Toxicity test of *Stenochlaena palustris* extract based on kidney histopathology examination

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ABSTRACT

**Background:** Kalimantan’s people consume *Stenochlaena palustris* leaf extract as food and for traditional medicine. The bioactive components of *Stenochlaena palustris* leaf extract are flavonoids, alkaloids, saponins, and tannins. An in vitro study shows that the leaf extract has no toxic effect, so it can be used as an alternative drug in oral health, such as in mouthwashes or topical ulcer drugs.

**Purpose:** This study aims to analyze the toxic effects of *Stenochlaena palustris* leaf extract based on the bleeding and lesions resulting from necrosis in kidney by using histopathology examination.

**Methods:** The *Stenochlaena palustris* leaves were extracted using 95% ethanol and then given to male Wistar strain (Rattus norvegicus) with a 2,000, 2,500, and 3,000 mg/kg/body weight two times a day for fourteen days. The kidneys were collected and subjected to histopathology examination.

**Results:** There are higher bleeding and necrosis lesion rates in the 2,500 and 3,000 mg/kg/body weight of *Stenochlaena palustris* leaves extract group compared to the control and 2,000 mg/kg/body weight of *Stenochlaena palustris* leaves extract group (p<0.05).

**Conclusion:** *Stenochlaena palustris* leaf extract showed no toxic effect at doses of 2,000 mg/kg/body weight.

**Keywords:** antioxidant; bleeding; necrosis; *Stenochlaena palustris* leaves; toxicity test; medicine

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INTRODUCTION

South Kalimantan has various types of ferns that are potentially medicinal plants. Based on empirical data, *Stenochlaena palustris* is eaten as a vegetable and used as a breast milk enhancer and alternative medicine to cure anemia and allergies and relieve fever.¹ The public demand for herbal ingredients is increasing because of science and technology’s rapid development and progress, which is encouraging the development of naturally derived alternative medicines. In addition, these natural ingredients are more affordable and easier to obtain compared to drugs made with chemicals and are considered to have minimal side effects.² *Stenochlaena palustris* leaves contain various secondary metabolites. Based on the results of phytochemical screening by Debnath et al.,³ the ethanolic extract of *Stenochlaena palustris* leaves contains flavonoids, saponins, alkaloids, steroids, and tannins. Flavonoids have a content of 503.56 mg QE/g. Alkaloids, saponins, steroids, tannins, and total phenol were 11.56%, 8.06%, 3.43%, 23 mg GAE/g, and 51.69 ± 1.28 mg GAE/g, respectively. Tannins are antioxidants that accelerate the epithelialization process. Steroids and saponins are anti-inflammatory and antiseptic.³–⁵

There have been several studies on the extract of *Stenochlaena palustris* leaves. One tested the toxicity of BHK-21 fibroblast cells and concluded that 10%, 20%, and 30% concentrations of the *Stenochlaena palustris* leaf extract were toxic to BHK-21 fibroblasts. In comparison, the concentrations of 40%, 50%, 60%, 70%, 80%, and 90% were proven to be non-toxic to BHK-21 fibroblast cells. This study concluded that the extract of *Stenochlaena palustris* leaves could increase cell viability because it contains flavonoids with non-enzymatic antioxidant functions that inhibit cell damage caused by reactive oxygen species (ROS).⁶

Therefore, antioxidants in *Stenochlaena palustris* leaves have the potential to heal wounds because of their
granular tissue formation ability, fibroblast proliferation, and collagen fiber production. Research has not found the toxicity or side effects of *Stenochlaena palustris* herbal products used as medicine to heal wounds. It has been widely used in the community. Several factors, including the accuracy of the dose, timeliness, and method of use, must be considered when taking natural treatments, such as *Stenochlaena palustris* leaves, as a drug to heal wounds. Therefore, it is necessary to carry out further testing to protect the user community from potentially detrimental effects and to ensure the safety of its use. Hazard data is obtained using a toxicity test that detects the poisonous effect of a substance on a biological system and receives specific dose-response data from the test preparation. The test detects the presence of toxins in a substance and determines the toxin’s target organ and its sensitivity after the acute administration of the compound for 14 days. This toxicity test’s parameters include clinical symptoms, subject death, and organ histopathology.

The kidneys often receive the unwanted effects of drugs. Suppose the content of the extract that enters the body exceeds normal conditions. In that case, it will decrease the kidneys’ ability to concentrate xenobiotic substances in the cells, resulting in the accumulation of toxic substances that cause damage to the kidneys. The relatively high blood flow to the kidneys exposes them to substances carried in the circulatory system, so toxic substances can quickly damage the kidney, causing changes in structure and function. Damage to the nephrons can occur in the tubules, renal corpuscles, and capillaries. The conditions of toxicity commonly found in the kidneys include cell necrosis, cell degeneration, and bleeding.

