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The Effect of *Moringa Oleifera* Lam Leaf Extract on the Kidney and Liver of Rat Exposed to Lead Acetate

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KEYWORDS

Degeneration, environmental pollution, malondialdehyde, necrosis, superoxide dismutase

ABSTRACT:

Introduction: Lead (Pb) is an environmentally friendly and toxic heavy metal, characterized by high stability and ease of transportation

Objectives: The purpose of this study was to evaluate the protective effect of leaf extract from *Moringa oleifera* on the kidney and liver of rats exposed to lead acetate.

Methods: Twenty-five rats were divided randomly into five groups. The control (C) group: rats were exposed to distilled water and 0.5% carboxymethyl cellulose sodium (CMC Na) orally at four h intervals. The rats of T0, T1, T2, and T3 group were exposed to 50 mg/kg BW/day lead acetate, followed by administration of 0.5% CMC Na, Moringa leaf extract 200, 316, and 500 mg/kg BW/day, respectively 4 h later for 20 days. On the 21st day, the rats were sacrificed, for evaluate the hepatic and renal histopathologic slides and functional chemicals marker.

Results: The results showed that rats in the T0 group have the higher ($p<0.05$) of malondialdehyde (MDA) and lower ($p<0.05$) of superoxide dismutase (SOD) activity than on the C group. These changes were followed by the higher scores of renal degeneration, renal necrosis, hepatic degeneration, hepatic necrosis, levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyrufic transaminase (SGPT), leucocyte and basophils profile and the lower ($p<0.05$) of monocyte profile. Administration of Moringa leaf extract in groups of T1, T2, and T3 were followed by restore MDA levels and SOD activity, and briefly, repaired the damages.

Conclusions: It could be concluded that *Moringa oleifera* leaf extract increased SOD activity, reduced MDA levels and ameliorate histological damage and chemicals marker of hepatic and renal function of rats exposed to lead acetate.

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1. Introduction

Pollution from engineering, traffic, agriculture, and trash causes hazardous materials such heavy metals to contaminate the air, land, food, and water, endangering human health **[1]**. Heavy metals are considered the most toxic ecological contaminants. Heavy metals affect humans neurotoxically and carcinogenically, even at low concentrations **[2]**. The most common heavy metals that are toxic to fish, plants, animals, and humans include mercury, cadmium, arsenic, chromium, and lead (Pb) **[3]**. Furthermore, the harmful effects of these heavy metals on various organs vary. Pb exposure was predicted to be the cause of 0.6% of the world's illness problems in 2016, with 540,000 fatalities worldwide attributed to Pb exposure, mostly in developing nations **[4]**.

Lead (Pb) is an environmentally friendly and toxic heavy metal, characterized by high stability and ease of transportation **[5]**. Pb is used in various industrial activities and applications due to its physicochemical properties. This metal can be found in both organic and inorganic forms. Lead (Pb) is utilized in a wide range of industrial operations, such as the production of lead-based paint, toys, gasoline, paint, solder, pipes, X-ray protection devices, ammunition, and cosmetics **[6]**. Lead poisoning (Pb) can occur through integumentary absorption; it is primarily absorbed from the respiratory and digestive tracts. Pb can cause immunological, neurological, embryonic, renal, cardiovascular, respiratory, bone, hematological, and reproductive disorders by upsetting the balance of antioxidant-antioxidant agents and inflaming different tissues **[7]**.

The global level of Pb poisoning in blood is 10 lg/dl. In rats exposed to lead, Melebary and Elnaggar **[8]** found a significant rise in the serum concentrations of urea, creatinine, uric acid, ALT, and AST. According to Andjelkovic et al. **[9]** mice subjected to Pb treatment had higher levels of urea, bilirubin, creatinine, and uric acid. Furthermore, Pb-exposed mice had higher TG, LDL, HDL, and total cholesterol levels in addition to higher levels of the enzymes ALP, GPT, and GOT. $Pb(C_2H_3O_2)_2$ alters hematological and biochemical profiles and manifests renal and hepatic damage by causing a notable increase in reactive oxygen species (ROS) generation, which results in oxidative stress (OS), increased lipid peroxidation, and decreased glutathione (GSH) levels in tissues **[10]**.

