

Acute Toxicity Test of Ekor Naga (*Rhaphidophora pinnata* (L.f) Schott) Leaf Extract in Mice and Kidney Histological Examination

Fathnur Sani K*, Dian Magfirah, Yuliawati, Elisma, Uce Lestari

Pharmacy Study Program, Faculty of Medicine and Public Health Sciences, Jambi University, Jambi, 36361, Indonesia

Abstract

Ekor Naga (*Rhaphidophora pinnata* (L.f) Schott) is a traditional plant used by the community. Previous studies have shown that this plant has pharmacological effects, including as anthelmintics, antioxidant, and anti-inflammatory agent as well as promotes wound healing. Thus, it is necessary to do a toxicity test. The study aimed to determine the effect of the extract on histopathology of the kidney through a toxicity test. The research used an experimental design. The test animals were divided into 5 groups, each of which consisted of 5 test animals: negative control (0.5% Na. CMC), Treatment 1 (Extract of ekor naga leaves at a dose of 200 mg/Kg BW), Treatment 2 (Extract of ekor naga leaves at a dose of 600 mg/Kg BW), Treatment 3 (Extract of ekor naga leaves at a dose of 1800 mg/Kg BW), and Treatment 4 (Extract of Ekor Naga Leaves at a dose of 5400 mg/Kg BW). The parameters observed were the Letal Dose (LD₅₀) and the histopathology of the kidney. Data were analyzed using One Way ANOVA test, followed with Duncan's test. The results showed no significant difference in the weight of the kidney ($p > 0.05$), and the histopathology of the kidney after 14 days of acute administration of ekor naga leaf extract in all the treatment was normal. The administration of ekor naga leaf extract at therapeutic doses and larger doses of single administration did not have a bad effect on the histology of the kidney.

Keywords: Ekor Naga Leaves, Kidney, Histopathology, Acute Toxicity.

INTRODUCTION

The histopathological examination serves to ensure that there is organ damage after the administration of a drug at high doses (Olayode, et al., 2020). One of the organs of the excretory system is the kidney. Kidneys play an important role in maintaining body fluid, electrolyte, and acid-base stability by filtering the blood. The kidneys excrete metabolic wastes from proteins such as urea, creatinine, ammonia, the hormone renin, and erythropoietin formation. In addition, the kidneys also produce red blood cells, absorbing toxic substances

in the body. The blood circulating in the kidneys is 25%-30% cleared by the kidneys. Toxicity in the kidney is often encountered in cell necrosis (cell death) and cell degeneration. Necrosis occurs due to toxins, metabolic disorders, and viral infections.

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*Corresponding author: fathnursanik@unja.ac.id

Meanwhile cell degeneration occurs due to cell abnormalities caused by minor injuries that can cause disturbances in metabolic processes. Kidney damage must be avoided, so prevention is needed to minimize damage (Braun & Khayat, 2021; Kellum, *et al.*, 2021).

Traditional medicine is a mixture of ingredients derived from plants, animals, galenic, or mixtures which are used for generations for treatment and can be applied according to community norms. Indonesia is a tropical country that is rich in various types of plants that can be used as medicinal plants (Yuan, *et al.*, 2016).

Ekor naga leaf extract is a plant based extract that has been tested to have pharmacological effects, including as antidiuretic, antihyperuricemic, anti-inflammatory, antibacterial, anticancer, and others. Drug safety is one of the main requirements for developing traditional medicines into herbal products that the public can enjoy through pharmaceutical preparations. Acute toxicity is the most important part of information about symptoms of poisoning to death after an administration at large doses (Masfria, *et al.*, 2017; Tarigan, *et al.*, 2021).

Based on the abovementioned problems, the researchers conducted an examination of the effects of acute toxicity on the histology of kidney damage in white female mice.

METHODS

Materials

The materials used in this research were ekor naga (*Rhaphidophora pinnata* (L.) Schott) leaf, 70% ethanol, Na-CMC, Mg powder, Dragendroff's reagent, Mayer's reagent, FeCl₃, acetic acid, sulfuric acid, 0.9% NaCl, formalin, graded alcohol (70%, 80%, 90%, 95%, and absolute), paraffin, xylol, and Haemotoxylin Eosin (HE).

Animals

The test animals used in this study were 25 female white mice weighing of 20-30 grams healthy,

and aged 2-3 months. The animals were acclimatized for 2 weeks before treatment and randomly selected as the control and experimental groups. The mice were housed in groups in polypropylene cages with rice husks as bedding and reared according to the standard procedures at a room temperature with a 12 h light/dark cycle. The animals were given standard pellets and water *ad libitum*. All procedures have passed an ethical review conducted by the Research Ethics Commission Team at the Faculty of Medicine, Andalas University Number 976/UN.16.2/KEP-FK/2022.

