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Original Research Article

Effects of Porang (*Amorphophallus oncophyllus*) Residue on Blood Urea Nitrogen and Creatinine Levels and Kidney Histopathology

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ABSTRACT

Amorphophallus oncophyllus contains glucomannan, a bioactive polysaccharide. However, it has a high calcium oxalate content, posing potential health hazards. Treatment of *A. oncophyllus* with *Strobilanthes crispus* ethanol macerates (PMS) reduced the calcium oxalate levels. A 28-day subchronic toxicity test was conducted to assess the impact of PMS on blood urea nitrogen (BUN), creatinine, and kidney histology. Twenty-four male Wistar rats (*Rattus norvegicus*) were split into 4 groups: the control that did not receive any PMS, and the treatment groups that received PMS of 1000, 2000, and 4000 mg·kg^{-1} body weight. The rats were observed daily for toxic symptoms. Their blood was analyzed for BUN and creatinine levels, and hematoxylin-eosin staining was used to access the kidney's histopathology. The administration of PMS showed no symptoms of toxicity but resulted in increased BUN and creatinine levels, indicating a decline in kidney function. This finding is consistent with the histopathological results, which showed kidney damage, particularly interstitial nephritis and tubular epithelial necrosis, that may be attributed to the presence of calcium oxalate in raw *A. oncophyllus*.

***Keywords*:** Blood urea nitrogen, Creatinine, Kidney, Subchronic, Toxicity.

Introduction

Amorphophallus oncophyllus (commonly known as porang in Indonesia) is a tuber plant widely used as a staple food and snack due to its high glucomannan content. Glucomannan, a bioactive polysaccharide, consists of beta bonds connecting glucose and mannose,[1] and it possesses antidiabetic, anti-obesity, laxative, prebiotic, anticancer, and anti-inflammatory qualities. [1–3]

The extraction of glucomannan is expensive and requires considerable time, leading to the preference for consuming raw *A. oncophyllus*. Despite its nutritional and functional advantages, *A. oncophyllus* should be integrated into the diet with caution due to the presence of calcium oxalate. When ingested in high amounts, calcium oxalate can cause itching[4] and lead to the development of kidney stones.[5]

Traditionally, various methods have been used to reduce calcium oxalate levels in *A. oncophyllus* include soaking in salt, boiling, blowing, ball milling, and immersion in graded ethanol.[6] Treatment of *A. oncophyllus* with traditional herbal *Strobilanthes crispus* ethanol macerates (PMS) reduced calcium oxalate content.[7,8] The use of ethanol and the potassium and flavonoid content in *S. crispus* facilitates calcium oxalate dissolution in *A. oncphyllus*.[9,10] Furthermore, immersion in *S. crispus* enhances functional benefits in vivo, primarily due to its antioxidant compounds, such as terpenoid, polyphenols, alkaloids, and saponins, which help regulate blood glucose levels.[11]

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A recent acute toxicity study assessing PMS safety showed no fatalities.[4] Further investigation is required to determine the effects of prolonged PMS consumption. Therefore, this study aimed to investigate the effect of PMS on blood urea nitrogen (BUN), creatinine, and kidney histology in a 28-day subchronic toxicity study.

Materials and Methods

Plant collection and identification

This study was conducted using dried, ground, and 40-mesh-sieved *A. oncophyllus* flour. Two year-old *A. oncophyllus* tuber was purchased in June 2022 from East Java farmers in Madiun (coordinate: -7.476968,111.811514). *S. crispus* powder was obtained from a local commercial brand (MSH). Both plants were identified by the taxonomist Ichsan Luqmana Indra Putra, S. Si., M.Sc. from the Biology Learning Laboratory, Universitas Ahmad Dahlan, Indonesia with the deposit number of 462/Lab.Bio/B/XII/2023.

Extraction of A. oncophyllus porang with Strobilanthes crispus (SC) ethanol macerates (PMS)

The PMS was prepared following Patent Protocol No. S00202006668.[12] PMS was produced from immersion of 50 g *A. oncophyllus* in a 100 mL ethanol macerates (%w/v). The ethanol macerates were prepared by soaking 50 g of *S. crispus* leaves simplicia in 100 mL of ethanol for 24 hours.