Pb(C2H3O2)2 affects the histology and biochemistry of blood, liver, kidney, and brain tissues **[11]**. Recently, the world has paid more and more attention to the use of natural products to improve livestock health. Chemical preparations can be dangerous. Therefore, the demand for making antioxidant ingredients is very high. Numerous tropical and subtropical regions of the world are home to moringa species. A common and adaptable therapeutic plant is *Moringa oleifera* Lam **[12]**. The tree's almost whole length has numerous industrial applications. This plant's high concentration of potent bioactive chemicals is linked to its nutritional, medicinal, and preventative qualities, making it a high-value crop **[13]**. *M. oleifera* possesses a number of medicinal qualities, including antioxidant, anti-inflammatory, anti-cancer, and ulcer-healing effects **[14]**.

2. Objectives

The study revealed a dose-dependent impact of *M. oleifera* extract on rat kidney tissue. In their estimation of *M. oleifera's* effect on acute arsenic poisoning in rats, Nurhayati et al. **[15]** discovered that the extract lessened the harmful effects of arsenic. Furthermore, *M. oleifera* extract decreased $Pb(C_2H_3O_2)2$-induced histological alterations in the kidney and liver of rabbits **[16]**. Consequently, the purpose of this study was to ascertain if *M. oleifera* leaf extract protected the kidneys and liver of mice that were exposed to lead acetate.

3. Methods

Ethics Statements

This research has been approved by the Ethical Clearance Committee for preclinical research at Airlangga University with ethical permit No.158/HRECCFODM/IV/2022.

Research design

Moringa leaves (*M. oleifera* L.) originating from UPT Herbal Materia Medica Laboratory Batu Malang with determination letter number 074/238/102.20-A/2022. Extraction of Moringa leaves is made using the maceration method. The maceration process uses Moringa leaf powder as a base ingredient and 96% ethanol as a solvent **[17]**. Maceration is carried out by soaking Moringa leaf powder using 96% ethanol solvent

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for 3 x 24 hours, then filtering to obtain the result in the form of macerate. After that, the maserate is placed in a votary evaporator and run at 40°C and 50 rpm until all of the solvent and water content of the moringa leaves have been extracted. Determining the dose of Moringa leaf extract refers to the research of Stohs and Hartman **[18]** with a division of doses, namely the minimum dose and maximum dose. The minimum dose is 200 mg/kgBW/day given to group P1, and the maximum dose given to group P3 is 500 mg/kgBW/day, while the P2 group is given interval doses based on dose interval calculations. times the minimum dose.

Lead acetate ($Pb(C_2H_3O_2)_2$) was obtained from Sigma-Aldrich (USA; Cat. No. 6080-56-4). The treatment is made by diluting lead acetate in distilled water which is given in a single dose of 50 mg/kg body weight (BW)/day **[19]**. Moringa leaf extract is obtained through extraction via maceration using 96% ethanol, followed by freeze drying, and subsequent dilution in 0.5% sodium carboxymethyl cellulose (CMC Na) **[20]**.

Treatment of experimental animals

Male rats (*Rattus norvegicus* strain Wistar), weighing approximately 200–250 g and 2.5–3.0 months old, were housed individually in plastic cages in a climate-controlled environment. The temperature was maintained at 26°C ± 2°C in a 12/12 h alternating light/dark cycle. Standard commercial food and drinking water were provided to the mice on an as-needed basis. Twenty-five rats were divided randomly and evenly into five groups, namely the negative control group, C–, consisting of rats that were exposed to distilled water (used as a lead acetate solvent) and given 0.5% CMC Na. The C+ positive control group was exposed to 50 mg/kg BW/day lead acetate and 0.5% CMC Na solution. Groups T1, T2, and T3 were exposed to 50 mg/kg BW of lead acetate and given Moringa leaf extract at doses of 200, 316, and 500 mg/kg BW/day, respectively. Mice were orally exposed to 0.5 mL lead acetate solution at 0700 hours before feeding, and 4 h later, they were orally administered 0.5 mL Moringa leaf extract solution. This regimen is continued for 20 days.

All mice were anesthetized with 40 mg/kg BW intraperitoneal ketamine and euthanized on day 21. Blood is taken from the heart via subcostal loop laparotomy. Serum was also collected to measure MDA levels and SOD activity.

