Acute Toxicity of Earthworm (*Pheretima javanica* K.) Powder on Renal Histopathological Description of Rat (*Rattus norvegicus* B.)

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Abstract

Earthworm (Pheretima javanica K.) is a common soil biofertilizer. This repulsive animal for some has been widely used for remedial medicine as well. Simplicia being used in this study was obtained from dried and blended earthworm along with its intact internal organs. The aim of the study was to determine the toxicity effect of earthworms powder to renal histopathology in rats (Rattus norvegicus). A total of 50 rats were divided into five groups consisting of a negative control group (2 ml 1% CMC Na), P1 earthworms group at dose of 0.4 grams, P2 earthworms groups at dose of 0.8 grams, P3 earthworms group at a dose of 1.6 grams, and P4 earthworms group at a dose of 3.2 grams. The rats were acclimated for about a week, then orally induced by earthworm powder. The treatments were conducted for 14 consecutive days to see any toxic symptoms developed. ANOVA results showed the administration of earthworm (Pheretima javanica K.) powder did not significantly affect renal physiology and histopathology. There was no damage observed microscopically.

Keywords: *Pheretima javanica K. powder, Rattus norvegicus B., renal histopathology.*

1. INTRODUCTION

The abundance of earthworms (*Pheretima javanica* K.) has been utilized by local people as biofertilizer, source of protein for livestock and as traditional remedies especially as an alternative raw material for treatment of typhoid fever. Earthworms are quite familiar especially for rural communities. They seem weak as if having no benefit, but they have tremendous potential for human life and well-being (Waluyo, et al., 1994).

In general, the earthworm contains proteins, amino acids and various enzymes. Several studies have also proved the antibacterial capacity of earthworm especially of *Lumbricus rubellus* and *Pheretima* sp to inhibit the growth of Gram-negative bacteria Escherichia coli, *Shigella dysenteriae*, *Staphylococcus aureus* and *Salmonella typhi* (Waluyo, 2006). The only toxic effect of earthworms is that they can accumulate heavy metals present in the soil and store it in their bodies. Earthworms can tolerate heavy metals in high concentrations.

Earthworms greatly influence the transformation of nitrogen in the soil, increasing in nitrogen mineralization will also increase the availability of nitrates (Ansyori, 2015). Earthworms also increase the nitrogen content of the vermicompost by excretion of ammonia, nitrogenase enzymes and mucous fluid.

The hyperaccumulation of nitrogen will have an impact on humans who consume earthworms as ingredients of medicine. Excessive nitrogen content in human body can cause a decrease in the oxygen carrying capacity when reacted with hemoglobin, it also decreasing thyroid gland and inducing vitamin A deficiency.

It can be concluded that earthworm is a high protein source which is used as a substitute drug of traditional chemicals. The consumption of the earthworm products

should be considered safe and free from toxic effects. However, any substance has toxic potential depends on the dose in the body. Toxic effects are an effect that can lead to toxic symptoms in varying degree of disturbances from mild to death (Farmacia, 2008 in Nuridayanti, 2011).

The use of a drug by humans benefit begins with pre-clinical trials. Pre-clinical test is an experimental study using test animals, such as mice and rats. Through pre-clinical test, information such as pharmacological effects, pharmacokinetic profile, and the safety of a drug can be obtained (Sukandar, 2000). Toxicity test is carried out to examine the safety of a drug.

Testing of earthworm products as drug is done due to its ability to accumulate heavy metals. Toxicity tests are performed to determine the toxic effects to vital organs such as the liver and kidneys (Price and Wilson, 2005), since both organs are likely to be affected by high doses of drugs. According to McPherson (2004) in Nuridayanti (2011) the level of urine and creatinine in blood are the parameters of renal function. If renal function is impaired, urine and creatinine concentration in the blood surpass their normal value.

Since the administration of earthworm (*Pheretima javanica* K.) powder is done within certain long period, an acute toxicity test is performed to determine its toxic effects. This will affect the function of kidney, so it is necessary to observe renal physiology and histopathology.

2. RESEARCH METHOD

This research was conducted in Pharmacology Laboratory Faculty of Dentistry and Laboratory of Biology Education, University of Jember. Totals of 50 samples used in this study consist of males and females rats (*Rattus norvegicus* B.) aged 3-4 months and weigh araound 120-200 grams. Each treatment repeated 5 times.

Animal samples were placed in cages with temperature of ± 25˚C and humidity 80%. Dry husk in the base of cages was replaced every 3 days. Animal samples were acclimated for 7 days to gain uniform state prior to treatment. Food and water was given on daily basis.

The powder was obtained after several procedurs. Fresh earthworm were washed with running water then rinsed for 6 days. After being ovened at 40°C for 4 hours, it was blended and weighed for each treatment doses that are 0.4 grams; 0.8 grams; 1.6 grams; and 3.2 grams.