Experimental animals

This study was approved by the Alma Ata University Ethics Committee (No. KE/AA/IX/10924/EC/2022). The experiment used 24 male Wistar rats (*Rattus norvegicus*), weighing between 150 and 200 g and aged 8-12 weeks. Each rat was housed in a wire cage at room temperature (22-30 °C) with a 12-hour light-dark cycle and provided with unlimited water and a standard commercial laboratory diet. Subsequently, all rats received the treatments for 28 days after 3-day adaptation period.

Subchronic toxicity study

The Indonesia Food and Drug Supervisory Agency's Regulation No. 7 of 2014 regarding the Guidelines for *in Vivo* Non-Clinical Toxicity Tests serves as the foundation for the implementation of subchronic toxicity study.[13] Four groups of rats were used: Group C (control) received no PMS, and Groups P1, P2, and P3 received PMS at doses of 1000, 2000, and 4000 mg·kg^{-1} body weight (BW), respectively. The rats were acclimated for 3 days, and treatments were administered orally once daily for 28 days.

During this period, observations were made for toxic symptoms and clinical signs, including changes in skin, fur, eyes, mucous membranes, secretions, excretions, gait changes, and odd behaviour such as walking backwards and seizures. Additionally, BW was monitored every 7 days to evaluate the amount of test preparation and the impact of PMS consumption.

Collection of samples and biochemical analysis

A total of 2 mL venous blood was drawn from the retro-orbital plexus of live animals on the final day using the microcapillary technique. The blood was centrifuged at 5000 rpm for 5 min to separate the plasma from the serum. The urease-GLDH (glutamate dehydrogenase) method was used to assess BUN levels [13,14] and the Jaffe kinetic test without deproteinization method was applied to analyze creatinine levels. [13,15]

Each rat was euthanized by cervical dislocation, followed by careful removal of the kidney, which were preserved in 10% buffered formalin for histological analysis. Tissue sections were subjected to hematoxylin and eosin (H&E) staining and examined microscopically at 400× magnification.

Statistical analysis

Data are presented as means ± standard deviation. Statistical analysis was performed using SPSS version 16. Duncan's multiple range test was used along with analysis of variance to assess differences between groups, with significance of $P < 0.05$.

Results and Discussion

Oral subchronic toxicity studies

The observation results did not show any toxic symptoms in the form of changes in the skin, fur, eyes, mucous membranes, secretions, excretions, gait; strange behaviour such as walking backwards; or seizures. Furthermore, no deaths were found after administering PMS, indicating it safe consumption at a concentration of 4000 mg·kg^{-1} BW. Subchronic oral toxicity test was conducted as a follow-up to a previous acute toxicity study, which demonstrated safe consumption based on a 72-hour observation period.[4] Toxic compounds may cause adverse effects, whether immediately (within minutes to hours at low doses) or after prolonged exposure (days to years). The classification may vary based on latency and dose-response, but harm at any stage defines toxicity.[16]

Body weight of rats

Figure 1 shows that rats in all treatment groups experienced weight gain. P2 and P3 showed markedly higher weight changes, which differed significantly from both the control and P1 ($P > 0.05$). This result demonstrated that PMS supplementation for 28 days had no bad effect on appetite. In addition, the extract may not obstruct the body's ability to absorb nutrients necessary for growth. Similarly, previous study on acute toxicity in which PMS samples were only given once showed no significant decrease in BW after 72 hours of administration.[4] *A. oncophyllus* without *S. crispus* maceration treatment was also reported an increase in the BW of rats during 6 days of treatment due to its starch, protein, fat, minerals, and glucomannan content.[17]

Blood urea nitrogen (BUN) levels

Figure 2 shows a significant increase in BUN levels in all treatment groups compared with the control group ($P < 0.05$), with the increase ranging from 10% to 28%. This indicates that PMS administration for 28 days significantly affected BUN levels in rats ($P < 0.05$). However, increasing the dose beyond 2000 mg·kg^{-1} BW did not result in a further significant elevation in BUN levels.