Malondialdehyde (MDA)

MDA levels in testicular tissue were determined using the thiobarbituric acid reactive substance test via the Thiobarbituric Acid Kit (NWLSS, USA, Cat. No. NWK-MDA01). Absorbance was measured using a UV-1601 spectrophotometer at a maximum wavelength of 535 nm **[21]**.

Superoxide Dismutase (SOD)

SOD activity in testicular tissue was assessed using a test kit from Cayman Chemicals (USA, Cat. No. 706.002). The wavelength at which absorbance was measured was 550 nm **[22]**.

Blood sample, BUN, Creatinine, SGOT, SGPT

On the 21st day, all white mice were anesthetized using ketamine at a dose of 40 mg/kg BW and xylazine at a dose of 40 mg/kg BW, then euthanized using the cervical dislocation method. Then intracardiac blood was taken from the mice until no more blood could be taken. The blood that has been obtained is then put into a plain vacuum tube measuring 3.5 ml. To check BUN, Creatinine, SGOT and SGPT levels, the blood is first centrifuged at 3000 rom for 10 minutes to take serum samples. Next, the serum will be sent to the Surabaya Central Health Laboratory (BBLK).

Counting leukocyte types

Counting the types of leukocytes (differential counting) is done using a Hematology Analyzer.

Kidney and liver histology

Histopathological examination of kidney and liver preparations using an Olympus® CX-22 microscope was carried out at the Laboratory of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Airlangga University. Examination of the preparations using 400x magnification in five different fields of view for each slide according to Sufarnap et al. **[23]**. Kidney histopathology data was determined using a scoring method according to the modified Klopfleisch **[24]** scoring method. Changes and change scores in histopathological kidney preparations can be seen in the table. Meanwhile, liver histopathology scoring used the Brunt method **[25]** (Tables 1 and 2).

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4. Results

MDA levels in mice exposed to lead acetate (group T0) were higher ($p<0.05$) compared to mice exposed only to placebo (group C). Administration of Moringa leaf extract to mice exposed to lead acetate resulted in lower MDA levels ($p<0.05$) compared to mice exposed to lead acetate alone (T0). Rats given 500 mg/kg BW/day of Moringa leaf extract after being exposed to lead acetate for 20 days did not exhibit substantially different MDA levels ($p>0.05$) from normal rats (group C). The opposite happened to SOD levels. SOD levels in mice exposed to lead acetate (group T0) were lower ($p<0.05$) compared to mice exposed only to placebo (group C). Administration of Moringa leaf extract at doses of 200, 316, and 500 mg/kg BW/day for 20 days to rats exposed to lead acetate (groups T1, T2, and T3) was followed by higher SOD levels ($p<0.05$) compared to in rats exposed to lead acetate alone (T0). Rats exposed to lead acetate and treated with Moringa leaf extract at a dose of 500 mg/kg BW/day for 20 days still had lower SOD levels ($p<0.05$) than normal rats (group C) (Table 3).

Renal tissue degeneration and necrosis scores, and BUN levels in mice exposed to lead acetate (group T0) were higher ($p<0.05$) compared to mice exposed only to placebo (group C). Administration of Moringa leaf extract to rats exposed to lead acetate resulted in lower scores of kidney tissue degeneration and necrosis, and BUN levels ($p<0.05$) compared to rats exposed to lead acetate alone (T0) (Table 4). In rats exposed to lead acetate and treated with Moringa leaf extract at a dose of 500 mg/kg BW/day for 20 days, kidney tissue necrosis scores and BUN levels were not significantly different ($p>0.05$) compared to normal rats (C), however the kidney tissue degeneration score was still lower ($p<0.05$) compared to normal mice. Creatinine levels were not significantly different ($p>0.05$) between all treatment groups (Figures 1 and 2).

Hepatic tissue degeneration and necrosis scores, SGOT and SGPT levels in mice exposed to lead acetate (group T0) were higher ($p<0.05$) compared to mice only exposed to placebo (group C). Administration of Moringa leaf extract to rats exposed to lead acetate resulted in lower liver tissue degeneration and necrosis scores, SGOT and SGPT levels ($p<0.05$) compared to rats exposed to lead acetate alone (T0) (Table 5). Mice exposed to lead acetate and treated with Moringa leaf extract at a dose of 500 mg/kg BW/day for 20 days still had liver tissue degeneration and necrosis scores, SGOT and SGPT levels were lower ($p<0.05$) compared to normal mice.