Furthermore, the study analyzes the toxic effects of three different *Stenochlaena palustris* leaf extracts (2,000, 2,500, and 3,000 mg/kg/body weight) based on the bleeding and necrosis found in kidney tissue using a histopathology examination.

**MATERIALS AND METHODS**

The test used 16 male Wistar rats (*Rattus novergicus*) aged 8–12 weeks, with body weights between 200–250 g, and all in good health. The Wistar rats usually ate 20% of their total body weight, so in a laboratory adaptation, they were fed 40 g once daily for one week. Boiled water was given in 500 ml bottles. The subjects were grouped by simple random sampling and divided into four groups, each consisting of four rats. The protocol of this study was approved by the ethical committee of the Faculty of Dentistry, Universitas Lambung Mangkurat, with registration number 016/KEPKG-FKGULM/EC/III/2022.

Greenish mature leaves (12 kg) of *Stenochlaena palustris* were taken in the Anjir Barito Kuala Region. The leaves were washed, cut into small pieces, and dried in an oven at 40°C for four hours. After, the leaves were crushed in a blender and macerated with 95% ethanol for 72 hours. Upon finishing, the solution was filtered with WH40 filter paper until a clear brownish liquid was obtained. In the next stage, a vacum rotary evaporator evaporated the solvent for four to six hours. It was then heated with a water bath to get a brownish liquid residue. Ethanol-free was tested with potassium dichromate (K$_2$Cr$_2$O$_7$) on 3 ml of ethanol. The extract was then stored in a 10°C refrigerator to prevent oxidation. The doses of the extract were prepared by diluting the extract with distilled water.

The toxicity test was performed by giving the *Stenochlaena palustris* per-oral to the subject with a gastric probe two times a day for 14 days. The subjects were divided into four groups listed in Table 1. At the end of the day’s treatment, the subjects were fasted and given only water for 8–12 hours.

On the 15th day, the subjects were sacrificed by giving intraperitoneal injection using 0.5 ml of ketamine. The kidneys were collected, cleaned, wrapped in a white cloth, and buried at a depth of ± 50 cm. The kidneys were then washed with NaCl and soaked with 10% BNF solution for four to six hours. It was then heated with a water bath and buried at a depth of ± 50 cm. The kidneys were then washed with NaCl and soaked with 10% BNF solution for tissue fixation and process for further processing.

The bleeding and necrosis lesions were analyzed using histopathology with hematoxylin–eosin (HE) staining. The bleeding lesions were characterized by red blood cells migrating from the vasculature into the tissue between the proximal tubular spaces. In contrast, the necrosis lesions were characterized by changes in shape and cell nucleus (darker nucleus), indicating the presence of pyknosis. The bleeding and necrosis lesions on kidney cells were counted and scored as listed in Table 2.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>The distribution of subjects in the toxicity test</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Dose</strong></td>
</tr>
<tr>
<td>Control</td>
<td>1 ml of distilled water</td>
</tr>
<tr>
<td>P1</td>
<td>2000 mg/kg/body weight <em>Stenochlaena palustris</em> extract (1 ml)</td>
</tr>
<tr>
<td>P2</td>
<td>2500 mg/kg/body weight <em>Stenochlaena palustris</em> extract (1 ml)</td>
</tr>
<tr>
<td>P3</td>
<td>3000 mg/kg/body weight <em>Stenochlaena palustris</em> extract (1 ml)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Table 2.</th>
<th>Bleeding and necrosis lesion scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleeding Appearance</strong></td>
<td><strong>Score</strong></td>
</tr>
<tr>
<td>No bleeding lesion</td>
<td>0</td>
</tr>
<tr>
<td>Mild bleeding (&lt;25% area)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate bleeding (25–50% area)</td>
<td>2</td>
</tr>
<tr>
<td>Severe bleeding (&gt;50% area)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Necrosis Lesion</strong></td>
<td><strong>Score</strong></td>
</tr>
<tr>
<td>No necrosis lesion</td>
<td>0</td>
</tr>
<tr>
<td>Mild necrosis (&lt;25% area)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate necrosis (25–50% area)</td>
<td>2</td>
</tr>
<tr>
<td>Severe necrosis (&gt;50% area)</td>
<td>3</td>
</tr>
</tbody>
</table>
RESULTS

The histopathology analysis of the bleeding lesion was characterized by red blood cells migrating from the vasculature into the tissue between the proximal tubular spaces (black arrow) (Figure 1). The kidney tissue showed mild intratubular bleeding (< 25% area) in groups that were given *Stenochlaena palustris* extract with 2,500 and 3,000 mg/kg/body weight (Figure 1C–D). The type of bleeding lesion was petechial, measuring 1–2 mm. There were no histopathological changes in bleeding in control and 2,000 mg/kg/body weight *Stenochlaena palustris* extract groups (Figure 1A–B).