Earthworm (*Pheretima javanica* K.) powder were administered on the first week after acclimation and continue for 14 consecutive days. Before administration the level of blood creatin and urine viscosity was measured. This measurement was repeated post powder administration or after 2 full weeks. The treated kidney then being microscopically observed its histopathology.

The preparation of histopathologic observation was performed through several procedures. First, kidney was fixed using a 10% Neutral Buffer Formalyn solution then cut and put specimens into plastic container. Dehydration process then carried out in alcohol concentration of 70%, 80%, 90%, alcohol absolute I, absolute II for 2 hours each. Xylol was added to clarify the preparation. It then embedded into molten paraffins and stored in refrigerator after solidified. The paraffin blocks were then cut thinly 5-6 μm using a microtome. The cuts were floated in warm water at 60° C to prevent the tissue from folding. The preparations are then removed and placed in a glass object and stained with Hematoxylin and Eosin (HE).

The histopathological observations were performed by comparing treatment groups with placebo groups. The parameters observed were changes in fatty degeneration (vacuolization), necrosis and hydrophilic degeneration. Scoring was done on every change observed. Histopathological scoring for normal = 0, presence of hydrophic degeneration = 1, presence of degeneration of fat = 2, and presence of necrosis = 3.

Anova was carried out to analyze the differences in serum creatinine and urin level before and after treatments, followed by LSD test to determine the least significant difference between treatments. The observation results analyzed descriptively.

3. RESULTS AND DISCUSSION

The parameters observed in this study included the difference of creatine level and urine viscosity before and after the administration of earthworm (*Pheretima javanica* K.) powder. From the results of laboratory tests, the values of creatinine in blood before and after induction are as follows.

Table 1. Creatinine level before and after induction on Rat (*Rattus norvegicus*)

Treatments	Average	
	Before	After
Control/C (-)	0.5	0.57
0.4 grams (T1)	0.55	0.55
0.8 grams (T2)	0.525	0.55
1.6 grams (T3)	0.425	0.6
3.2 grams (T4)	0.475	0.6

The result of Anova test indicated that there was no significant difference of powder administration to renal physiology of rats before treatment and after treatment (p> 0,05 Psig: 0,570). Earthworm powder (*Pheretima javanica* K.) has been shown to be non toxic based on the results of a blood creatinine test and is also evidenced by the results of initial urine observation and the end of treatment. The results of urine examination are provided in table form as follows.

Table 2. Urine Density Before and After Treatments

Treatments	Average	
	Before	After
Control (-)	1,0005	1,007
0.4 grams	1,001	1,001
0.8 grams	1,0005	1,002
1.6 grams	1,0005	1,001
3.2 grams	1,0005	1,000

The results of histopathologic examination of renal can be seen in Table 3.

Table 3. Renal Histopathology Examination Result

Treatment	Repetition					Score
	1	2	3	4	5	
C- male	-	-	-	0	-	0
C- female	-	0	-	-	-	0
T1 male	0	-	-	-	-	0
T1 female	-	-	0	-	-	0
T2 male	0	-	-	-	-	0
T2 female	0	-	-	-	-	0
T3 male	-	-	-	-	0	0
T3 female	-	0	-	-	-	0
T4 male	0	-	-	-	-	0
T4 female	-	-	-	-	0	0

The results of histopathology examination found no hydrophic degeneration, necrosis nor fatty degeneration. The renal structure in general does not show any histopathological changes of degeneration or necrosis. The results of microscopic observations representing each treatment group can be seen in Figures 1 through 10.

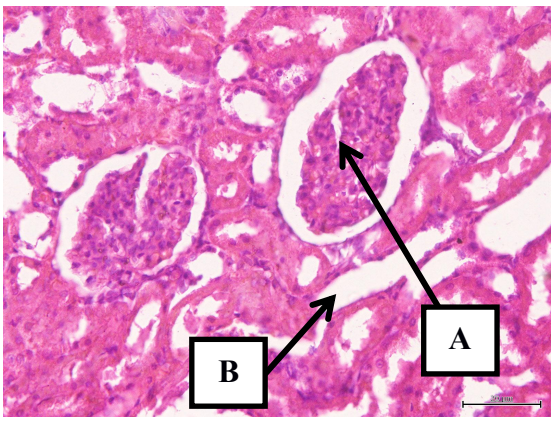

Figure 1. Histopathology features of white rats in the C-group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

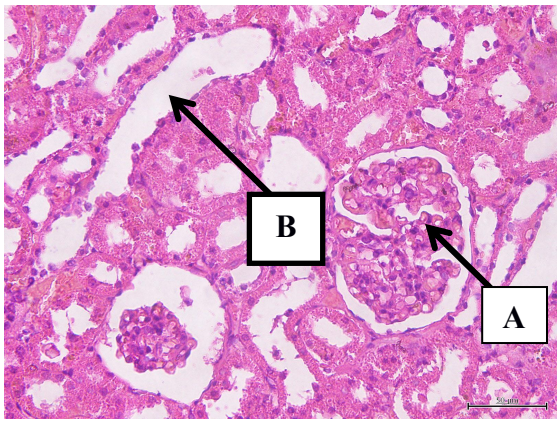

Figure 2. Histopathology features of white rats in the C-female group with a magnification of 400x (A: Glomerolus, B: Proximal Tubulus).

