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KIDNEY MICROANATOMY OF WHITE RATS ON THE ADMINISTRATION OF ETHANOL EXTRACT OF *Spatholobus littoralis* STEM ACUTE DOSE

Mikroanatomi Ginjal Tikus Putih Pada Pemberian Ekstrak Etanol Batang *Spatholobus littoralis* Dosis Akut

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ABSTRACT

Tampala bajakah stem (Spatholobus littoralis Hassk) is empirically used by the Indonesian people to recover from disease and maintain health, but the use of tampala bajakah has not been tested for doses that are safe for consumption. The purpose of this study was to determine the type of damage caused by acute doses of bajakah tampala stem extract on white rat kidney microanatomy (Rattus norvegicus Berkenhout). This study used bajakah stem derived from Ambawang, extraction using ethanol solvent, and Wistar strain white rat (± 150g) test as a test animal. The method used a Complete Randomized Design with 4 treatments namely normal control using distilled water, treatment of doses of 300, 2000, and 5000 mg/kg BW of bajakah tampala stem extract. Each group was given 5 replicates. The extract was shown in a single dose orally and observations were made for 14 days. The results obtained that doses of 2000 and 5000 mg/kg BW caused damage to white rat kidney tissue in the form of microanatomy dilatation of tubules and loss of the brush border, and doses of 300, 2000, and 5000 mg/kg BW caused necrosis and haemorrhage. The most severe damage to kidney microanatomy is the dose of 2000 and 5000 mg/kg BW. The administration of acute doses of bajakah extract has the potential to cause damage to kidney tissue.

Keywords: *Acute Dose, Kidney Microanatomy, Spatholobus littoralis, Secondary Metabolism*

ABSTRAK

Batang bajakah tampala (*Spatholobus littoralis* Hassk) secara empiris digunakan oleh masyarakat Indonesia sebagai upaya pemulihan penyakit dan menjaga kesehatan, namun penggunaan bajakah tampala belum ada pengujian dosis yang aman dikonsumsi. Tujuan penelitian untuk mengetahui bentuk kerusakan yang ditimbulkan dari pemberian oral ekstrak batang bajakah tampala dosis akut terhadap mikroanatomi ginjal tikus putih (*Rattus norvegicus* Berkenhout). Penelitian menggunakan batang bajakah tampala berasal dari Ambawang, ekstraksi menggunakan pelarut etanol, dan tikus putih betina galur wistar (±150g) sebagai hewan uji. Metode penelitian menggunakan Rancangan Acak Lengkap dengan 4 perlakuan yaitu kontrol normal menggunakan akuades, perlakuan ekstrak batang bajakah tampala dosis 300, 2000, serta 5000 mg/kg BB. Setiap perlakuan terdiri atas 5 ulangan. Ekstrak diberikan secara oral pada dosis tunggal dan pengamatan respon dilakukan selama 14 hari. Hasil yang diperoleh bahwa dosis 2000, dan 5000 mg/kg BB menyebabkan dilatasi tubulus dan kehilangan brush border, serta

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kerusakan pada perlakuan 300, 2000, dan 5000 mg/kg BB berupa nekrosis dan hemoragi. Kerusakan mikroanatomi ginjal yang paling parah berada pada dosis 2000 dan 5000 mg/kg BB. Pemberian ekstrak bajakah dosis akut berpotensi menyebabkan kerusakan jaringan ginjal.

Kata Kunci: Dosis Akut, Metabolit Sekunder, Mikroanatomi Ginjal, *Spatholobus littoralis*

INTRODUCTION

The use of natural materials as an effort to cure diseases and keep the body always fit has become a habit of the world population. World Health Organization (2018), the use of traditional herbal medicines is used in public health maintenance, prevention, and treatment of diseases, especially chronic diseases. Basic Health Research (2023), the use of traditional herbal medicine by the Indonesian population amounted to 32.5% which is mostly given by traditional healers. Ethnobotanical studies in the Kapuas Hulu community, West Kalimantan, empirically show the use of bajakah tampala *S. littoralis* boiled water as a stamina recovery and cancer treatment (Yusro & Mariani, 2021).

The use of bajakah tampala as a traditional herbal medicine is known to maintain body fitness and can cure several diseases (Ayuchecaria et al., 2020). The bajakah tampala plant can be found in the interior of Kalimantan, whose status has not been widely spread to other regions (Saputera & Ayuchecaria, 2018). Bajakah tampala stem extract contains secondary metabolite compounds such as flavonoids, saponins, steroids, terpenoids, tannins, and phenols (Saputera & Ayuchecaria, 2018). Several studies have shown that bajakah tampala stem extract can be anticancer (Maulina, 2019), antidiabetic (Ayuchecaria et al., 2020), and antibacterial E. coli (Saputera et al., 2019).