The description of the count of the types of white blood cells in this study produces an irregular pattern. The profile of leukocytes, eosinophils, neutrophils, and lymphocytes in mice exposed to lead acetate (group T0) was not significantly different ($p<0.05$) compared to mice exposed only to placebo (group C). Differences identified ($p<0.05$) were in the number of basophil cells (higher) and the number of monocyte cells (lower in mice exposed to lead acetate (group T0) compared to mice only exposed to placebo (group C). Administration of Moringa leaf extract to rats exposed to lead acetate was followed by no change ($p>0.05$) in the number of eosinophils and lymphocytes. In rats exposed to lead acetate and treated with Moringa leaf extract at a dose of 500 mg/kg BW/ days for 20 days still had a higher number of leukocyte and monocyte cells ($p<0.05$), but the number of neutrophil cells was lower ($p<0.05$) compared to normal mice (Table 6).

5. Discussion

Recently, the use of naturally occurring therapeutic herbs has grown in importance in contemporary medicine. There is confidence in the usage of herbal products since they are readily accessible, socially acceptable, affordable, and safe. Thus, in both developed and developing nations, there is now a serious issue with the efficacy, safety, and quality of plant-based medications **[26]**. Antioxidants, flavonoids, amino acids, isothiocyanates, vitamins, alkaloids, carotenoids, glucosinolates, polyphenols, minerals, phenolic acids, tannins, and saponins are all abundant in Moringa **[14]**. Therefore, the use of natural components such as peptides, nano-fertilizers, bio-fertilizers in improving the growth and quality of Moringa trees is very important for the trend of using pure medicine throughout the world.

In this study there were real differences in malondialdehyde levels. Kidney and liver toxicity due to lead causes increased formation of free radicals reactive oxygen species (ROS) and oxidative stress (OS) **[27]**. As free radicals increase, cell membrane lipid peroxidation also increases, producing the final product in the form of malondialdehyde (MDA) **[28]**. Antioxidants are required to lessen damage brought on by free radicals. Catalase,

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superoxide dismutase (SOD), and glutathione peroxidase are examples of the naturally occurring antioxidant defense system enzymes in the body. Superoxide dismutase (SOD) has been described as the first line of defense against free radical chemicals based on earlier research **[29]**.

Administration of *M. oleifera* can suppress free radicals due to its antioxidant properties. The flavonoid activity in Moringa leaf extract, quercetin, has the potential to be an antioxidant because it can lower levels of free radical formation enzymes and stimulate internal antioxidant enzymes, thereby preventing and eliminating oxidative damage to target molecules **[12]**. Quercetin is a member of the flavonoid group that has the ability to seek for and sequester reactive oxygen species (ROS) and then chelate them by either donating an electron or transferring one **[30]**. The quercetin compound can also be used to chelate transition metal elements because flavonoids have chelating properties which are activated to bind metal ions in the human body such as chelating Fe^{2+} and Cu^{2+} metal ions which play an important role in the formation of free radicals **[31]**.

In the kidneys, this study showed real differences in Blood Urea Nitrogen (BUN) levels. The heavy metal lead that enters through the digestive tract will be distributed to tissues, one of which is the kidneys **[32]**. Lead in the kidney will form vacuolization of the proximal tubule cells, then a bulge (bleb) will form from the cytoplasm of the proximal tubule cells **[33]**. Next, the bleb ruptures so that the microphilia disappears. The bleb fragments will block the proximal tubule resulting in obstruction of the proximal tubule, this situation results in tubular necrosis **[34]**. In tubular necrosis due to nephrotoxicity, the kidneys are swollen, red, and vacuolization of the cytoplasm of the tubular epithelial cells is often found. Most damage occurs in the proximal tubule, rarely in the distal tubule. There is degeneration of the proximal tubule containing debris, but the basement membrane is intact **[34]**.