This finding is in contrasts with a previous acute oral toxicity study, which reported no significant effect on BUN levels when PMS was administered as a single dose with observation over 72 hours.[7] Similarly, a subacute oral toxicity study using a single dose of the ethanol extract of *S. crispus* at doses ranging from 150 to 600 mg·kg^{-1} BW also showed no significant effect on BUN levels in female Wistar rats after 14 days of treatment.[18] The elevation in BUN observed in this study may be attributed to the higher doses and prolonged administration of PMS.

BUN level a biomarkers of kidney function,[19] reflecting the glomerular filtration rate and other influencing factors, such as systemic and renal hypoperfusion, low cardiac output, and neurohumoral activation.[14] It is a waste product of protein catabolism formed in the liver and reabsorbed in the kidneys.[20] In renal impairment, elevated serum urea levels occur when urea production exceeds the renal clearance capacity.

Many herbal medicines can cause renal toxicity, particularly in the case of long-term administration. The substances responsible for kidney toxicity include aristolochic acid, matrine, and genistein from the alkaloid group; cinnamaldehyde; heavy metals such as arsenic, mercury, and lead; flavonoid glycosides; and saponins.[21]

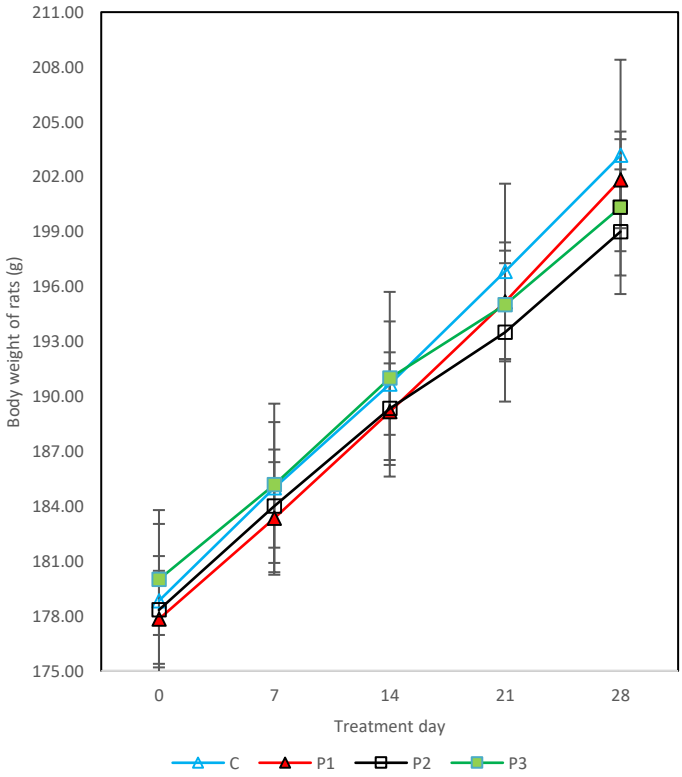

Figure 1: Body weight of rats in oral sub-chronic toxicity study of *A. oncophyllus*) residue produced from maceration process in *S. crispus* ethanol macerates (PMS). P1, P2, and P3 represented the treatment groups administered with 1000, 2000, and 4000 mg·kg^{-1} BW of PMS, respectively, while C represented the control group. ANOVA test was conducted to assess differences in weight gain across the rat groups, with statistical significance set at $P < 0.05$.

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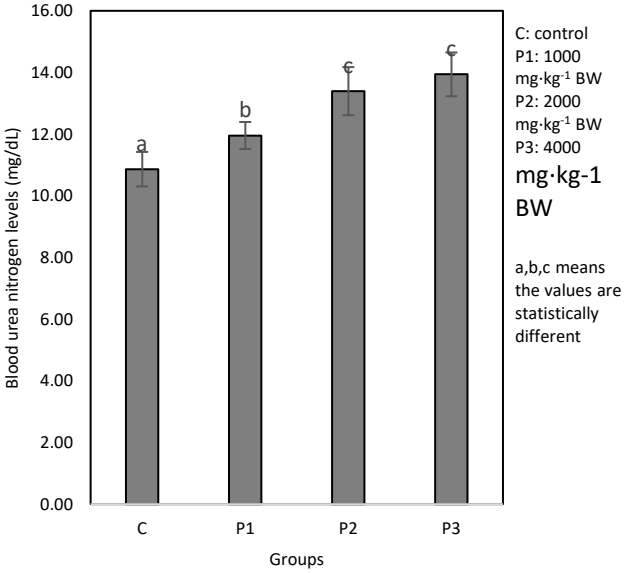