BPOM data (2012) states that there were 41 cases of poisoning due to the use of traditional medicine. One of the organs targeted for poisoning is the kidney. The kidney is an organ that is sensitive to toxic effects (Rahman et al., 2020). The kidneys play a role in removing toxins, and metabolic waste from foodstuffs, and drugs. Research by Muthmainnah et al. (2015), on 70% ethanol extract of karamunting leaves (*Rhodomyrtus tomentosa* (Aiton) Hassk) at a dose of 600 mg/kg BW caused narrowing of the tubule lumen, the presence of intratubular casts, and fatty degeneration. In line with the increase in dose, damage in the form of degeneration, necrosis of tubular cells, and tubular damage occurs getting bigger in the kidney organ.

Saponin is a content possessed by bajakah tampala stem extract and this compound is indicated as a factor in kidney damage. Saponins have the ability to lyse erythrocytes by forming pores due to phospholipid interactions on cell membranes (Setiawan et al., 2022). Lysed erythrocytes cause the presence of free hemoglobin in the plasma inducing damage to the tubule epithelium. According to Shafaei et al. (2012), stated that the administration of *Citrullus colocynthis* extract containing saponins caused kidney damage in the form of brush border loss, glomerular haemorrhage, and haemorrhage in the kidney cortex.

The toxicity of herbal medicines can be caused by the content of toxic metabolite compounds in plant tissues. Toxic compounds can increase the formation of free radicals and reduce the ability of antioxidants in the body. Oxidative stress causes free radicals to react with fats, proteins, and nucleic acids that make up cell components resulting in local damage and dysfunction of certain organs (Sinaga, 2016). The use of bajakah tampala as an herb requires further research on the safety of its use.

MATERIALS AND METHODS

Location and time

This research was conducted from January 2023 at the Wood and Forest Products Workshop Laboratory, Forest Products Chemistry Laboratory, Organic Chemistry Laboratory, Biology Laboratory, Zoology Laboratory, and Microtechnical Laboratory of Tanjungpura University.

Materials and tools

The tools used are rat drinking bottles, glass bottles, plastic bottles, surgical scissors, measuring cups, Beaker glasses, needles, object glasses, rat cages, filter paper, light microscope, micrometre, refrigerator, tweezers, scalpel, rotary evaporator, sonde, syringe, staining jar, thermometer, analytical balance, and vacuum. The materials used were distilled water, graded alcohol (70-100%), bajakah tampala stem extract, CMC 0.5%, ethanol, eosin, ethanol, physiological saline (NaCl 0.9%), hematoxylin, rat food, NBF (neutral buffer formalin) 10%, paraffin, xylol, and Wistar strain female white rats (*Rattus norvegicus* Berkenhout) with nulliparous/virgin conditions (±150 grams).

Methods

This research is an experimental study using the Fixed Dose Method with a grouping of female test animals. The design used is a Completely Randomized Design (CRD) consisting of 4 treatments and 5 replicates (BPOM, 2014). The treatments were carried out as follows; NC: Test animals were given distilled water, P1: Test animals were given bajakah tampala stem extract at a dose of 300 mg/kg BW dissolved in 2 ml of 0.5% CMC, P2: Test animals were given bajakah tampala stem extract at a dose of 2000 mg/kg BW dissolved in 2 ml of 0.5% CMC, P3: Test animals were given a dose of 5000 mg/kg BW of bajakah tampala stem extract dissolved in 2 ml of 0.5% CMC.

The extract was given orally in a single dose once. Then the behavioral response and mortality of the rats were observed. On the 14th day, all rats are sacrificed and the kidney organ is dissected and cut to make a histological preparation. Kidney organs were weighed to determine the relative organ weight with the following equation (BPOM, 2014):

$$\text{Relative Organ Weight (\%)} = \frac{\text{absolute organ weight (g)}}{\text{total body weight (g)}} \times 100\%$$

Preparation of kidney organ incision by paraffin method or embedding. Incision preparations were made at 10 intervals with 5 slices each slide series arrangement of 5 micron thickness. The staining method with Hematoxylin-Eosin dye.