Administration of *M. oleifera* showed a protective effect on the kidneys due to lead toxicity. This shows that the content of Moringa leaf extract which is rich in antioxidants such as flavonoids, kaempferol, rhamnetin, quercitin, chlorogenic acid, routine, apigenin can prevent damage to rat kidney cells **[35]**. In addition, Moringa leaves have nephroprotective abilities in overcoming heavy metal exposure by improving kidney function and inhibiting oxidative stress and inflammation in kidney tissue **[36]**. The flavonoids in Moringa leaves can downregulate the formation of TGF-β and reduce the level of oxidative stress in kidney cells **[16]**. Apart from that, one of the flavonoids, namely icariside II, functions to increase endogenous EdU+ kidney progenitor cells **[37]**. Increasing progenitor cells can reduce oxidative stress and fibrosis thereby improving renal function **[38]**.

In the liver, this study did not show any real differences in SGOT and SGPT levels. The liver is an organ that contains a lot of metallothionein protein which can bind heavy metals, making it very susceptible to toxic effects **[2]**. This shows that the liver has a better ability to accumulate metals compared to meat, because the liver is a vital organ that functions as a detoxifier and secretes chemicals used for the digestive process **[39]**. The process of detoxifying heavy metals in the liver is through the process of binding metals (metallothionein) in the tissue. The liver's detoxification ability is relatively limited, so excessive heavy metals in the body will be distributed throughout the fish's body tissues through the blood vessels **[40]**. The lower the liver's ability to detoxify, the more heavy metal Pb will be distributed to other tissues **[41]**.

Administration of *M. oleifera* showed liver protective effects due to lead toxicity. Moringa leaves act as a hepatoprotector against acute liver injury that induces hepatotoxins. Moringa leaf extract is significantly able to reduce liver cells that experience necrosis and reduce SGOT and SGPT levels **[42]**. Moringa leaf extract can alleviate the incidence of hepatotoxicity due to APAP induction through anti-oxidative mechanisms and restore GSH levels and is rich in antioxidants due to the bioactive components contained therein **[43]**. The chemical compounds contained in Moringa leaf extract, especially flavonoids, can suppress the action of the CYP 450 enzyme isoform, inhibit oxidative stress, and regulate the expression of pro- and anti-inflammatory cytokines to reduce the occurrence of liver damage **[44]**.

In blood, this study showed real differences in basophil and neutrophil levels. Lead has a strong attraction for red blood cells. After being absorbed from the digestive tract, the heavy metal lead enters the blood circulation and more than 99% will bind to erythrocytes **[45]**. In erythrocytes, 80% of lead is found in the cell cytoplasm

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and the remaining 20% is found in the membrane **[46]**. Several factors such as high oxygen concentrations, auto-oxidation of Hb and the sensitivity of membrane components to lipid peroxidation cause erythrocytes to be sensitive to oxidative stress caused by lead **[47]**. Reactive Oxygen Species (ROS) can react and cause damage to many molecules in cells **[48]**. Phospholipids, which are the main elements in plasma membranes and cell organelle membranes, are often subject to lipid peroxidation **[28]**.

Administration of *M. oliefera* can return the blood profile to normal. Moringa leaf extract can reduce the accumulation of metals, especially lead, in cells due to the gallic acid content and the formation of chelation compounds by hydroxyl, carboxyl, amino and phosphate groups in Moringa leaf extract which occurs in yeast cells **[49]**.

Conclusion

Administration of *M. oleifera* shows a protective effect on the kidneys due to lead toxicity and can return the blood profile to normal.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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Table 1. Histopathological lesions were observed in the kidneys

Score	Degeneration of tubular epithelial cells of the renal cortex	Necrosis of renal cortex tubular epithelial cells
0	No degenerative changes occur	No necrotic changes occurred
1	If the number of degenerative cells is <25% of the visual field	-
2	If the number of degenerative cells is between 26-50% of the visual field	If the number of necrotic cells is <25% of the visual field
3	If the number of degenerative cells is between 51-75% of the visual field	-
4	If the number of degenerative cells is >76% of the visual field	If the number of necrotic cells is 26-50% of the visual field
5	-	-
6	-	If the number of necrotic cells is 51-75% of the visual field
7	-	-
8	-	If the number of necrotic cells is >76% of the visual field