Figure 2: Blood urea nitrogen levels of rats in oral sub-chronic toxicity study of *A. oncophyllus* residue produced from maceration process in *S. crispus* ethanol macerates (PMS). P1, P2, and P3 represented the treatment groups administered with 1000, 2000, and 4000 mg·kg^{-1} BW of PMS, respectively, while C represented the control group

Creatinine levels

The blood serum creatinine levels of rats after the oral administration of PMS for 28 days are shown in Figure 3. The results showed that treatment with PMS at different doses had a significant effect on creatinine levels ($P < 0.05$). A significant increase in creatinine levels was observed in groups P1, P2, and P3, with values of 1.14 ± 0.08, 1.63 ± 0.11, and 1.80 ± 0.09 mg· dL^{-1}, respectively, compared with the control group, which had a level of 0.77 ± 0.07 mg· dL^{-1}. According to the reference values, the normal range for creatinine levels is 0.2–0.7 mg·dL^{-1}.[22] Therefore, the control group remained within the normal range, while the treatment groups exceeded the normal limit.

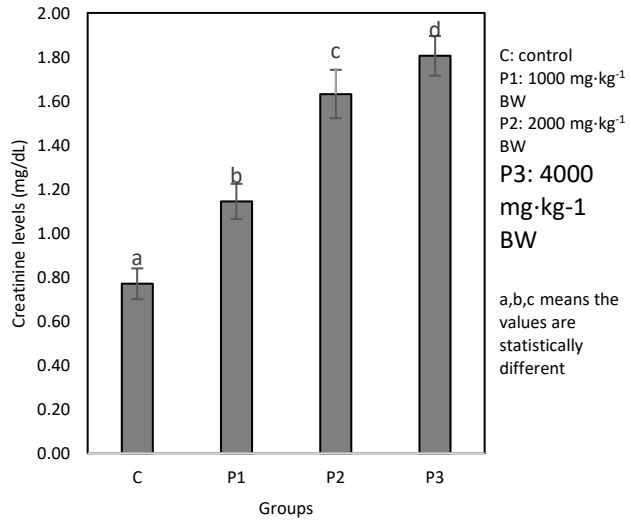

Figure 3: Levels of creatinine of rats in oral sub-chronic toxicity study of *A. oncophyllus* residue produced from maceration process in *S. crispus* ethanol macerates (PMS). P1, P2, and P3 represented the treatment groups administered with 1000, 2000, and 4000 mg·kg^{-1} BW of PMS, respectively, while C represented the control group

These findings differ from a study in which PMS was administered as a single dose. In that study, there was no significant changes in the creatinine levels at a dose of 2000 mg·kg^{-1} BW, but a significant increase was observed at 5000 mg·kg^{-1} BW[8], likely due to the high concentration of *A. oncophyllus*. Similarly, a single dose of *S. crispus* given to rats for 14 days at a concentration of 600 mg·kg^{-1} BW did not affect creatinine levels.[18] These results suggest that the elevation in creatinine levels following *A. oncophyllus* consumption may be attributed to the presence of calcium oxalate, indicating impaired kidney function.[8,23]

Similar to BUN levels, creatinine is commonly measured to assess kidney function. Urea is a waste product formed during protein metabolism, whereas creatinine is a nitrogenous compound generated from the breakdown of phosphocreatine and creatine and primarily excreted via glomerular filtration.[24] Creatinine levels typically increase in proportion to muscle mass. Additionally, blood creatinine concentration is influenced by the tubular secretion of creatinine. Other factors affecting blood creatinine levels include age, sex, diet, body composition, and physical activity. [25]