Analysis data

Observations included glomerular diameter, Bowman gap width, lumen diameter of proximal cortical tubules and distal cortical tubules, and observation of damaged cells. Examination of preparations with 5 field of view 400× magnification. The diameter calculation was then calibrated with the provisions of microscope magnification,

$$\frac{D1+D2}{2} \times 2{,}5 = \mu m$$

Observation data were tested for normality and homogeneity. Normally distributed and homogeneous data are then analyzed using one-way ANOVA (<0.05), but if the data are not normally distributed and not homogeneous then the data are analyzed using non-parametric Kruskal-Wallis. The results of the analysis obtained significant differences from Anova, then continued with the Duncan Test with a confidence level of 95% (Rinaldi & Mujianto, 2017).

RESULTS AND DISCUSSION

The test was followed by weighing the body weight of the test animals on day 14 and the relative kidney weight after treatment.

Table 1. Body weight of rats treated with Bajakah Tampala Stem Extract

Perlakuan	Weight D-14 (g±SD)	Relative Kidney Weight (%)	
		Right	Left
Normal Control (NC)	148,60±8,47	0,42±0,08^{ns}	0,42±0,08^{ns}
Dose 300 mg/kg BB (P1)	160,60±13,37	0,36±0,05^{ns}	0,36 ±0,05^{ns}
Dose 2000 mg/kg BB (P2)	166,60±12,89	0,40±0,00^{ns}	0,42 ±0,04^{ns}
Dose 5000 mg/kg BB (P3)	168,40±13,37	0,40±0,00^{ns}	0,40 ±0,00^{ns}

The normality and homogeneity tests showed that the data were not normally distributed and not homogeneous. The data were tested by non-parametric Kruskal-Wallis (Table 1). The mean relative kidney weight in Kruskal-Wallis testing showed no difference between treatments (α > 0.05), this indicates that the administration of bajakah tampala stem extract does not affect the weight of the kidney organs.

Measuring the body weight of rats 14 days after administration of an acute dose of *S. littoralis* showed that all treatments experienced weight gain. Observation of the body weight of rats was carried out to determine changes in body weight which was used as an indicator of the side effects of the extract. Data collection was then continued with the measurement of relative organ weight and the results obtained in each treatment did not affect the relative organ weight. Caturizani's research (2016) in testing the acute toxicity test of sand leaf extract (*Ilex cymosa* Blume) in female Wistar strain rats at doses of 2000 mg/kg BW and 5000 mg/kg BW did not cause death, weight loss, and the effect of relative organ weight on test animals. No deaths were found in the test animals at the highest dose indicating that the test preparation was classified as practically non-toxic criteria (Sianturi et al., 2020), so the LD50 value of bajakah tampala stem extract is >5000 mg/kg BW.

Observations of the effect of bajakah tampala stem extract on white rat kidney microanatomy, including glomerular diameter, Bowman capsule gap, proximal cortical tubule diameter, and distal cortical tubule diameter are shown in Table 2.

Table 2. Effect of bajakah tampala stem extract on white rat kidney microanatomy

Treatment	Parameter			
	Glomerular Diameter (µm)	Bowman's Capsula Gap (µm)	Proximal Cortical Tubule Diameter (µm)	Distal Cortical Tubule Diameter (µm)
Normal control (NC)	65,18±6,12^{ns}	8,58±3,37^{ns}	8,74±3,17^{a}	12,00±2,73^{a}
Dosis 300 mg/kg BB (P1)	69,22±6,70^{ns}	12,42±1,32^{ns}	8,92±2,86^{a}	13,02±3,18^{a}
Dosis 2000 mg/kg BB (P2)	72,22±7,00^{ns}	13,98±2,95^{ns}	14,42±2,09^{b}	18,28±2,27^{b}
Dosis 5000 mg/kg BB (P3)	63,10±6,90^{ns}	11,80±3,52^{ns}	14,65±1,14^{b}	17,60±3,96^{b}

The smallest renal glomerular diameter size is normal control which is 65.18 ± 6.12 and the widest diameter size at a dose of 2000 mg/kg BW which is 72.22 ± 7.00. The smallest Bowman's capsule gap size was the normal treatment which was 8.58 ± 3.37 and the widest gap size at a dose of 2000 mg/kg BW was 13.98 ± 2.95. The lowest diameter size of the proximal cortical tubule is the normal control which is 8.74 ± 3.17 and the highest diameter size at a dose of 5000 mg/kg BW which is 14.65 ± 1.14. The lowest diameter size of distal cortical tubules is normal control which is 12.00 ± 2.73 and the highest diameter size at a dose of 2000 mg/kg BB which is 18.28 ± 2.27 (Figure 1).