Table 2. Numerical Scoring of Liver Biopsy Specimens

Score	Periportal +/— Bridging Necrosis	Intralobular Degeneration* and Focal Necrosis	Portal Inflammation	Fibrosis
0	None	None	No portal inflammation	No fibrosis
1	Mild piecemeal necrosis	Mild (acidophilic bodies, ballooning degeneration and scattered foci of hepa-tocellular necrosis in <1/3 of lobules or nodulles)	Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	Fibrous portal expansion
3	Moderate piecemeal necrosis (involves less than 50% of the circumference of most portal tracts)	Moderate (involvement of 1/3-2/3 of lobules or nodules)	Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	Bridging fibrosis (portal-portal or portal-central linkage)
4	Marked piecemeal necrosis (involves more than 50% of the circumference of most portal tracts)	Marked (involvement of >2/3 of lobules or nodules)	Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	Cirrhosis†
5	Moderate piecemeal necrosis plus bridging necrosis‡	-	-	-
6	Marked piecemeal necrosis plus bridging necrosis‡	-	-	-
10	Multilobular necrosis§	-	-	-

Note: Score is the combined scores for necrosis, inflammation, and fibrosis.

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*Degeneration—acidophil bodies, ballooning; focal necrosis-scattered foci of hepatocellular necrosis; †Loss of normal hepatic lobular architecture with fibrous septae separating and surrounding nodules.
‡Bridging is defined as ≥2 bridges in the liver biopsy specimen; no distinction is made between portal-portal and portal-central linkage.
§Two or more contiguous lobules with panlobular necrosis. Modified and reprinted with permission.

Table 3. Malondialdehyde (MDA) and superoxide dismutase (SOD) in blood

Group	MDA	SOD
C	5.36±0.47^{a}	84.11±2.30^{e}
T0	10.53±1.58^{d}	25.97±1.31^{a}
T1	9.59±1.38^{c}	39.93±2.04^{b}
T2	7.55±0.98^{ab}	61.71±3.62^{c}
T3	6.25±0.51^{a}	73.58±1.66^{d}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$)

Table 4. Renal degeneration, necrosis and metabolite scores

Group	Degeneration	Necrosis	BUN	Creatinin
C	0.48±0.11^{a}	0.68±0.23^{a}	16.30±3.42^{a}	0.28±0.06^{a}
T0	1.76±0.26^{c}	3.40±0.62^{c}	19.95±1.40^{b}	0.31±0.06^{a}
T1	1.36±0.17^{c}	1.88±0.63^{b}	14.80±1.30^{a}	0.24±0.04^{a}
T2	0.88±0.11^{b}	0.96±0.22^{a}	17.88±0.72^{ab}	0.28±0.05^{a}
T3	0.52±0.11^{a}	0.64±0.26^{a}	18.34±2.92^{ab}	0.27±0.04^{a}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$)

Table 5. Hepatic degeneration, necrosis and metabolite scores

Group	Degeneration	Necrosis	SGOT	SGPT
C	0.44±0.09^{a}	0.28±0.11^{a}	118.96±23.45^{a}	49.48±6.92^{a}
T0	3.04±0.22^{e}	2.52±0.39^{e}	158.84±9.72^{b}	89.48±7.31^{c}
T1	2.64±0.17^{d}	1.96±0.26^{d}	157.20±21.16^{b}	73.00±6.04^{b}
T2	2.12±0.18^{c}	1.44±0.26^{c}	187.20±31.35^{b}	75.92±9.28^{b}
T3	1.16±0.09^{b}	0.92±0.11^{b}	190.68±23.84^{b}	89.13±8.34^{c}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$)

Table 6. Blood profile

Group	Leukocytes	Eosinophils	Basophils	Neutrophils	Lymphocytes	Monocytes
C	5.88±0.36^{ab}	3.60±0.55^{a}	0.20±0.45^{a}	19.40±3.13^{c}	71.80±2.49^{a}	8.20±1.79^{b}
T0	6.90±0.47^{b}	4.40±1.52^{a}	1.20±0.45^{b}	21.40±4.16^{c}	70.40±2.19^{a}	3.40±1.82^{a}
T1	4.62±0.47^{a}	4.00±1.73^{a}	0.60±0.55^{ab}	15.20±3.27^{ab}	72.40±2.19^{a}	15.60±4.56^{c}
T2	11.54±2.11^{c}	3.00±1.22^{a}	1.40±0.55^{b}	18.60±2.61^{bc}	71.80±2.17^{a}	9.20±3.70^{b}
T3	20.24±1.05^{d}	2.80±1.92^{a}	1.00±0.71^{ab}	*12.00±3.16^{a}*	72.80±2.39^{a}	15.60±5.13^{c}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$)

