BULETIN VETERINER UDAYANA

pISSN 2085-2495
eISSN 2477-2712

Received: 3 February 2026; Accepted: 27 February 2026; Published: 30 April 2026

KIDNEY HISTOPATHOLOGICAL FEATURES OF WHITE RATS *(RATTUS NORVEGICUS)* ADMINISTERED MORINGA (*MORINGA OLEIFERA*) LEAF SIMPLICIA AFTER LEAD EXPOSURE

Gambaran Histopatologi Ginjal Tikus Putih (*Rattus norvegicus*) yang diberikan Simplisia Daun Kelor (*Moringa oleifera*) Setelah Dipapar Logam Berat Timbal

Winda Audia Ananda[1]*, I Ketut Berata[2], Ida Bagus Oka Winaya[2], Ni Luh Eka Setiasih[3], Samsuri[4], Ni Nyoman Werdi Susari[5].

[1]Bachelor Program in Veterinary Medicine, Faculty of Veterinary Medicine, Udayana University, Jalan Raya Kampus Unud Jimbaran, Badung, Bali 80361, Indonesia

[2]Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University, Jalan Raya Kampus Unud Jimbaran, Badung, Bali 80361, Indonesia

[3]Veterinary Histology Laboratory, Faculty of Veterinary Medicine, Udayana University, Jalan Raya Kampus Unud Jimbaran, Badung, Bali 80361, Indonesia

[4]Laboratory of Veterinary Physiology, Pharmacology and Pharmacy, Faculty of Veterinary Medicine, Udayana University, Jalan Raya Kampus Unud Jimbaran, Badung, Bali 80361, Indonesia

[5]Laboratory of Veterinary Anatomy and Embryology, Faculty of Veterinary Medicine, Udayana University, Jalan Raya Kampus Unud Jimbaran, Badung, Bali 80361, Indonesia

*Corresponding author email: windaaudiaananda@gmail.com

How to cite: Ananda WA, Berata IK, Winaya IBO, Setiasih NLE, Samsuri S, Susari NNW. 2026. Kidney histopathological features of white rats (*Rattus norvegicus*) administered moringa (*Moringa oleifera*) leaf simplicia after lead exposure. *Bul. Vet. Udayana.* 18(2): 407-415. DOI: https://doi.org/10.24843/bulvet.2026.v18.i02.p05

Abstract

Lead (Pb) exposure can induce nephrotoxicity through oxidative stress and inflammation, resulting in histopathological alterations in the kidneys. Moringa (*Moringa oleifera*) leaves contain antioxidant and anti-inflammatory compounds with potential nephroprotective effects. This study aimed to describe renal histopathological lesions in lead-exposed white rats and evaluate the effects of Moringa leaf simplicia administration following lead exposure. Twenty male Wistar rats were divided into four groups (n=5): P0, placebo; P1, Pb acetate 0.5 mg/rat/day for 3 days; P2, Pb acetate for 3 days followed by Moringa leaf simplicia 0.1 g/rat/day for 14 days; and P3, Pb acetate followed by Moringa leaf simplicia 0.6 g/rat/day for 14 days. On day 15 after administration of the test substance, the kidneys were collected and processed into histopathological slides stained with hematoxylin and eosin. Congestion, inflammation, and necrosis were assessed in five fields of view using a 0–3 scoring system for each. Significant differences were observed among the groups for all parameters. At 0.6 g/rat/day, the degree of congestion and inflammation decreased compared to the Pb-only group,

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https://doi.org/10.24843/bulvet.2026.v18.i02.p05

whereas the 0.1 g/rat/day dose showed no difference. Necrotic lesions were still observed in all Pb-exposed groups and did not differ significantly among the groups receiving simplicia. It was concluded that Moringa leaf simplicia at 0.6 g/rat/day can improve congestion and inflammation following Pb exposure but has not yet improved necrotic lesions. Further studies with longer durations and higher doses are required.

Keywords: kidney histopathology, lead (Pb), *Moringa oleifera*, simplicia, white rats.