Table 3. Kidney microanatomy of white rats after administration of bajakah tampala stem extract descriptively

Damage	Treatment			
	Normal control	Dose 300 mg/kg BB	Dose 2000 mg/kg BB	Dose 5000 mg/kg BB
Glomerular dilation	-	+	+	+
Dilataon of Bowman's capsule gap	-	+	+	+
Dilation of tubules	-	+	+	+
Presence of brush borders	+	+	-	-
Necrosis	-	-	+	+
Hemorrhage	-	+	+	+

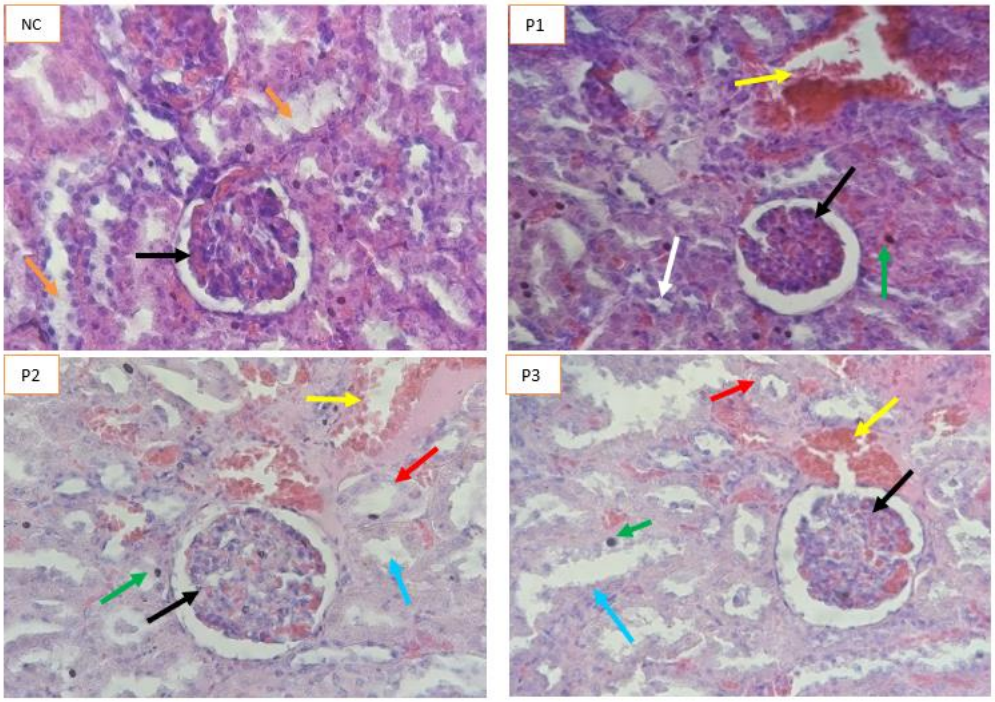

Figure 1. Histology of white rats after treatment (magnification 400x) description: black = normal glomerulus, yellow = haemorrhage, brown = normal tubules, white = closed tubules, red = missing brush border, blue arrow = dilated tubules, green = necrosis (Source: Personal Documentation, 2023).

Cell damage was found in the form of necrosis in the treatment dose of 300, 2000, and 5000 mg/kg BW. Another damage in the form of haemorrhage was found in the dose treatment of 300, 2000, and 5000 mg/kg BW (Figure 1). Dilatation of tubules characterized by widening the lumen of the tubules and loss of brush border characterized by the absence of epithelial cells constituent of the proximal tubules. The brush border decays and accumulates in the tubule lumen. The resulting haemorrhage includes haemorrhage in the organ tissue appearing erythrocytes and blood plasma around the tubules. Cell necrosis is characterized by darkened cell nuclei.

Based on the results of the study, the administration of bajakah stem extract can cause some damage to kidney microanatomy. Renal microanatomy damage in the form of tubule dilatation, loss of brush border in proximal contortus tubules, necrosis, and haemorrhage, but the observation of the diameter and gap of Bowman's capsule tends to dilate at high doses (Figure 1 and Table 3). Research by Anzini et al. (2014) on the acute toxicity test of the ethyl acetate fraction of stems and leaves of water henna against female white rats found that kidney microanatomy damage was the loss of brush border in the proximal cortical tubules.

Glomerulus and Bowman's capsule gap tended to widen at doses of 300, 2000, and 5000 mg/kg BW (Figure 1 and Table 3), but statistically, the data did not show any significant differences between treatments. According to Mayori et al. (2013), glomerular dilation is caused by increased filtration workload on the glomerulus so that there is an increase in glomerular cell volume so that the glomerulus appears larger. Research by Septiva et al. (2019) stated that the administration of neem leaf ethanol extract caused an increase in glomerular diameter due to a form of cellular adaptation. The widening of the Bowman capsule gap is also caused by the glomerular work system which has increased filtrate output. According to research by Fahriyansyah et al. (2021) stated that the increase in the amount of filtrate entering the Bowman capsule chamber from the glomerulus is due to hyperfiltration.