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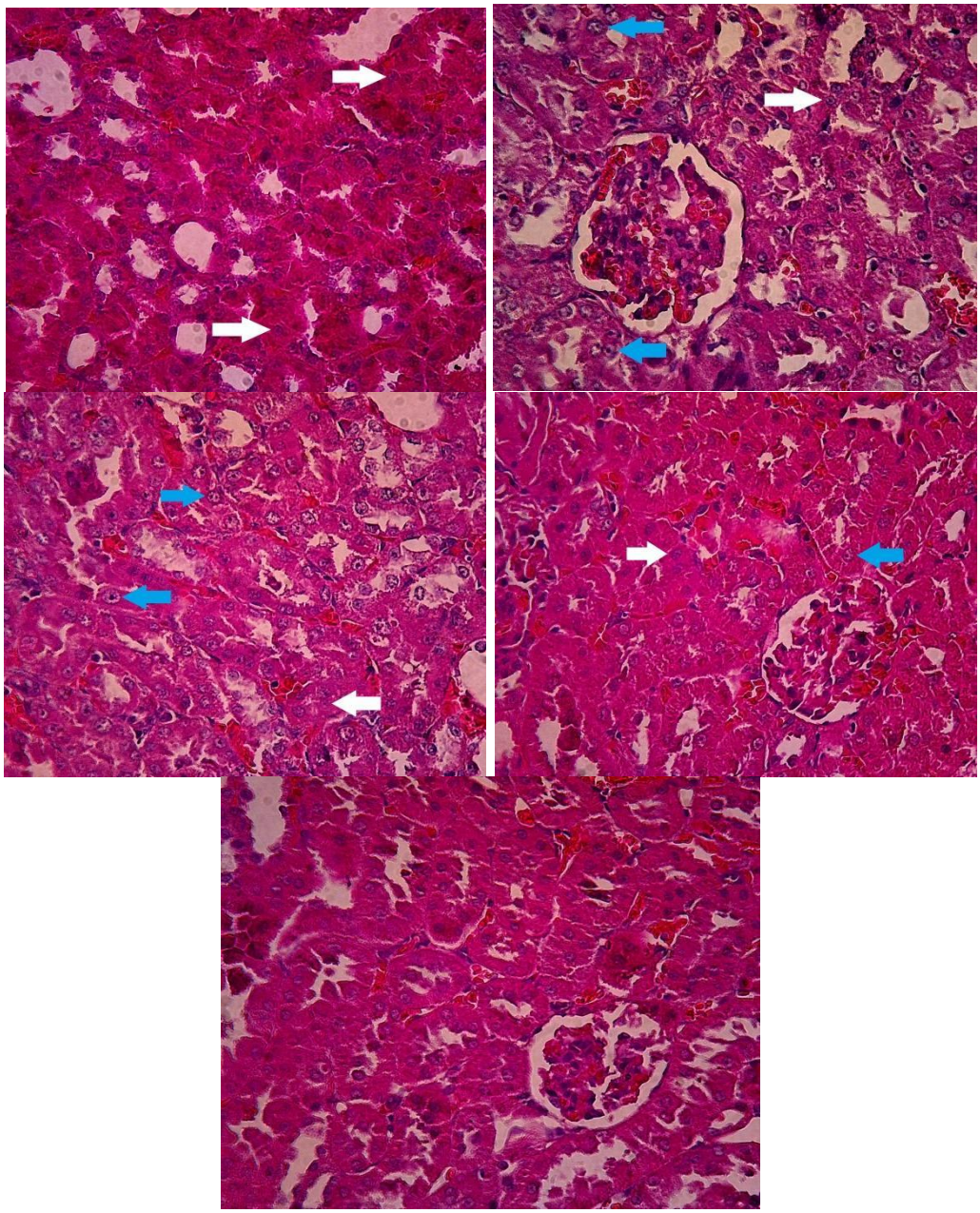

Figure 1. Histopathology of kidney cell degeneration of white rats (*Rattus norvegicus*), 400x magnification, HE staining; White arrow: normal kidney cell nuclei; Blue arrows: degenerated kidney cell nuclei.