Abstrak

Paparan logam berat timbal (Pb) dapat menimbulkan nefrotoksisitas melalui stres oksidatif dan peradangan yang berujung pada perubahan histopatologi ginjal. Daun kelor mengandung senyawa antioksidan dan antiinflamasi yang berpotensi sebagai agen nefroprotektif. Penelitian ini bertujuan menggambarkan lesi histopatologi ginjal tikus putih yang dipapar timbal serta mengevaluasi pengaruh pemberian simplisia daun kelor setelah paparan timbal. Dua puluh ekor tikus jantan galur Wistar dibagi menjadi empat kelompok (n=5): P0 plasebo; P1 Pb asetat 0,5 mg/ekor/hari selama 3 hari; P2 Pb asetat selama 3 hari dilanjutkan simplisia daun kelor 0,1 g/ekor/hari selama 14 hari; dan P3 Pb asetat dilanjutkan simplisia daun kelor 0,6 g/ekor/hari selama 14 hari. Pada hari ke-15 setelah pemberian simplisia, ginjal diambil dan dibuat preparat histopatologi dengan pewarnaan hematoksilin-eosin. Kongesti, peradangan, dan nekrosis dinilai pada lima lapang pandang dengan skoring 0-3. Terdapat perbedaan signifikan antarkelompok pada seluruh parameter. Pada dosis 0,6 g/ekor/hari, derajat kongesti dan peradangan menurun dibanding kelompok Pb saja, sedangkan dosis 0,1 g/ekor/hari tidak berbeda. Lesi nekrosis masih dijumpai pada seluruh kelompok terpapar Pb dan tidak berbeda signifikan antarkelompok yang diberi simplisia. Disimpulkan bahwa simplisia daun kelor dosis 0,6 g/ekor/hari mampu memperbaiki lesi kongesti dan peradangan pasca paparan Pb, namun belum memperbaiki lesi nekrosis; diperlukan penelitian dengan durasi lebih lama dan dosis lebih tinggi.

Kata kunci: histopatologi ginjal, *Moringa oleifera*, simplisia, tikus putih, timbal

INTRODUCTION

Lead (Pb) poisoning remains an environmental health problem that affects both animals and humans adversely. Pb is non-essential and toxic, and exposure can trigger oxidative stress and dysfunction in various organs. The kidneys are the main target organs for Pb accumulation because they are excretory organs responsible for filtering and eliminating toxic substances from the blood (Flora *et al.*,2012; Luby *et al.*,2024). Lead accumulation in the kidney tissue can cause progressive structural and functional damage, particularly in the proximal tubules, which are segments of the nephron with high metabolic activity and intensive reabsorption capacity.

The mechanism of Pb nephrotoxicity involves complex and interrelated pathophysiological processes. Pb exposure triggers an increase in the production of reactive oxygen species (ROS) that exceeds the capacity of the endogenous antioxidant defense system, resulting in oxidative damage to cell membrane lipids, proteins, and DNA (Gurer & Ercal, 2000). Prolonged oxidative stress causes vascular endothelial dysfunction and activates inflammatory pathways. This condition contributes to renal microcirculatory disorders and inflammatory cell infiltration into the interstitium. This exacerbates damage to tubular epithelial cells and can lead to irreversible cell necrosis. Renal histopathological changes commonly observed in animals exposed to Pb include congestion, interstitial inflammation, and tubular necrosis (Berata *et al*., 2017). Prevention and supportive therapy strategies for Pb poisoning generally focus on

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administering antioxidants to suppress free radical formation and strengthen the endogenous antioxidant defense system (Dominika, 2023).

Moringa leaves (*Moringa oleifera*) are known to contain various bioactive compounds that are rich in antioxidants. Moringa leaves contain various phytochemicals, especially phenolics and flavonoids, such as quercetin, kaempferol, and chlorogenic acid, which have high antioxidant capacities and protective effects on various organs (Leone *et al.*,2015). Several studies have shown that Moringa leaves have a protective effect on organs undergoing oxidative stress, including those induced by Pb (Saputra & Sulistyarsi, 2019; Usman *et al*., 2022). However, although the toxic effects of Pb and the nephroprotective potential of moringa leaves have been widely reported, most studies have used extracts that contain more standardized active compounds (Vergara-Jimenez *et al*., 2017). Studies using moringa leaf crude extracts are limited; therefore, their effectiveness in repairing kidney damage after Pb exposure has not yet been confirmed. Therefore, there is an urgent need to fill this knowledge gap and provide a scientific basis for the potential use of crude Moringa leaf extracts as a supportive therapy for reducing kidney damage due to Pb exposure.