Tubular dilatation occurred in the treatment of 2000 and 5000 mg/kg BW (Figure 1 and Table 3). Tubular dilatation is the widening of the tubule structure due to urine retention. Dilation of tubule diameter is due to the interaction between free radicals with nitric oxide (NO) to form peroxynitrite. Peroxynitrite compounds can accelerate lipid peroxidation in the form of increased production of malondialdehyde (MDA) which causes vascular smooth muscle relaxation and dilation of the tubule lumen (Ismail et al., 2003). Dilation of the tubules was found as well as research by Ichsan et al. (2022) in the form of a tubule lumen that is wider than normal controls.

Dilated tubules found the loss of brush border on the proximal tubules such as the treatment dose of 2000 and 5000 mg/kg BW (Figure 1 and Table 3). Brush border that sheds on the tubule epithelium then collects in the lumen of the tubule. Tandi et al. (2020) suggest that damage to cuboidal epithelial cells is followed by damage to the brush border, then within a certain period, it undergoes compaction to form an intraluminal cast in the tubule lumen. Brush border loss caused by free haemoglobin and ischemia in response to reduced oxygen to the tissue triggers calcium entry across the plasma membrane (Kumar et al., 2012). Increased intracellular calcium can cause loss of tubule cell integrity in the form of brush border loss (Muthmainnah et al., 2015).

Other cell damage encountered in this study is necrosis, indicated by cell nuclei experiencing a change in colour to darker and shrinking cell nuclei. Necrosis occurs in epithelial cells that make up the proximal cortical tubules and distal cortical tubules treated with 300, 2000, and 5000 mg/kg BW (Figure 1 and Table 3). The characteristics of necrosis cells are in line with the research of Assiam et al. (2014) which showed that pyknotic-type necrosis cells experience discolouration, cell nuclei shrink, and chromatin clumped. Research by Anzini et al. (2014) showed cell damage in the kidney caused by changes in blood pressure and impaired mitochondrial function.

Increased blood flow to the glomerulus due to peroxynitrite compounds can then cause haemorrhage in kidney tissue. The normal glomerular capacity for filtering blood is 1.2L/min, but the high blood flow can cause blood to escape from the blood vessels into the tissue (Pringgoutomo, 2006). Hemorrhage occurred in the treatment doses of 300, 2000, and 5000 mg/kg BW (Figure 1) around the tubules and erythrocytes were also seen. This is similar to the research of Assiam et al. (2014) which found haemorrhage and visible erythrocytes in the tissue.

According to Saputera and Ayuchecaria (2018), bajakah tampala stems contain secondary metabolite compounds in the form of flavonoids, saponins, steroids, terpenoids, tannins, and phenols which have the potential as compounds that damage tissue microanatomy if the amount is too large. Research by Sari et al. (2017) mentioned that alkaloids, terpenoids, and flavonoids contained in sipatah-patah stem extract at a dose of 105 mg/kg BW can cause degeneration to necrosis in renal tubular cells. Saponins can lyse erythrocytes (McMurry and Fay, 2004). Research conducted by Shafaei et al. (2012), stated that saponins cause kidney damage in the form of brush border loss, glomerular bleeding, and bleeding in the kidney cortex. Saponins can cause hemolysis of the lipid bilayer in erythrocytes by forming pores in the cell membrane. Lysed erythrocytes cause the release of free haemoglobin into the plasma (Gutierrez et al., 2012).

Free haemoglobin binds to plasma haptoglobin previously secreted by the liver and becomes a hemoglobin-haptoglobin dimer (Muthmainnah et al., 2015). Unbound haemoglobin will still be processed by the kidney. Free haemoglobin that is also filtered into the vascular system in the glomerulus can cause epithelial cell damage and cell death, induce protein deposition, and act as a strong inhibitor of nitric oxide that triggers internal vasoconstriction (Sacher & McPherson, 2004).

CONCLUSION

The form of damage caused by acute doses of bajakah tampala stem extract on white rat kidney microanatomy in the form of tubular dilatation and loss of brush border in proximal cortical tubules at doses of 2000 and 5000 mg/kg BW. Other damage caused

is necrosis of the epithelial cells that make up the proximal cortical tubules and distal cortical tubules and the presence of haemorrhage found at doses of 300, 2000, and 5000 mg/kg BB. This study suggests that it is necessary to know more about cellular and molecular after the administration of bajakah tampala stem extract.

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