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Original Research Article

EFFECT OF LEAD ACETATE TOXICITY ON THE HISTOLOGICAL AND BIOCHEMICAL CHANGES IN LIVER AND KIDNEY OF FISH ROHU (*LABEO ROHITA*)

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Abstract *The objective of this study was to investigate the histological alterations in the kidneys and liver of the fish known as Rohu (Labeo rohita). For seven days, 100 L. rohita fingerlings were kept in a glass aquarium to acclimate. The fingerlings were split into four groups, with 25 fingerlings in each group. Lead acetate was created in four concentrations: Nil; 1/5th LC50 (6.86 ppm); 1/10th LC50 (3.43 ppm); and 1/20th LC50 of 1.71 ppm. These concentrations were then randomly assigned to 4 groups: 1; (control); 2; 3 and 4 in that order. After 10, 20, 30, and 40-days fillets were dissected and the Liver and kidney were separated for histopathology analysis. The major histopathological changes observed in the liver were melano-macrophage canters, leukocyte infiltration, cytoplasmic degeneration, and pyknosis, while the changes in the kidney were renal tubule atrophy, aggregation of inflammatory cells, loss of cellular integrity, renal tubule degeneration, uneven renal tubule diameters, and few necrotic regions. The fingerlings in G-2, which received a higher dose of lead acetate during the fourth week of the research, had the most lesions. As the concentration of lead acetate dropped during this time, as it did in the fingerlings of G-3 and G-4, fewer lesions were observed in the liver and kidneys. However, compared to the fourth week of the trial, fewer lesions were seen in the kidneys and liver in the first, second, and third weeks. The fingerlings' protein and moisture contents demonstrated a declining tendency as compared to the group-1 (control) (1.75 vs. 2.20), and the differences in the protein contents were not statistically significant ($P<0.05$). In a similar vein, the liver and kidneys' moisture contents trended downward when compared to the control group. Overall, the study's findings showed that greater concentrations of lead acetate had a negative impact on the kidneys and liver of the fish Rohu; as a result, it is important to limit fish exposure to this chemical in order to maintain the fishes' overall health.*

Keywords: *Labeo rohita; Lead acetate; Fingerlings; concentration; Liver; Kidneys; Toxicity*

Introduction

Heavy metals pollution is a serious threat to aquatic organisms and the environment if the concentration of these metals is higher than permitted levels. Heavy metals are hazardous to fish population because they are not biodegradable and have a lengthy environmental persistence. Lead has the potential to be harmful to aquatic animals. Polluted environments cause fish to accumulate lead, which is then spread throughout their tissues. Whether the lead is consumed by food or is present in water, the amount of lead that accumulates in various tissues varies regardless of the method of exposure, lead builds up substantially more in the kidneys, liver, and gills of fishes. South Asian rivers are the homes of fish rui or rohu (*Labeo rohita*); the species of common carp. It is a large omnivore that is used extensively in the aquaculture industry and is thought to be an important fish for the economy. The negative effects of lead on -fish must be evaluated because they are a significant part of aquatic ecosystems and a significant source of white meat.

Heavy metals, such as lead acetate, affect fish physiology, and immunological parameters particularly enzymes, hormones, hemato-biochemical properties, and histopathology of various organs (Shahjahan et al., 2022). All of these factors have been recognized as crucial bio-monitoring means for assessing the toxicity of heavy metals since they are all considerably altered by the exposure of these metals. Major fish organs, including the gills, liver, and kidneys, showed a variety of pathologies in these organs in both acute and chronic heavy metal exposure.

High amounts of toxic minerals and chemicals in water pollution have been an important issue both for the environment and public health. The water bodies have recently become heavily polluted with dangerous levels of many contaminants severely

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harming both aquatic and human life (Mustafa, Al-Rudainy and Al-Samawi,2020). Heavy metal pollution is extremely toxic both for the fish population and human tissues. Heavy metal accumulation harms the muscles, kidneys, gills, liver, and other tissues of fish, which can be studied through histopathology.