RESEARCH METHOD

Animal Testing Ethics

The use of test animals in this study was approved by the Veterinary Faculty Ethics Committee No. B/208/UN14.2.9/PT.01.04/2025.

Research Subjects

This study used clinically healthy Wistar strain white rats (*Rattus norvegicus*) with an average weight of 160–180 g, male, and 2 months old. The research samples were the kidneys of white rats (*Rattus norvegicus*) that were exposed to heavy metal lead (Pb) and administered Moringa leaf simplisia.

Research Design

This study used a completely randomized design (CRD) consisting of four treatment groups: P0 (negative control), P1 (positive control with 0.5 mg/animal/day of lead acetate), P2 (lead + 0.1 g/animal/day of moringa leaf extract), and P3 (lead + 0.6 g/animal/day of moringa leaf extract). Five replicates were performed for each group (n = 5). The sample size was determined based on Federer's formula $(t)(n-1) \geq 15$, with $t = 4$, resulting in a minimum of five animals per group.

Lead acetate was administered for three consecutive days, followed by moringa leaf extract for 14 days via oral gavage. The moringa leaf extract (*Moringa oleifera*) used in this study was a commercial product packaged in 150 g jars under the Daribumi brand, produced by PT. Solusi Pangan Kita. After the entire treatment series was completed, the rats were euthanized under anesthesia using a combination of 0.2 mL of xylazine-ketamine intramuscularly. Necropsy was then performed to remove the kidneys, which were fixed in 10% Neutral Buffered Formalin (NBF) solution for histopathological processing.

Preparation of Renal Histopathology Specimens

Histopathological specimens were prepared at the Denpasar Veterinary Center Pathology Laboratory using the Kiernan (1990) method. The process began with tissue dehydration using a series of graded alcohol solutions (70%, 80%, 90%, 95%, and absolute) for 60 min each. After dehydration, the tissue was immersed in xylene solution three times for 45 min for the clearing process. The next stage was paraffin infiltration at 60 °C, carried out three times for 60 min each, and the tissue was then embedded in a liquid paraffin block. After the paraffin

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hardened, the tissue was cut with a rotary microtome to a thickness of 5 µm. The tissue sections were flattened in warm water (45 °C) and attached to coated slides. The preparations were dried vertically at room temperature and then horizontally on a 37 °C slide warmer for 12 h.

Hematoxylin-Eosin (HE) Staining

Staining of the specimen began with deparaffinization in xylene for 5–10 min twice. Rehydration was performed by immersing the specimen in absolute alcohol twice, followed by 95% and 70% alcohol for 2–3 min each. The specimen was then rinsed under running water for 2–3 min before being immersed in a hematoxylin solution for 5–10 min. After rinsing with water, the specimen was immersed in Scott's blue or ammonia solution for "bluing," and then stained with eosin for 1–2 min. The specimen was rinsed again with water, followed by gradual dehydration with absolute alcohol, clearing with xylene, and mounting with adhesive. The specimens were dried and were ready for observation under a microscope.

Research Variables

The research variables included independent, dependent, and control variables. The independent variables were exposure to lead (Pb) solution and administration of moringa leaf simplicia at two different doses. Renal histopathological changes were the dependent variable, which were assessed based on the degree of congestion, inflammation, and necrosis. The control variables were rat strain, age, body weight, sex, feed type, drinking water, and environmental conditions.

Data Collection Method

Data for renal histopathological analysis were obtained through macroscopic examination of the white rat kidney tissue. Kidney samples were processed for histopathological examination using hematoxylin and eosin (H&E) staining. The prepared slides were observed under a light microscope in five microscopic fields of view to evaluate histopathological changes, namely congestion, necrosis, and inflammation.

Renal histopathological lesions were assessed using a 0–3 scoring system for three types of lesions: congestion, necrosis, and inflammation. A score of 0 indicated normal conditions without lesions. A score of 1 indicated mild focal lesions. A score of 2 indicated moderate multifocal lesions, and a score of 3 indicated severe diffuse lesions. These criteria were consistently applied to assess congestion, necrosis, and inflammatory lesions.