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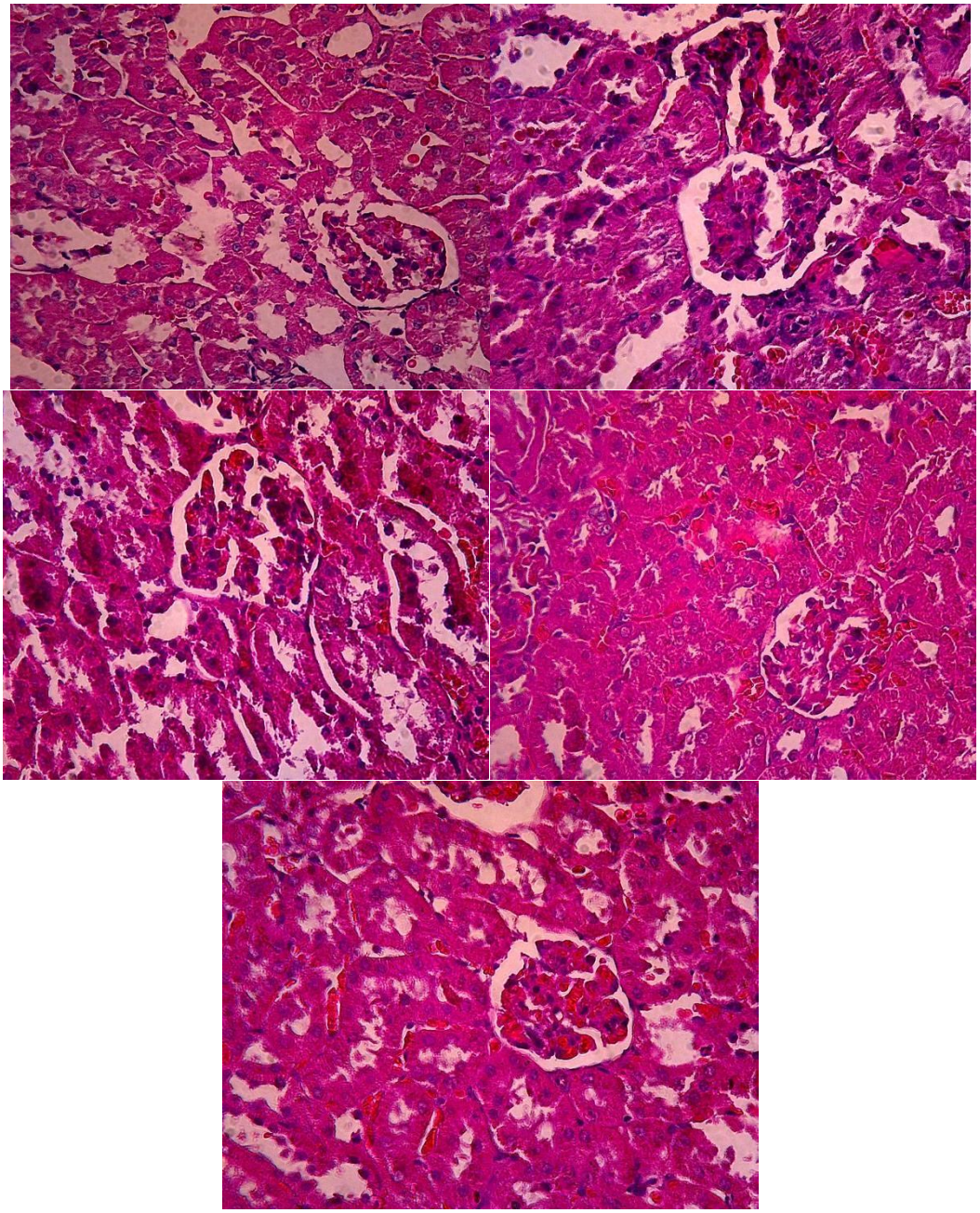

Figure 2. Histopathology of kidney cell necrosis of white rats (*Rattus norvegicus*), 400x magnification, HE staining; White arrow: normal kidney cell nuclei, yellow arrow: pyknotic kidney cell nuclei, green arrow: karyorrhexic kidney cell nuclei, blue arrow: karyolysis kidney cell nuclei.