Extract Preparation

Ekor naga leaves were extracted using the maceration method. The ekor naga (*Rhaphidophora pinnata* (L.) Schott) leaves were obtained from Mendalo Indah Village, Jambi Province. The identification of the ekor naga leaves obtained was validated by the Jatinagor Herbarium Laboratory of Plant Taxonomy, Department of Biology FMIPA Padjadjaran University with identification number 35/HB/07/2022. A total of 500 grams of ekor naga leaves powder was dissolved in 5 liters of 70% ethanol to submerge all the powder. Soaking was carried out for 6 h with stirring, then left to stand for 18 h. The macerate was separated from the pulp using a funnel lined with filter paper. Remaceration was performed with 2.5 liters of 70% ethanol. After that the macerate, was collected and evaporated using a rotary evaporator at a temperature of 60°C to obtain a thick extract.

Phytochemical Screening

Phytochemical screening of the extract ekor naga leaves employed standard procedures to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, and saponins. This result is in line with the results of research by Astika, *et al.* (2021).

Acute Toxicity Test

Acute Toxicity Testing referred to the *in vivo* non-clinical acute toxicity testing guidelines

Table 1. Content of secondary metabolites of ekor naga leaves extract.

Phytochemical Test	Results
Alkaloids	
1. Mayer	+
2. Dragendorff	+
Tannins	+
Flavonoids	+
Terpenoids	-
Steroids	+
Saponins	+

Notes :

+ Positive results

- Negative result

set by the Food and Drug Administration (BPOM RI, 2014). The five treatment groups were treated using the ethanol extract of the ekor naga leaf extract at different doses, each group consisted of 7 mice, and given the solution orally, with the following treatment:

1. Negative Control: Not given the ethanol extract of the ekor naga leaves.
2. Treatment 1: Given ethanol extract of the ekor naga leaves at a dose of 200 mg/kg BW.
3. Treatment 2: Given ethanol extract of the ekor naga leaves at a dose of 600 mg/kg BW.
4. Treatment 3 : Given ethanol extract of the ekor naga leaves at a dose of 1,800 mg/kg BW.
5. Treatment 4: Given ethanol extract of the ekor naga leaves at a dose of 5,400 mg/kg BW.

The symptoms of toxicity in mice was observed after 14 days. The number of dead mice was counted, and the Letal Dose (LD₅₀) value was calculated using the *Thompson-Weil* calculation.

Kidney Histology

At the end of the observation, *i.e.*, on the 14th after oral administration, the test animals were sacrificed intraperitoneally by administering the anesthetic ketamine-xylazine euthanasia (0.1 ml/100 grams containing 4-10 mg of ketamine and 0.5-1.3 mg of xylazine). Then the kidneys were taken (Putri, *et al.*, 2022). The kidney organs were observed microscopically following the stages of organ histology test and observed for protein precipitate, glomerular atrophy, karyopyknosis, karyorekrosis, and karyolysis. The histological stages are first, the organs were washed using 0.9% NaCl after which the weight of the organs was weighed. The organs were fixation using a 10% formalin solution. Then proceed with the dehydration process using graded alcohol with concentrations of 70%, 80%, 90%, and 95% for 24 h each and continued with absolute alcohol (100%) for one hour and repeated three times. After that, it was purified using xylol for one hour three times and followed by infiltration

Table 2. Percentage of test animal mortality within 24 h after single oral administration.

Treatment Group	Number of Dead Animals ±SEM	%death ±SEM
C- (Na CMC 0.5%)	0±0	0±0
T1 (Extract 200mg/KgBW)	0±0	0±0
T2 (Extract 600mg/KgBW)	0±0	0±0
T3 (Extract 1800mg/KgBW)	0±0	0±0
T4 (Extract 5400mg/KgBW)	0±0	0±0