Research design and methodology

Selection of the Samples

This study included one hundred (100) similarly sized fish fillets of the Rohu (Labeo *rohita*) species, that had been from the Fisheries and Training Institute Complex Manawan Lahore. The experiment was conducted in the School of Zoology laboratory of Minhaj University Lahore. The fish fillets were acclimated for seven days in a glass aquarium that held twenty-four liters of the non-chlorinated tap water. The fingerlings were fed a lead-free food at a daily rate of three percent of their body weight during this time, along with proper aeration. The fish fillets were stopped (24) hours before the experiment began and the water of the aquarium was changed so that there were no leftovers, before putting fish fingerlings in them. The aquarium water parameters which were maintained in all four aquariums and the results of water analysis obtained from the water testing laboratory in Manawa, Lahore are shown in table 1.

Table 1 showing the water parameters and the results of water analysis.

Parameters	Recommended range	Results
Temperature	18- 35ºC	23.2 ºC
Ph	6.5- 8.7	7.7
Total alkalinity	50- 400 mg/l	284 mg/l
Total hardness	50- 400 mg/l	251.6 mg/l
Chloride	10- 400 mg/l	140 mg/l
Electrical conductivity	Up to 1650 µS/cm	745 µS/cm
Free CO_2	0- 15 mg/l	11 mg/l

The stock solution of the lead acetate was prepared. The lethal dose of lead acetate was determined using the method outlined by Prabha and Rajkumar (2016). The catastrophic concentration of the lead acetate was 34.40 ppm for the 96 hours. Considering the persistent lethal concentration of the lead acetate; all fillets were divided into 4 groups (G-1 (control), G-2, G-3, and G-4); each with 25 fillets. These groups were randomly assigned four different concentrations of lead acetate (0; 1/5th; 1/10th; and 1/20th of LC50). The fingerlings in each of the 4 groups were then kept in four glass aquariums to conduct the experiment for next 40 days. Four different groups and their concentrations used for experiment is shown at Table-2

Table 2 displaying the groups and designated concentration of the lead acetate (LC_{50})

Groups	Concentration of lead Acetate (LC_{50})
Group1 (G-1) (Control)	No lead acetate
Group 2 (G-2)	1/5th LC_{50} (6.86 ppm) of the Lead acetate
Group 3 (G-3)	1/10th LC_{50} (3.43 ppm) of the Lead acetate
Group 4 (G-4)	1/20th LC_{50} (1.71 ppm) of the Lead acetate

During 40 days of the trial, fingerlings of all four groups received their usual fish feed at a daily rate of 3 percent of the body weight. Working solution was prepared for each group by putting stock solution of the lead acetate of the lethal concentrations of 0/0 (00); 1/5th (6.86ppm); 1/10th (3.43ppm); and 1/20th (1.71ppm) for all 4 groups in the experiment.

Dissection of the Fish Samples

After 10th; 20th; 30th and 40th days of the experiment 3 fish fillets from every group were removed from the tank and then placed in sealed jars to be dissected to examine the histopathology of the kidneys and liver.

Histopathological Study

Liver and kidney tissues were removed from both the exposed and control fish fingerlings, and they were fixatively stored for 48 hours in a 10% formaldehyde solution. Tissues were processed using the method of Bernet et al. (1999) for histological investigations. Tissues were, in short, dehydrated in various gradations of ethanol alcohol, cleared in xylene, and then embedded in paraffin wax. Following that, a rotary microtome was used to cut the sagittal segments (6 µm thick), which were then placed on glass slides. For general histological analyses, segments were deparaffinized in xylene; hydrated in alcohol, and stained with hematoxylin & eosin (HE). Under a light microscope, photomicrographs of stained slices were taken. Images of treatment-induced alterations in the liver and kidney tissues were captured and examined with a 400X total magnification using an Optika digital camera.

Biochemical analysis

Protein samples were computed using the method outlined by Lowry et al., 1951, and the fillets underwent dissection from both control & experimental groups on the 10th; 20th; 30th and 40th days of exposure period before being analyzed for the biochemical alterations.

Results and Discussion

Under a microscope, the prepared slides were examined, and a 400X lens was used to take a

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photomicrograph of the slides to get their results on effect of lead acetate in liver and kidney.