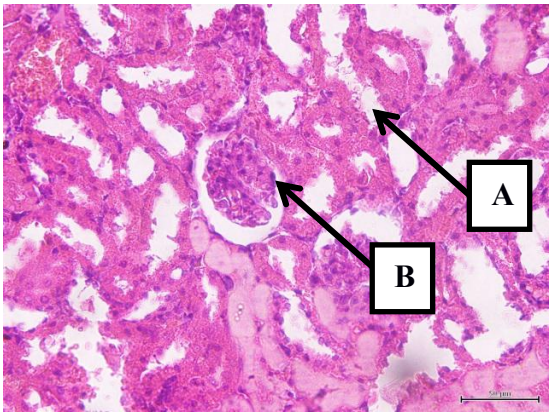

Figure 5. Histopathological features of white rats in the male T2 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

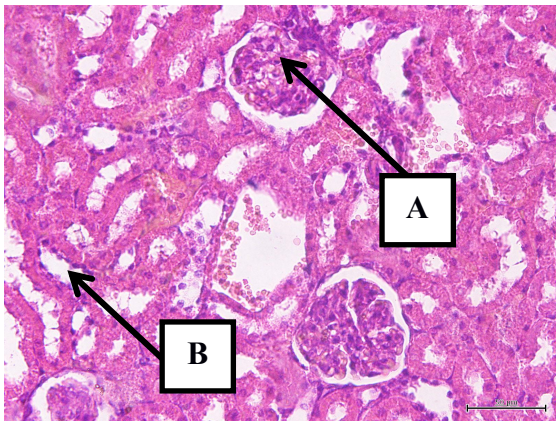

Figure 3 Histopathological features of rats of white rats in the male T1 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

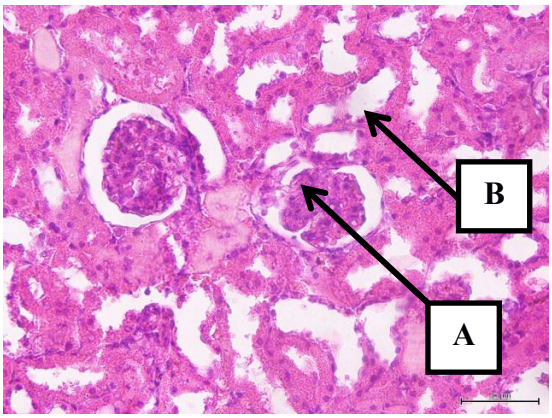

Figure 6 Histopathology of rats of white rat in female P2 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

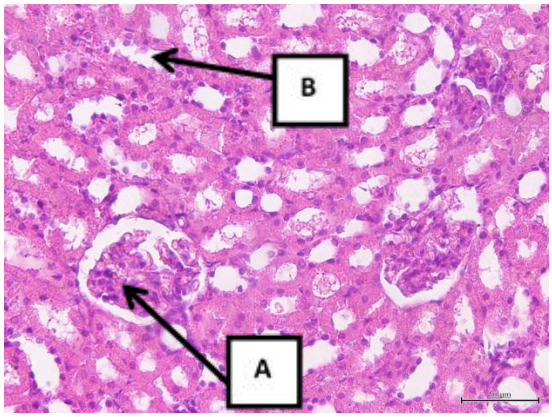

Figure 4. Histopathology features of white rats in the female T1 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

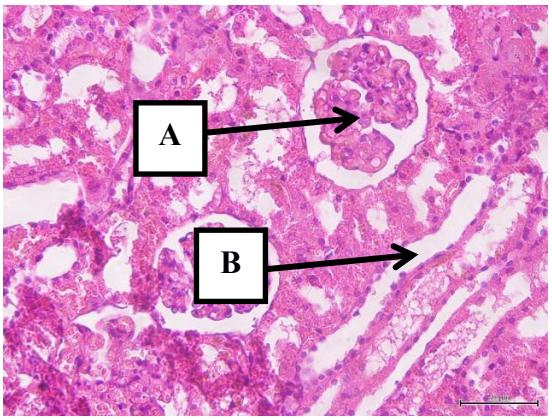

Figure 7. Renal histopathologic features of white rats in the male T3 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