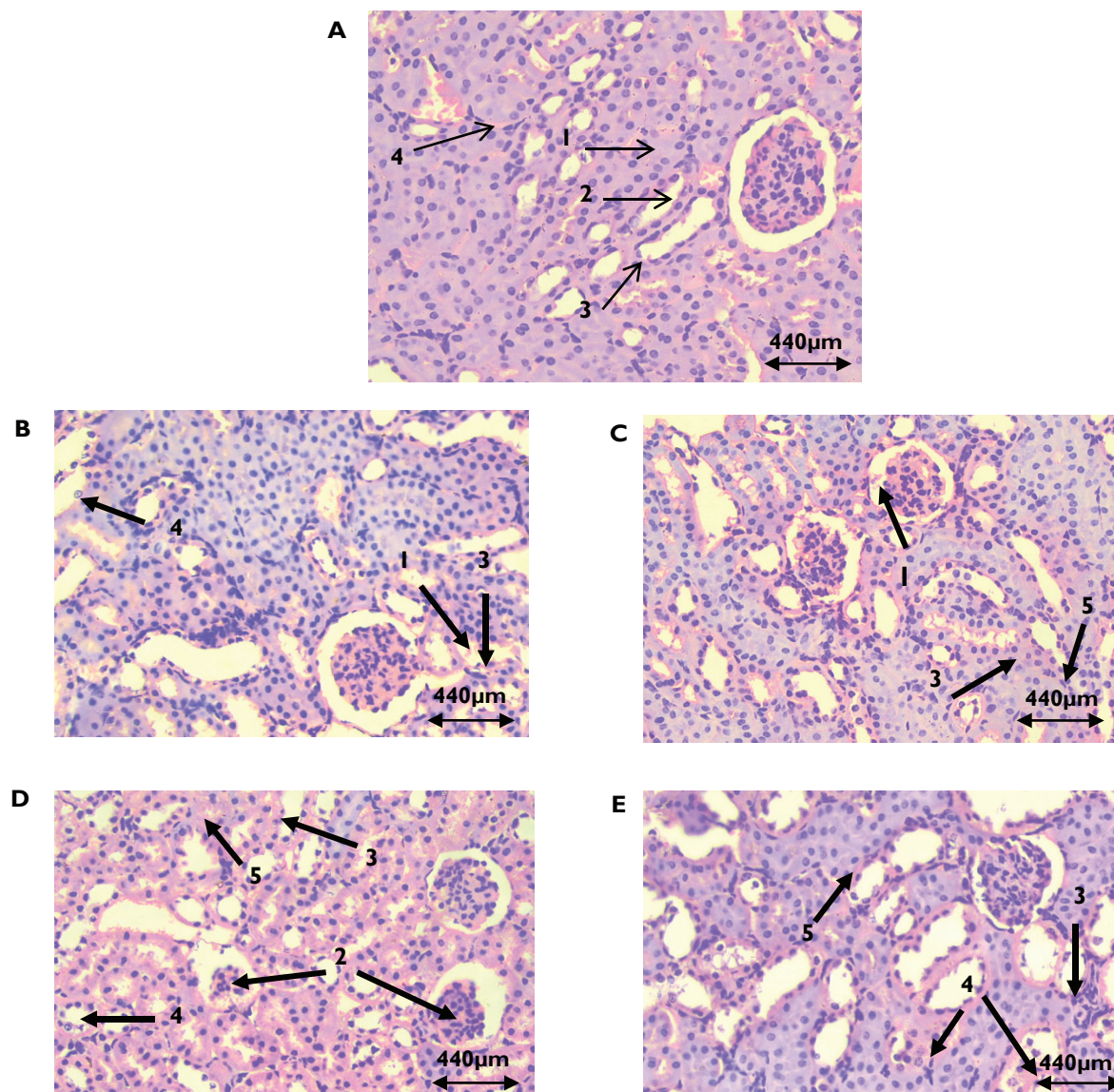


Figure 1. Histology of the kidney of mice (*Mus musculus*) Magnification: 400x with HE staining.
A. Control group; B. T1: (Extract 200 mg/KgBW); C. T2: (Extract 600 mg/KgBW); D. T3: (Extract 1800 mg/KgBW); E. T4: (Extract 200 mg/KgBW). Image A caption : 1. Bowman's capsule; 2. Glomerulus; 3. proximal tubule; 4. distal tubule. Image B,C,D, and E caption: 1. Protein precipitate; 2. Glomerular atrophy; 3. Karyopyknosis; 4. Karyorrhexis; 5. Karyolysis.

using paraffin. Organs were implanted into paraffin media and tissue incisions were made with a thickness of 4-5 µm. Then, the results of the incision were stained using Hematoxylin Eosin (HE). The preparations were labeled and their descriptions were then analyzed. The preparations were observed with a magnification of 40x10 in 5 fields of view.

RESULTS

Extract Yield

The Maceration of 750 grams of the sample, a thick extract of 77.496 grams was obtained with a percent yield of 10.33%.

Phytochemical Screening

The results of the phytochemical screening can be seen in Table 1. The results showed that the ethanol extract of the ekor naga leaves did not contain terpenoid secondary metabolites.

Acute Oral Toxicity Study

The results of the percentage of mortality in mice from the acute toxicity test on day 1 to day 14 after the administration of the ekor naga leaves extract are presented in Table 2.