Liver

After 1st period of 10 days inflammation necrosis with the nuclear piknosis and patchy degeneration with 6.86 ppm of lead acetate; disarrangement of the hepatic cord and patchy degeneration with 3.43 ppm of lead acetate & patchy degeneration with 1.71 ppm of lead acetate were examined. After 2nd period of 20 days inflammation disarrangement of hepatic cords & damage of hepatopancreas characterized by the loss of contact b/w pancerocyte & hepatocyte with 6.86 ppm of lead acetate; degeneration of the macrocytes & cytoplasmic vacuolation with 3.43 ppm of lead acetate; patchy degeneration & necrosis with the nuclear pyknosis with 1.71 ppm of lead acetate were observed. (The photomicrographs of lesions observed in the liver during these periods are shown at figures 1-2)

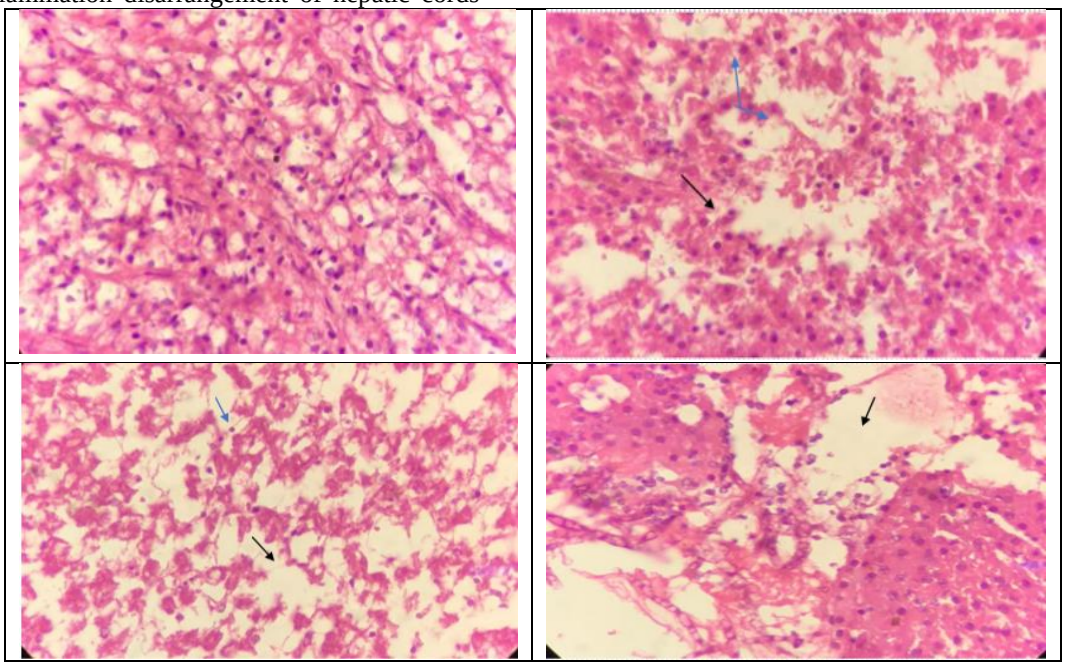

Figure 1 photomicrograph of the liver sections of L. rohita after 10 days with 0, 6.83 ppm, 3.433 ppm, 1.71 ppm lead acetate effect respectively