Histopathology of kidney

Table 1 shows histopathological observations of kidneys after 28 days of treatment with PMS. This indicates significant changes across all groups. In the treatment groups, higher PMS doses appeared to reduce kidney damage, as less rats exhibit interstitial nephritis. However, the severity of inflammation increased with higher PMS doses based on the random quantification of infiltrated lymphocyte cells per microscopic field of view. The numbers of inflammatory cells were 276 (45%) in the control group, 584 (60%) in the P1 group, and 639 (80%) in the P2 group. Interstitial nephritis is characterized by the infiltration of inflammatory cells (primarily lymphocytes) between the renal tubules and typically occurs in tissues exposed to infection or toxic substances. Similar pathological findings have been reported in rats exposed to monosodium glutamate, sodium fluoride, and 7,12-dimethylbenz[a]anthracene.[26,27]

Tubular epithelial necrosis was observed only in the P1 group (incidence: 20%). Similar changes have been reported in previous studies in which cell death in the renal tubules was attributed to exposure to toxic substances, including certain herbal plants.[28,29] Necrosis is an uncontrolled cell death process that often occurs in response to severe cellular injury. The major causes of cellular injuries include infection, inflammation, and ischemia.[28]

A representation of normal kidney histopathology is shown in Figure 4, which the renal corpuscle, proximal convoluted tubule, and distal convoluted tubule appear normal. Histopathological images of kidney exhibiting interstitial nephritis are shown in Figure 5. This figure shows the infiltration of inflammatory cell, whereas other structures, such as the distal convoluted tubule, proximal convoluted tubule, and collecting duct, remain normal under a microscope.

These histology changes were consistent with changes in BUN and creatinine levels, indicating kidney impairment. These findings also support previous research on acute toxicity studies using high doses of PMS (2000 and 5000 mg·kg^{-1} BW), which showed inflammation, haemorrhage, and congestion in kidney histhology.[4] The observed toxicity of PMS may be attributed to the use of high doses of raw *A. oncophyllus*, which still contains calcium oxalate. In contrast, other studies have shown that using glucomannan (the bioactive component from *A. oncophyllus*) and the ethanol extract of *A. oncophyllus* did not induce toxicity. On the contrary, these treatments were reported to reduce the occurrence of glomerulosclerosis after 50 days of treatment in diabetic rats,[4] and inflammation and cell necrosis after 28 days of treatment with *A. konjac* glucomannan in a single dose.[30] Additionally, the use of *S. crispus* did not result in signs of toxicity or kidney injury. [31]

Table 1: Histopathological observations of rat kidneys after 28 days of treatment with *A. oncophyllus* residue produced from maceration process in *S. crispus* ethanol macerates (PMS)

Groups	No pathological changes	Interstitial nephritis	Cyst	Tubular epithelial necrosis
C	2 (40%)	2 (40%)	1 (20%)	0 (0%)
P1	2 (40%)	2 (40%)	0 (0%)	1 (20%)
P2	4 (80%)	1 (20%)	0 (0%)	0 (0%)
P3	5 (100%)	0 (0%)	0 (0%)	0 (0%)

Notes: P1, P2, and P3 represented the treatment groups administered with 1000, 2000, and 4000 mg·kg^{-1} BW of PMS, respectively, while C represented the control group