The distribution of lesions was determined based on their tissue distribution. Focal lesions were localized to a small area, multifocal lesions were characterized by the presence of several separate lesion areas, and diffuse lesions spread widely over most of the observed area. These criteria were applied to assess congestion, necrosis, and inflammation in histopathological preparations. The scores obtained from each treatment group were averaged and used to compare the effectiveness of the treatments between groups, including the negative control, positive control, and treatment groups administered different doses of moringa leaf simplicia.

Data Analysis

The data obtained from renal histopathological observations were statistically analyzed using the Kruskal-Wallis nonparametric test to determine whether significant differences existed between the treatment groups. If the analysis showed significant differences ($P < 0.05$), the Mann-Whitney U test was used to determine which groups differed significantly. All data analyses were performed using IBM SPSS software.

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RESULTS AND DISCUSSION

Results

Histopathological examination of the kidneys of white rats (*Rattus norvegicus*) in five fields of view using a 0–3 scoring method revealed differences in the degree of congestion, inflammation, and necrosis between the treatment groups. The overall histopathological changes are shown in Figure 1 and Table 1.

Based on the histopathological examination data, the negative control group (P0) had a score of 0 for all parameters, indicating the absence of histopathological lesions. In the group exposed only to Pb (P1), congestive lesions were observed, characterized by glomerular capillary dilation and erythrocyte accumulation in the peritubular blood vessels. Inflammatory cell infiltration was primarily observed in the renal interstitium. Tubular epithelial necrosis was observed in the form of pyknosis, karyorrhexis, and desquamation of cells into the tubular lumen, especially in the proximal tubules, which showed high susceptibility to Pb. In group P2, similar histopathological lesions were observed, but to a lesser extent than in group P1. Congestion was still visible in some glomerular and peritubular capillaries, while inflammatory cell infiltration appeared to be reduced but was still present in the interstitial area. Tubular epithelial necrosis was still visible, especially in the proximal tubules, although the number of cells undergoing degeneration and desquamation appeared to be fewer than that in group P1. Group P3 showed more marked improvement. Congestion in the glomerular capillaries and peritubular vessels appeared to decrease significantly, and inflammatory cell infiltration in the interstitium decreased. However, necrotic lesions in the tubular epithelium were still found in some areas, indicating that irreversible tubular damage had not fully recovered despite the improvements in the vascular and inflammatory parameters.

The Kruskal-Wallis test results showed significant differences between the treatment groups for all three lesion parameters ($p<0.05$). Pb treatment and moringa leaf administration had a significant effect on the degree of histopathological lesions in the kidneys of white rats (Table 2); therefore, the analysis was continued using the Mann–Whitney U test to determine the pairs of groups that differed. Based on the Mann–Whitney test, group P3 showed a significant reduction in congestion and inflammation compared to group P1 ($p < 0.05$). Group P0 differed significantly from P1 and P2 in all parameters, whereas in P3, significant differences were found only in the necrosis parameter ($p < 0.05$). No other significant differences were observed between the groups.

Discussion

The results of this study indicate that exposure to heavy metal Pb acetate causes histopathological changes in the kidneys, including congestion, inflammation, and necrosis. The kidneys are the main target organs for Pb accumulation because they play a role in filtration and excretion; consequently, tubule tissues, especially the proximal tubules, are susceptible to damage due to Pb toxicity (Flora *et al.*,2012; Patrick, 2006) Pb can trigger oxidative stress through increased formation of reactive oxygen species (ROS), decreased endogenous antioxidant systems, and increased lipid peroxidation of cell membranes, which ultimately triggers cell injury and inflammatory responses (Gurer & Ercal, 2000; Patrick, 2006).

In the positive control group, congestion appeared as dilation of blood vessels/capillaries with erythrocyte accumulation in the blood vessels of the glomerulus and peritubular areas. Congestion can occur due to microcirculatory disorders and changes in vascular permeability, which are influenced by endothelial damage and increased inflammatory mediators following Pb exposure (Patrick, 2006). The observed inflammation consisted of inflammatory cell

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infiltration in the interstitium, which is likely a response to tissue damage and the release of pro-inflammatory mediators. These findings are consistent with the mechanism of Pb nephrotoxicity, which activates inflammatory pathways and exacerbates kidney injury (Patrick, 2006).