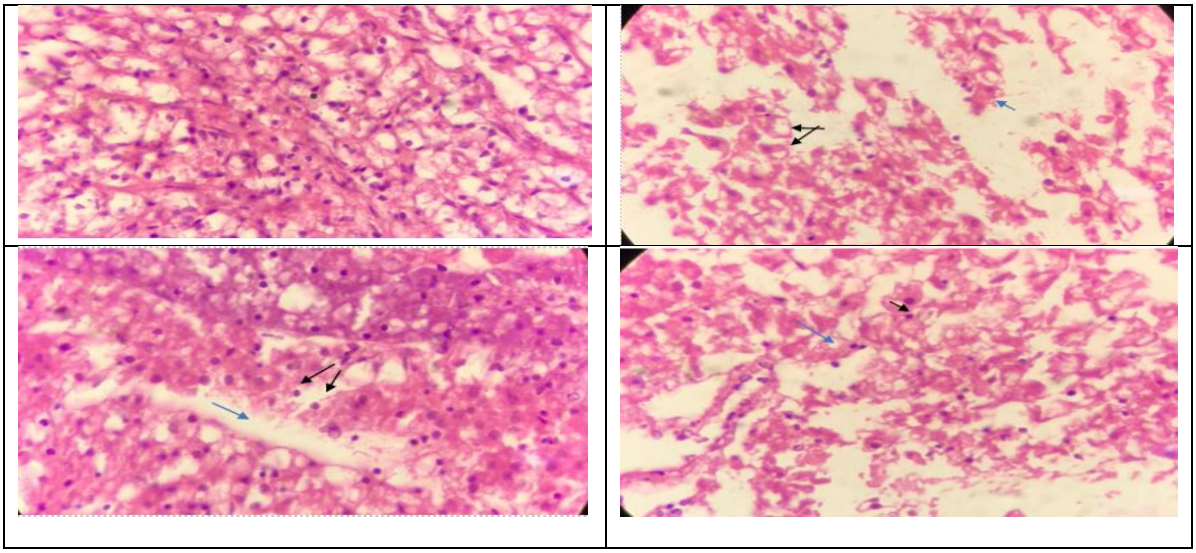

Figure 2 photomicrograph of the liver sections of L. rohita after 20 days with 0, 6.83 ppm, 3.433 ppm, 1.71 ppm lead acetate effect respectively

After 3rd period of 30 days inflammation intravascular haemolysis in the hepatoportal blood vessels, hepatocyte hypertrophy in the liver & melano macrophages aggregation with 6.86 ppm of lead acetate; cellular degeneration, congestion & dilation in sinusoids with 3.23 ppm of lead acetate; patchy degeneration & intravascular haemolysis in the hepatoportal blood vessels with 1.71ppm of lead

acetate were observed. After 4th period of 40 days inflammation congestion and dilation in sinusoids, in hepatocytes cytoplasmic vacuolation occurs & pancreocytes with 6.86 ppm of lead acetate; Milano microphages aggregation, cytoplasmic vacuolation in the hepatocytes & hepatocyte hypertrophy with 3.43 ppm of lead acetate; hepatopancreas distraction characterized by the loss of interaction b/w pancerocyte and hepatocyte & necrosis with 1.71 ppm of lead acetate were observed in liver of fish *L. rohita*. (The photomicrographs of lesions observed in liver during these periods are shown at figures 3-4).