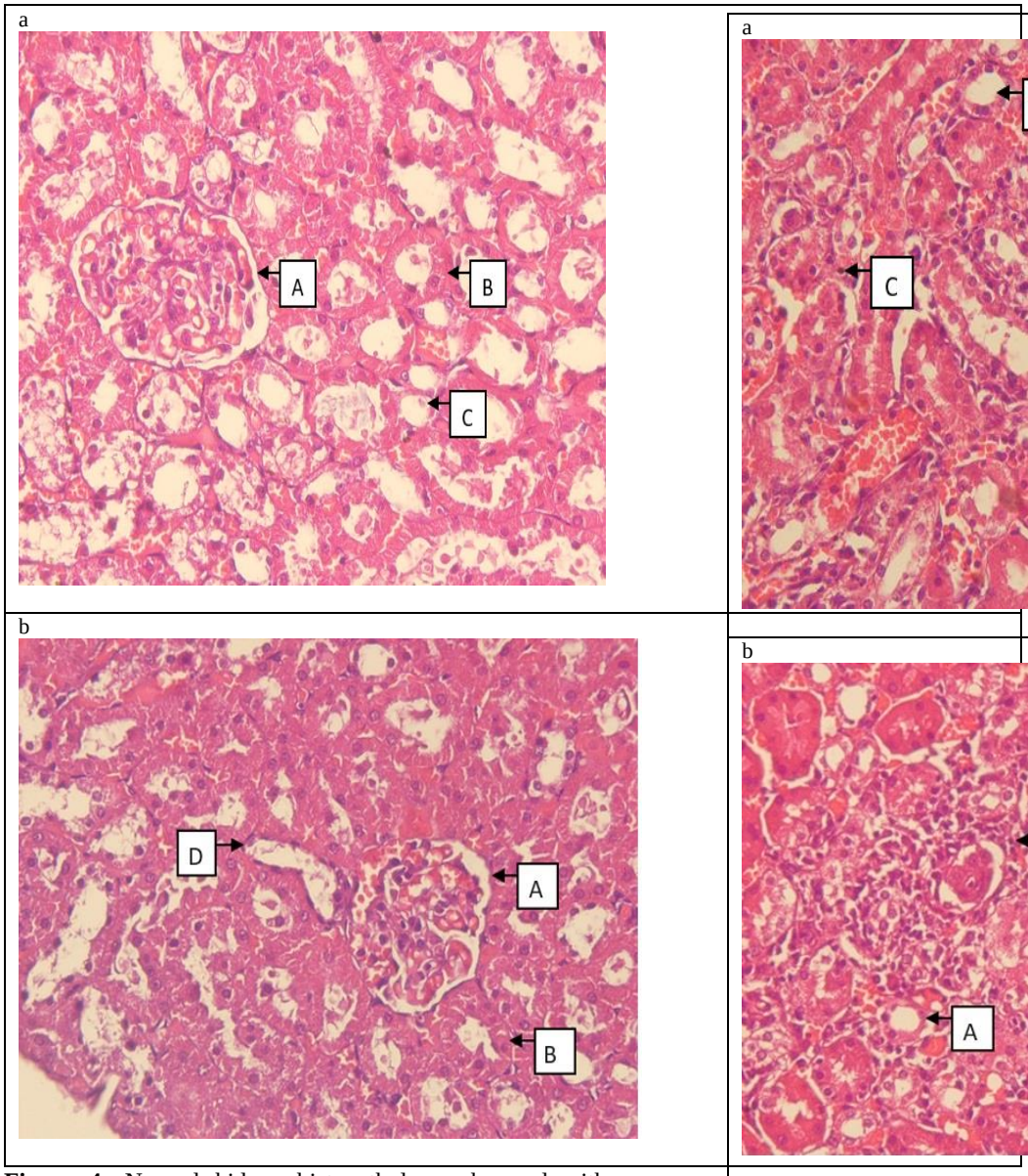

Figure 4: Normal kidney histopathology observed with a microscope at 400 times magnification in rats control without *A. oncophyllus* residue produced from maceration process in *S. crispus* ethanol macerates (PMS) (a) and P3 given 4000 mg·kg^{-1} BW of PMS (b). A = renal corpuscle, B = proximal convoluted tubule, C = distal convoluted tubule, D = blood vessels