The 2,500 and 3,000 mg/kg/body weight *Stenochlaena palustris* extract groups showed higher bleeding lesions than the control and 2,000 mg/kg/body weight *Stenochlaena palustris* extract (p<0.05). There is no significant difference in bleeding lesions in the control and 2,000 mg/kg/body-weight *Stenochlaena palustris* extract groups (p>0.05).

Figure 1. The histopathology of a bleeding lesion in the kidney tissue. (A) control; (B) 2,000 mg/kg/body weight *Stenochlaena palustris* extract; (C) 2,500 mg/kg/body weight *Stenochlaena palustris* extract; and (D) 3,000 mg/kg/body weight *Stenochlaena palustris* extract. Magnification 400x, hematoxylin–eosin staining.

Figure 2. The histopathology of a necrosis lesion in the kidney tissue. (A) control; (B) 2,000 mg/kg/body weight *Stenochlaena palustris* extract; (C) 2,500 mg/kg/body weight *Stenochlaena palustris* extract; and (D) 3,000 mg/kg/body weight *Stenochlaena palustris* extract. Magnification 400x, hematoxylin–eosin staining.
The necrosis lesion in histopathology analysis is characterized by changes in shape and cell nucleus (darker nucleus) indicating the presence of pyknosis (black arrow) (Figure 2). The kidney showed mild necrosis (<25% area) in groups with 2,500 and 3,000 mg/kg/body weight Stenochlaena palustris extract (p<0.05) (Figure 2C–D). No necrosis lesion was observed in the control and 2,000 mg/kg/body-weight Stenochlaena palustris extract groups (Figure 2A–B).

**DISCUSSION**

The histopathological changes in the kidneys are usually caused by substances that enter the bloodstream and that are toxic to the body. Inappropriate use of natural ingredients can potentially cause organ damage, such as the kidney. This is because the kidney is an organ that often accumulates unwanted effects from the use of drugs taken orally. Based on the results of statistical tests on the histopathological appearance of bleeding, it was found that the 2,000 mg/kg/body weight group had no significant difference from the control. In the treatment group at a dose of 2,500 mg/kg/body weight and the treatment group at a dose of 3,000 mg/kg/body weight, there was a significant difference with the control group, suggesting the treatment was potentially toxic to the kidneys of Wistar rats.

In the control group that was given distilled water, there was no histopathological appearance of bleeding. This indicates that the administration of distilled water is safe for the kidney of rats. According to research by Maliangkay et al., distilled water does not have the potential to have a toxic effect because it is not an irritant. The administration of a dose of 2,000 mg/kg/body weight of Stenochlaena palustris leaf extract did not cause any signs of bleeding. In the P1 group who were given the 2,000 mg/kg/body weight Stenochlaena palustris extract, no bleeding was seen. This is in line with the research by Alsawaf et al., who found that the use of flavonoids at the right dose will not cause toxic effects on the kidneys of Wistar rats. Sudira et al. stated that the presence of flavonoids strengthens blood vessels and inhibits lipid peroxidation through peroxidase activation of hemoglobin in anticipation of damage caused by free radicals. Flavonoids can prevent hemolysis of red blood cells caused by free radicals.

The treatment group at a dose of 2,500 mg/kg/body weight and the treatment group at a dose of 3,000 mg/kg/body weight had a significant difference from the control group. In both groups, there was a histopathological appearance of mild bleeding <25%. According to research by Rafe et al., tubular bleeding that resulted from toxic effects occurred in the treatment group that consumed a high dose of the extract. Saponin is one of the secondary metabolites that affect the occurrence of bleeding with the use of Stenochlaena palustris leaf extract. Saponins are membranolytic which causes disintegration of the capillary endothelial layer, causing bleeding in the rats’ kidneys. Saponins injure the lipid bilayer of the protein membrane of red blood cells, resulting in an inflammatory response. The two stages of inflammation are the vascular stage and the late stage. The vascular stage is associated with vasodilation and increased capillary permeability where blood substances leave the plasma. The late stage occurs when leukocytes infiltrate the inflamed tissue. This causes the release of various mediators such as histamine, prostaglandins, and leukotrienes which are produced from plasma. Prostaglandins have a vasodilating effect, relax smooth muscles, and increase capillary permeability. Histamine plays a role in the earliest changes, causing vasodilation in arterioles which is preceded by initial vasoconstriction and increased capillary permeability. This causes changes in the distribution of red blood cells, such as in slow blood flow when red blood cells clump and are consequently pushed to the edge. White blood cells stick to the walls of blood vessels with slower blood flow. Changes in permeability that occur cause blood to come out of the blood vessels and collect in the tissues.