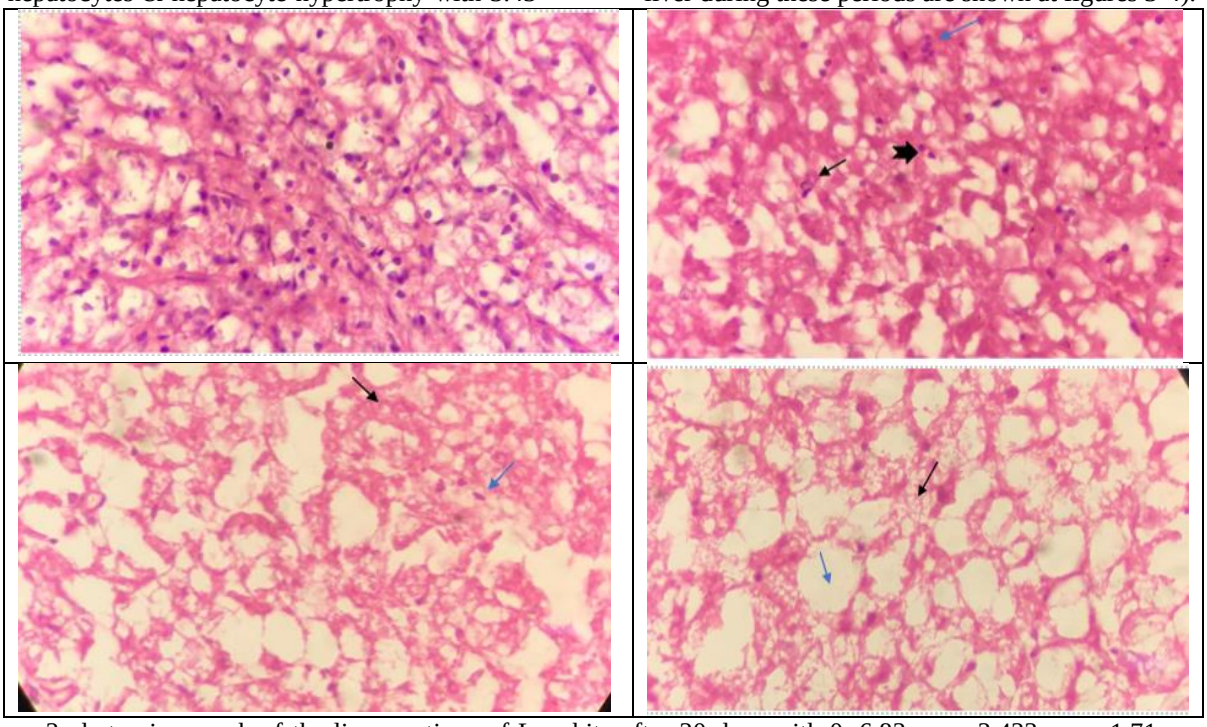

Figure 3 photomicrograph of the liver sections of L. rohita after 30 days with 0, 6.83 ppm, 3.433 ppm, 1.71 ppm lead acetate effect respectively

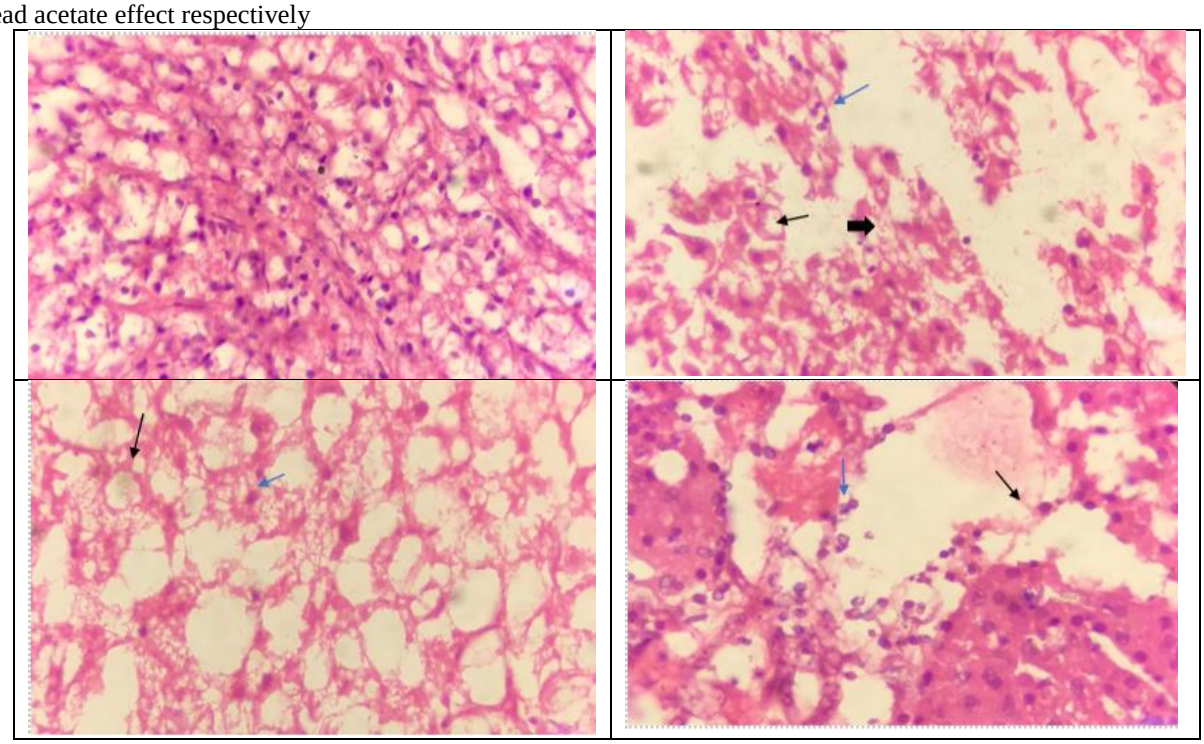

Figure 4 photomicrograph of the liver sections of L. rohita after 40 days with 0, 6.83 ppm, 3.433 ppm, 1.71 ppm lead acetate effect respectively