Necrotic lesions in the positive control group were characterized by morphological changes in tubular epithelial cells, such as pyknosis, karyorrhexis/karyolysis, and desquamation of cells within the tubular lumen. Necrosis is a more severe form of cell damage associated with mitochondrial dysfunction, enzyme inhibition, and cell membrane damage due to oxidative stress and interaction of Pb with protein sulfhydryl groups (Dominika, 2023). This explains why the group exposed to Pb showed necrosis with a high degree of severity.

Administration of moringa leaf simplicia after Pb exposure tended to improve the severity of lesions, especially at the 0.6 g/animal/day dose. Moringa leaves contain phenolic compounds, flavonoids, and vitamins with antioxidant properties, enabling them to scavenge free radicals and reduce lipid peroxidation (Leone *et al.* ,2015; Vergara-Jimenez *et al.,* 2017). In addition, several bioactive components of moringa leaves reportedly exert anti-inflammatory effects by suppressing the production of pro-inflammatory mediators, thereby reducing inflammatory cell infiltration in tissues (Abdel-Aty *et al.*, 2025).

Statistically, the 0.6 g/animal/day treatment group differed significantly ($p<0.05$) from the positive control group in terms of congestion and inflammation parameters, indicating that the 0.6 g/animal/day dose was more effective in reducing the degree of vascular stasis lesions and inflammatory response than the 0.1 g/animal/day dose. At a dose of 0.1 g/animal/day (P2), the reduction in lesion severity did not reach a significant difference ($p<0.05$) compared to P1, which may be influenced by the lower dose, biological variation between individuals, and the use of crude extracts, whose active ingredient content has not been standardized, compared to the use of extracts (Saputra & Sulistyarsi, 2019).

Although there was improvement in congestion and inflammation, necrotic lesions in the groups administered 0.1 g/animal/day and 0.6 g/animal/day of P3 did not show significant differences ($p<0.05$) compared to the positive control. This can be explained by the fact that necrosis is a more severe form of cell damage and can be irreversible in some cases, requiring a longer duration for tissue regeneration. The duration of moringa leaf administration for 14 days after Pb exposure may not have been sufficient to show significant structural improvement in necrotic lesions. In addition, Pb accumulated in the kidneys can continue to cause oxidative stress even after exposure has stopped (Patrick, 2006). The results of this study indicate that moringa leaf extract, especially at a dose of 0.6 g/animal/day, has the potential to reduce congestion and inflammation of the kidneys in white rats exposed to Pb.

CONCLUSION AND SUGGESTIONS

Conclusion

The administration of *M. oleifera* leaf extract affected the histopathology of the kidneys of white rats exposed to Pb. A dose of 0.6 g/rat/day was more effective in reducing congestion and inflammation than a dose of 0.1 g/rat/day, although neither dose was able to repair necrotic lesions to the same extent as the control group.

Suggestions

Further research is warranted on the effects of *M. oleifera* leaf extract on the kidneys of white rats exposed to Pb, using higher doses of *M. oleifera* leaf extract and longer administration periods. This study aimed to determine the long-term effects of *M. oleifera* leaf extract.

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ACKNOWLEDGMENTS

The author thanks the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University, and the Denpasar Veterinary Center for the facilities and assistance provided during this study. We also thank the supervisor and all parties involved.

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Tables

Table 1. Mean scores of renal histopathological lesions in white rats for each treatment group (score 0–3).

Group	Congestion	Inflammation	Necrosis
P0 (negative control)	0.0^{a}	0.0^{a}	0.0^{a}
P1 (Pb)	1.6^{b}	1.8^{b}	1.8^{b}
P2 (Pb + moringa 0.1 g)	1.0^{b}	1.4^{b}	1.8^{b}
P3 (Pb + moringa 0.6 g)	0.4^{c}	0.6^{c}	1.2^{b}

Note: Score 0 = normal; 1 = mild; 2 = moderate; 3 = severe. The values in the table represent the mean lesion scores. Different superscript letters in the same column indicate a significant difference ($p < 0.05$) based on the Mann–Whitney test (post-hoc Kruskal–Wallis).

Table 2. Results of the Kruskal–Wallis test on renal histopathological lesion scores.