The results of statistical tests on the histopathological appearance of necrosis showed that the treatment group at a dose of 2,000 mg/kg/body weight had no significant difference from the control group. In the treatment group at a dose of 2,500 mg/kg/body weight and the treatment group at a dose of 3,000 mg/kg/body weight, there was a significant difference with the control group. According to research, Makiyah et al., if the treatment group had a significant difference from the control group, the group was potentially toxic to the kidneys based on necrosis. In the microscopic observation of the control group that was only given distilled water, there is no histopathological change in necrosis because water is not an irritant, so it is not potentially toxic to organs.

The P1 group, which was given a dose of 2,000 mg/kg/body weight of Stenochlaena palustris leaf extract, did not show any histopathological appearance of necrosis. The right dose of flavonoids has antioxidant potential to inhibit oxidation reactions by neutralizing free radicals and preventing cell damage. This is due to the content of secondary metabolites possessed by the extracts of the Stenochlaena palustris leaves, such as flavonoids and phenolics, which have the ability to inhibit free radicals and oxidative reactions. As antioxidants, the direct mechanism of action for flavonoids is to stabilize free radicals by complementing their lack of electrons and inhibiting the chain reactions that form the cell-damaging free radicals. The indirect mechanism of action of antioxidants is the inhibition of the enzymes involved in the production of free radicals by neutralizing and suppressing the formation of ROS so that their number decreases and the activity of superoxide dismutase (SOD) enzymes increases. Antioxidants can modulate free radical reactions by reducing lipid hyper peroxides and hydrogen peroxide (H₂O₂) thereby preventing the occurrence of...
lipid peroxidation. SOD plays a role in catalyzing the dismutation reaction of superoxide anion free radicals (O-) into hydrogen peroxide and oxygen, thereby turning them into products that are more stable and harmless to cells.  

In the treatment group, a dose of 2,500 mg/kg/body weight and a dose of 3,000 mg/kg/body weight showed a histopathological appearance of necrosis. The results of statistical tests stated that the treatment group at a dose of 2,500 mg/kg/body weight and the treatment group at a dose of 3,000 mg/kg/body weight had significant differences from the control group so that both treatment groups were potentially toxic to the kidneys of rats. Tubular epithelial cell necrosis is caused by excessive intake of secondary metabolites contained in the extract that are nephrotoxic. Flavonoids is one of the potentially toxic contents in *Stenochlaena palustris* leaf extract. Flavonoids in high concentrations can be pro-oxidants. Quercetin is one of the compounds that can be prooxidant among these flavonoid compounds.

When there are too many free radicals, the endogenous antioxidants will deplete their rate of use compared to their rate of regeneration. An imbalance between pro-oxidants and antioxidants in the body will result in an increased rate of free radical formation, resulting in an increase in oxidative stress that causes lipid peroxidation, oxidized DNA, and misfolded proteins. This results in the accumulation of covalent bonds in the tubular membrane with free radicals, resulting in damage to the renal tubules. Pro-oxidants cause oxidative stress and mitochondrial dysfunction. Mitochondrial dysfunction is a condition characterized by impaired mitochondrial biogenesis, altered membrane potential, reduced number of mitochondria, and altered protein oxidative activity due to the accumulation of ROS in cells and tissues. Mitochondrial dysfunction causes hydropic degeneration due to impaired active transport. The disturbed osmosis process causes water to enter the cell so that the cell swells with the vacuole, and the cell nucleus enlarges. Cells undergoing hydropic degeneration and that are continuously exposed to toxic compounds will become necrotic.

It can be concluded that there was no toxic effect on the administration of 2,000 mg/kg/body weight dose of *Stenochlaena palustris* leaf extract to the kidney of Wistar rats based on the histopathological appearance of bleeding and necrosis. There was a toxic effect on the administration of the *Stenochlaena palustris* leaves extract at a dose of 2,500 mg/kg/body weight and a dose of 3,000 mg/kg/body weight on the kidney of Wistar rats based on the histopathological appearance of bleeding and necrosis.

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REFERENCES