Kidneys

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After 1st period of 10 days inflammation of tubular hemorrhage, congestion of blood cells, and vacuolated deterioration with 6.86 ppm of the lead acetate; de-shaped and edema in the renal tubule and aggregation of the inflammatory cells with 3.43 ppm of the lead acetate & amplification of Bowmans capsule and the distortion of the renal tubule with 1.71 ppm of lead acetate were observed. After 2nd period of 20 days inflammation kidney edema and loosing of the haemopioetic tissues with 6.86 ppm of lead acetate; tubular atrophy, necrosis and de-shaped of the Bowmans space with 3.43 ppm of the lead acetate; shrinkage of Bowmans space, vacuolated degeneration and mild necrosis with 1.71 ppm of the lead acetate were examined in kidney sections of fish *L. rohita.* (The photomicrographs of lesions observed in liver during these periods are shown at figures 5-6)

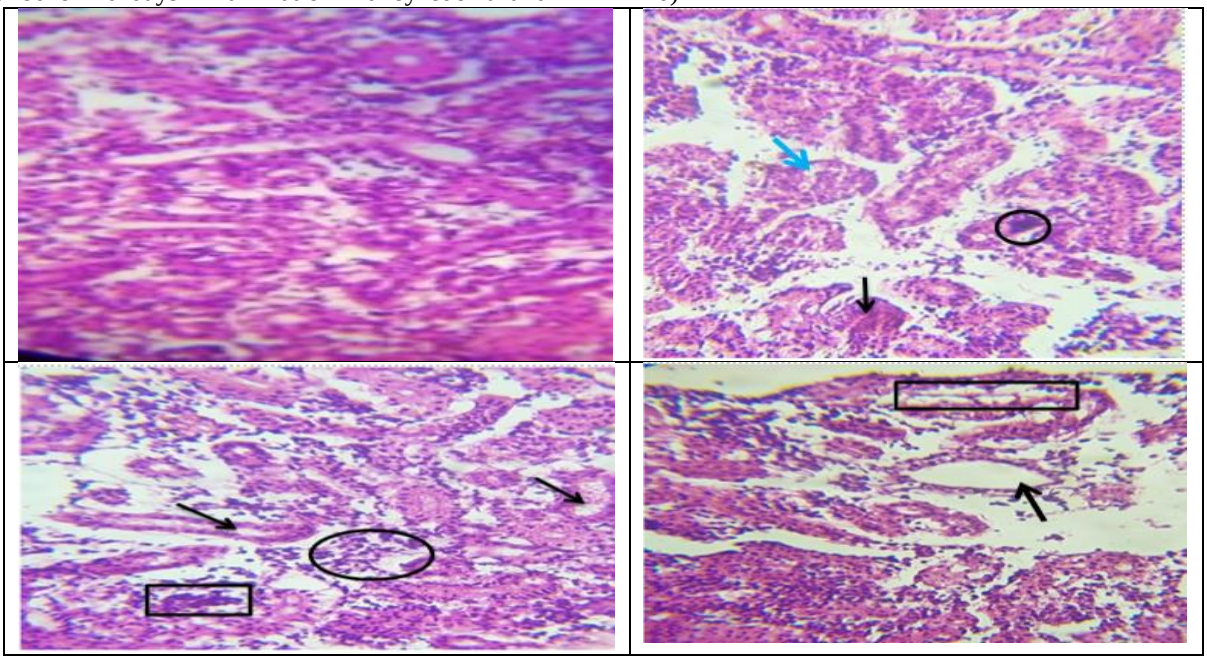

Figure 5 photomicrograph of kidney sections of L. rohita after 10 days with 0,6.83ppm, 3.433ppm, 1.71ppm lead acetate effect respectively