Statistic	Congestion	Inflammation	Necrosis
Chi-Square	14.601	14.397	14.558
df	3	3	3
Asymp. Sig.	0.002	0.002	0.002

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Figure

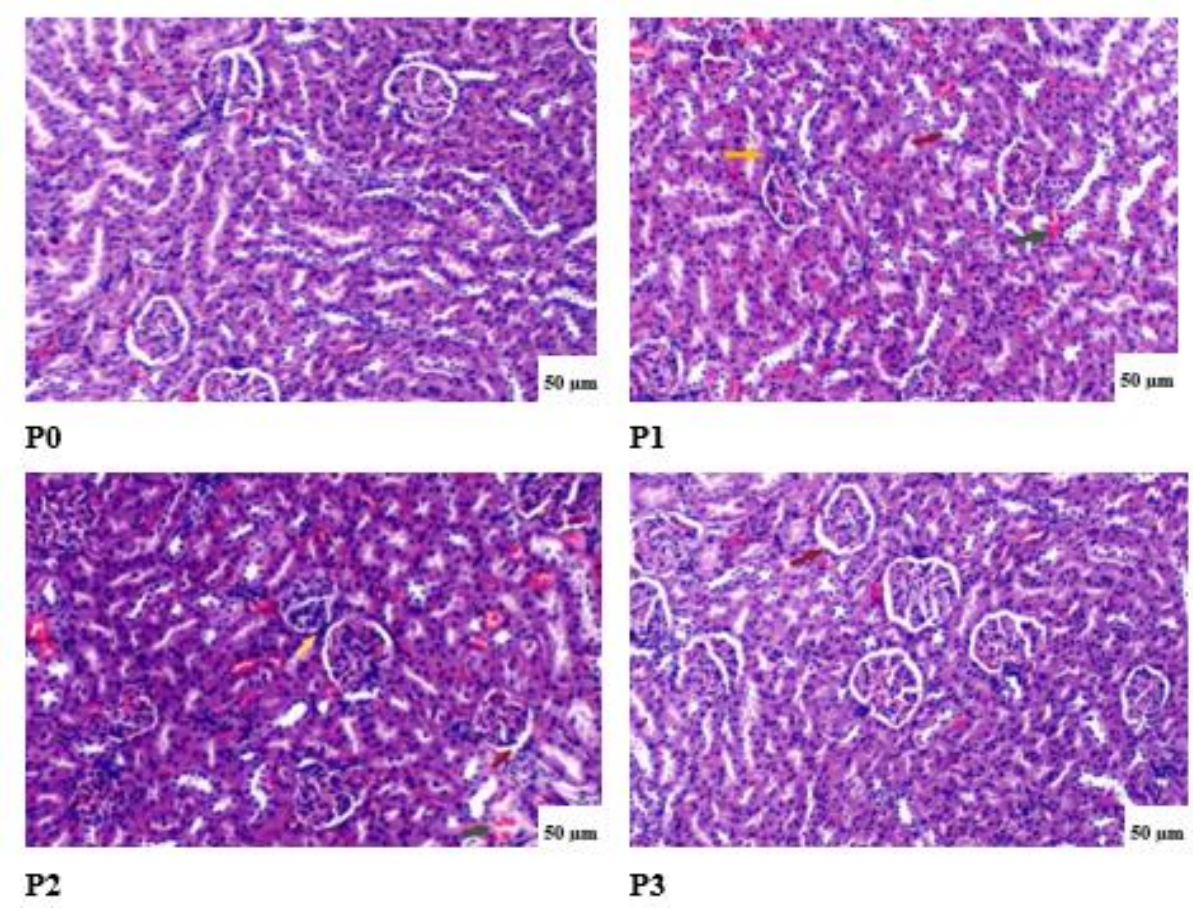

Figure 1. Histopathology of the kidneys of white rats (*Rattus norvegicus*) in each treatment group (HE, 200×). P0: Normal kidney structure. P1: Congestion (green arrow), inflammatory cell infiltration (yellow arrow), and tubular epithelial necrosis (red arrow) were observed. P2: Similar lesions were observed, but to a lesser degree. P3: Congestion and inflammation appeared to have decreased; however, tubular necrosis was still present.

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