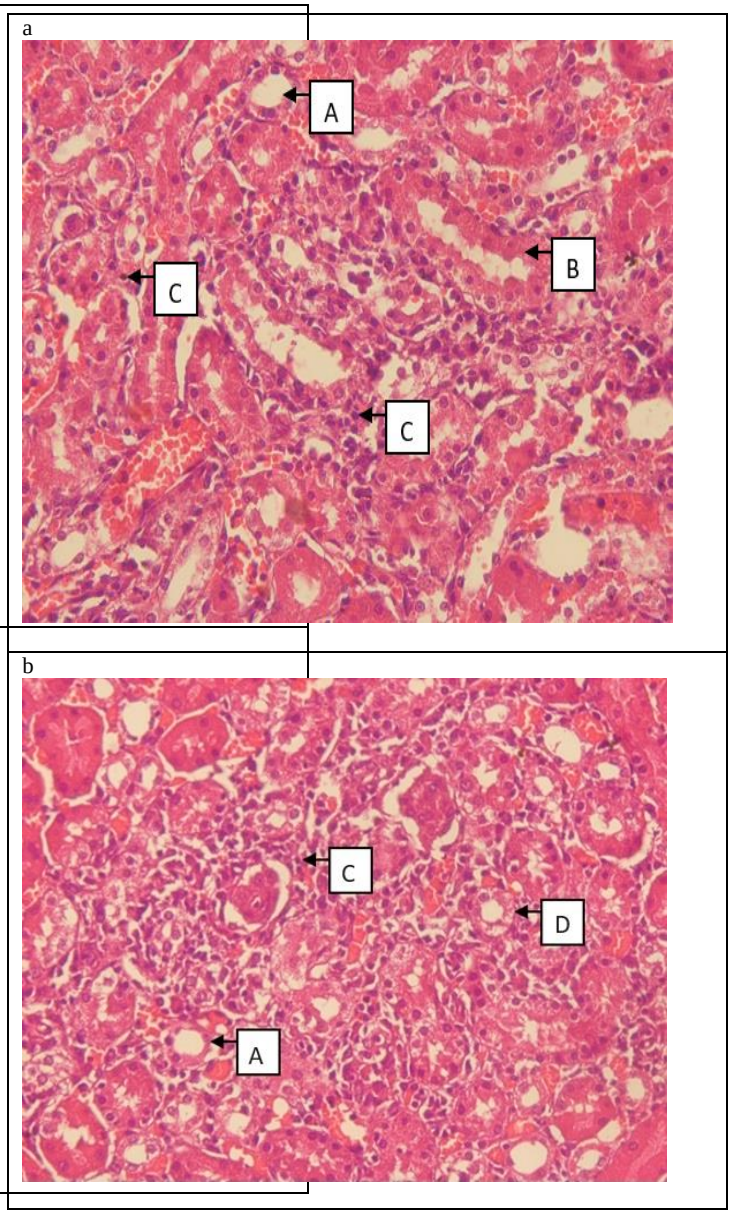

Figure 5: Histopathological figure of kidneys experiencing interstitial nephritis observed with a microscope at 400 times magnification in rats P1 given 1000 mg·kg^{-1} BW of *A. oncophyllus* residue produced from maceration process in *S. crispus* ethanol macerates (PMS) (a) and P2 given 2000 mg·kg^{-1} BW of PMS (b). A = normal distal convoluted tubule, B = normal proximal convoluted tubule, C = infiltration of inflammatory cells (lymphocytes) between the tubules, D = normal collecting duct

Conclusion

Subchronic oral administration of PMS (a combination of *A. oncophyllus* and *S. crispus*) at doses up to 4000 mg·kg^{-1} BW for 28 days caused no observable external toxic symptoms or mortality. However, biochemical and histopathological findings demonstrated renal impairment, as indicated by elevated BUN and creatinine levels and kidney lesions such as interstitial nephritis and tubular epithelial necrosis. These renal effects may be attributed primarily to the high concentration of calcium oxalate present in raw *A. oncophyllus*. Despite the absence of acute toxicity, prolonged exposure and high doses of PMS may pose a risk to kidney function. The nephrotoxic effects observed in this study underline the importance of evaluating not only the visible symptoms but also internal organ function in long-term toxicity assessments.

Future research should concentrate on dose optimization and long-term safety studies using lower and standardized doses, isolation or processing techniques to reduce calcium oxalate content in *A. oncophyllus*, comparative studies using purified glucomannan or *S. crispus* ethanol extracts versus raw plant materials to distinguish bioactive benefits from toxicity, and mechanistic studies to better understand the pathways of renal injury and inflammation induced by PMS in order to ensure the safe development of PMS as a potential nutraceutical or therapeutic agent.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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