Histological Observations of Kidney Organs

The results of the histological observations of the mice's kidneys using a light microscope with a magnification of 400x in the control and treatment groups can be seen in Figure 1.

Based on Figure 1, the kidney histology of the control group treated with 0.5% Na-CMC showed that the Bowman's capsule, glomerulus, proximal tubule, and distal tubule cells of the mice appeared normal. In treatment groups T1, T2, T3, and T4, which were given a single dose of the ethanol extract of the ekor naga leaves, the mice had kidney damage in the form of protein deposits, glomerular atrophy, karyopyknosis, karyorrhexis, and karyolysis.

DISCUSSION

An acute toxicity test is performed to detect the toxic effect of the administration of the test preparation. The principle of the acute oral toxicity test is that a test preparation in several different doses levels is given to several groups of test animals with one dose per group, followed by an observation of the toxic effects and death. To get more accurate results, the LD₅₀ calculation is used. The LD₅₀ is the dose that causes the death of 50% of the animals tested in a given time period (Alelign, *et al.*, 2020; Yuandani & Suwarso, 2017).

The LD₅₀ was calculated using the *Thompson-Weil* method. This method was chosen

because it has a fairly high level of confidence. In addition, this method does not require too many experimental animals. Based on the data in Table 2, by giving a single preparation orally to mice with variations in doses starting from 200, 600, 1800, to 5400 mg/kgBW, none of them caused death so the LD₅₀ was not obtained. However, the LD₅₀ was obtained at a dose of >5400 mg/kgBW (the highest dose used). Thus, it can be said that the ethanol extract of the ekor naga leaves had LD₅₀ at a dose greater >5400 mg/kgBW possibly causing toxicity (BPOM RI, 2014).

The presence of protein deposits in the tubular lumen and Bowman's space in groups T1 and T2 can be caused by a poor filtration process. According to McGavin and Zachary (2007), nephrosis is one of the factors causing protein deposits. Nephrosis is a degenerative change in the kidneys caused by disturbances in the process of exchanging substances. Nephrosis causes the glomerular capillaries to not function properly, causing protein deposits. Another factor that can cause protein deposition is a disturbance in the function of the tubular epithelial lysosomal enzymes caused by the accumulation of damaging substances. If the enzymatic protein degradation process is disturbed, it can cause protein deposits to appear in the lumen and epithelial cells. The protein deposits seen on the histological observations were marked by the presence of a red mass after staining with HE (Alkhunaizi, *et al.*, 2016; Bevilacqua, *et al.*, 2011).

Glomerular atrophy that was found in the T3 group was characterized by the widening of the space between the glomerulus and Bowman's capsule. Changes in the shape of the glomerulus are thought to be the effect of giving ethanol extract of the ekor naga leaves which caused ischemia in the glomerulus. Severe tubular damage can increase intraglomerular pressure, causing glomerular atrophy (El-Zoghby, *et al.*, 2009; Muthukumaravel, *et al.*, 2021). In the histological picture the necrotic kidney of the mice were is characterized by

the presence of nuclear changes (cell nuclei) which were divided into three, namely karyopyknosis, karyorrhexis, and karyolysis.

Karyopyknosis is characterized by shrinkage of the cell nucleus, irregular cell boundaries, and darker colored nuclei. Karyorrhexis is characterized by the destruction of the cell nucleus causing it to lose its original shape and form scattered chromatin fragments. Karyolysis is characterized by a very pale, and shapeless cell nucleus (Abdelhalim & Jarrar, 2011; Islam, *et al.*, 2019). Treatment groups T1, T2, and T3 showed in the mice had necrotic. On T1, karyopyknosis, necrosis and karyorrhexis were found. In T2, karyopyknosis, necrosis and karyolysis were found. In T3 and T4, three stages of necrosis were found, namely karyopyknosis, karyorrhexis, and karyolysis. In addition to the effect of the ethanol extract of the ekor naga leaves, cell damage can also be caused by other factors such as poor cage conditions, lack of variation in feeds and drinking water, and other diseases that can affect yields. Internal factors such as endurance and susceptibility of mice, can also influence the incidence of kidney damage in the mice being tested.

CONCLUSION

Based on the results of the research, the following conclusions were obtained. The administration of the ethanol extract of the ekor naga leaves (*Rhaphidophora pinnata* (L.) Schott) up to a dose of 5,400 mg/kgBW did not cause death in the test animals. The administration of the ethanol extract of the ekor naga leaves (*Rhaphidophora pinnata* (L.) Schott) had an effect on the histology picture of the mice's kidney, but it cause serious damage.

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