and has gone through the excretion process in the kidneys, thereby interfering with the reabsorption process and decreasing tubular function. Decreased tubular reabsorption function is due to the tubular epithelium having degenerated to apoptosis. The protein that escapes from the glomerulus cannot be completely absorbed by the tubular epithelium, so there is a buildup of protein in the tubular lumen. The protein deposits presence in the tubules also indicates that the insecticide formulation of cypermethrin is nephrotoxic.¹⁰

The proteins accumulation in the cytoplasm of cells is characterized by eosinophilic colored material. This change is simply a reflex of an increase in protein absorption by cells. Plasma protein escapes from the abnormal glomerular capillaries and is absorbed by pinocytosis from the tubular lumen. If the protein that escapes exceeds the cell's absorption capacity, it will show eosinophilic albumin in the tubular lumen. The



number of protein drops in the kidney tubules is due to the presence of toxic substances, causing these materials to remain in the tubular lumen.^{10,11}

Other things that give impact are stress factors for *Wistar* rats, the impact of other substances or diseases, and internal factors such as immunity and vulnerability of *Wistar* rats. The weakness in this study was that albuminose degeneration is a biased factor. In addition, the control group who looked healthy, it turned out that the condition of the kidneys on microscopic observation showed a histopathological description abnormality. The Anatomical Pathologist can eliminate bias that may occur in microscopic by doing an observations in 5 fields of view in each preparation which is considered to represent the overall description and observations and it is done for 2 observers.

DISCUSSION AND SUGGESTIONS

There was a significant difference between the control group and the group giving the dose of 250 mg/kgBW and 500 mg/kgBW, between the 125 mg/kgBW group giving the dose of 250 mg/kgBW and 500 mg/kgBW. The description of kidney damage can be in the form of injury to the tubules which include: albuminous degeneration accompanied by narrowing of the tubular lumen and hyaline cast. The mean rate of tubular injury will increase with increasing dose of pyrethroid.

Suggestions from this study are:

1. It is necessary to do a comparative study regarding the effect of orally pyrethroid administering at the same dose in this study so that the obtained results have higher objectivity.
2. For future studies, consider to use a lower dose than the most recent LD50 so that the demonstrated renal damage effect can be maximized, without causing premature death in rats.
3. It needs more attention in caring *Wistar* rats so that there are no abnormalities that cause bias in the study results.
4. It is necessary to do further regulations regarding restrictions on the use of pyrethroid pesticides in human life.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Writing-original draft preparation, Diaz Almayang; writing-review and editing, Saebani, S.KM, M.Kes

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