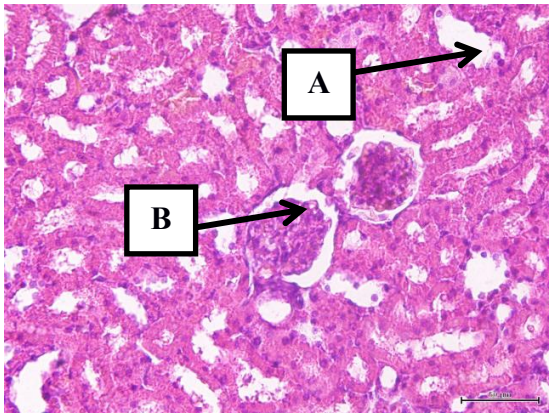

Figure 8. Histopathologic features of white rats in the female T3 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

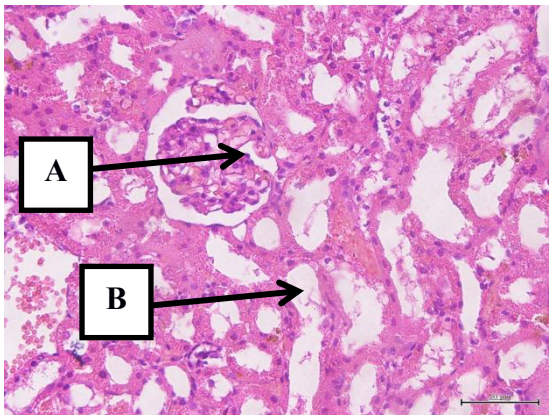

Figure 9. Histopathology features of white rats in the male T4 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus).

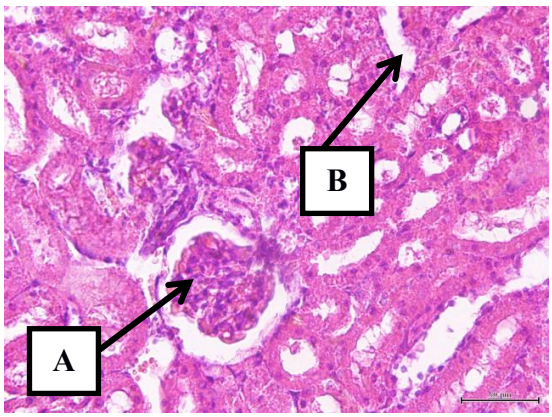

Figure 10. Renal histopathologic picture of white rat in female T4 group with 400x magnification (A: Glomerolus, B: Proximal Tubulus

The tests were performed to determine whether there was a significant difference from each dose group to the normal group. If there are significant differences, then proceed with BNT test. Data of laboratory examination results continued with ANOVA test. From the results of ANOVA testing there was no significant difference in serum creatinine.

In the renal histopathology of white rats (*Rattus norvegicus* B.) showed that the various doses of earthworm powder (*Pheretima javanica* K.) given did not cause significant changes in the histologic structure. Neither degeneration nor necrosis was found in all treatment groups showing that ground worm powder (*Pheretima javanica* K.) from the smallest dose of 0.4 g to the highest dose of 3.2 g did not cause nephrotoxicity.

Kidney is a vital organ. This is due to its function for excretion of metabolic wastes. Renal damage due to toxic substances can be identified based on changes in histologic structure, such as acute tubular necrosis (NTA) that morphologically characterized by proximal tubular epithelial decay. Proximal tubular epithelial cells are susceptible to anoxia and are easily destroyed by intoxication due to contact with ingredients excreted through the kidneys.

Epithelial cells in proximal tubular are susceptible to anoxia and are easily destroyed by poisoning if its in contact with substances excreted through the kidneys. These histologic changes in the kidney are certainly influenced by the amount of compounds that enter the body. Another factor that may cause kidney damage is the ability of the kidney to accumulate the xenobiotic substance in the cell. If a chemical is secreted actively from the blood to the urine, the chemical is first accumulated in the proximal tubule or if the chemical substance is reabsorbed from the urine it will pass through a high concentration of tubular epithelial cells. As a result of the concentration process, these toxic substances will accumulate in the kidneys and cause damage to the kidneys (Yuanita, 2008).

It can be infered from thr result that in the absence of serum creatinine differences, degeneration of fat, hydrophic degeneration, and necrosis, the administration of earthworm powder is safe from the smallest dose of 0.4 g / kgBW white rat to the largest dose of 3.2 g / kgbw of rat.

4. CONCLUSION

Based on the test results, the earthworm powder (Pheretima javanica K.) used as a substitute for chemical drugs as an alternative to healing typhoid fever does not cause toxic effects for the body of white rats, especially renal physiology when consumed within a certain period. And there is no change or renal damage through histopathologic features in white rats (Rattus norvegicus B.) after the administration of earthworm powder (*Pheretima javanica* K.).

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