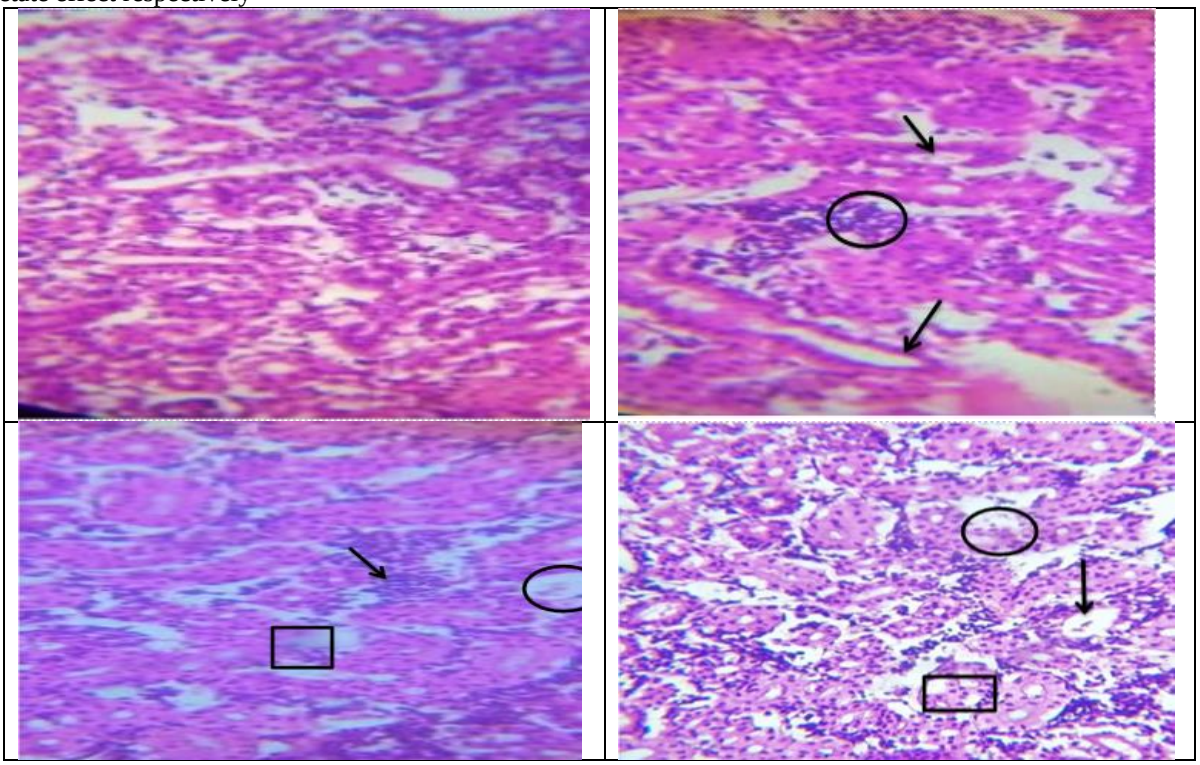

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Figure 6 photomicrograph of kidney sections of L. rohita after 20 days with 0,6.83ppm, 3.433ppm, 1.71ppm lead acetate effect respectively

After 3rd period of 30 days inflammation regenerating of kidney tissues and distortion in renal tubules with 6.86 ppm of the lead acetate; damaged the glomerulus and destruction in collecting ducts with 3.43 ppm of the lead acetate; renal tubules hemorrhages and contraction in Bowmans space with 1.71 ppm of the lead acetate were noticed. After 4th period of 40 days inflammation complete deterioration of the kidney tissues with 6.86 ppm of the lead acetate; renal tubules congestion & irregular structure and dead cells of the kidney with 3.43 ppm of the lead acetate meanwhile atrophy in renal tubules, contraction of Bowmans space and vacuolated degeneration with 1.71 ppm of the lead acetate were observed in kidney sections of fish *L. rohita*. (The photomicrographs of lesions observed in kidney during these periods are shown at figures 7-8)

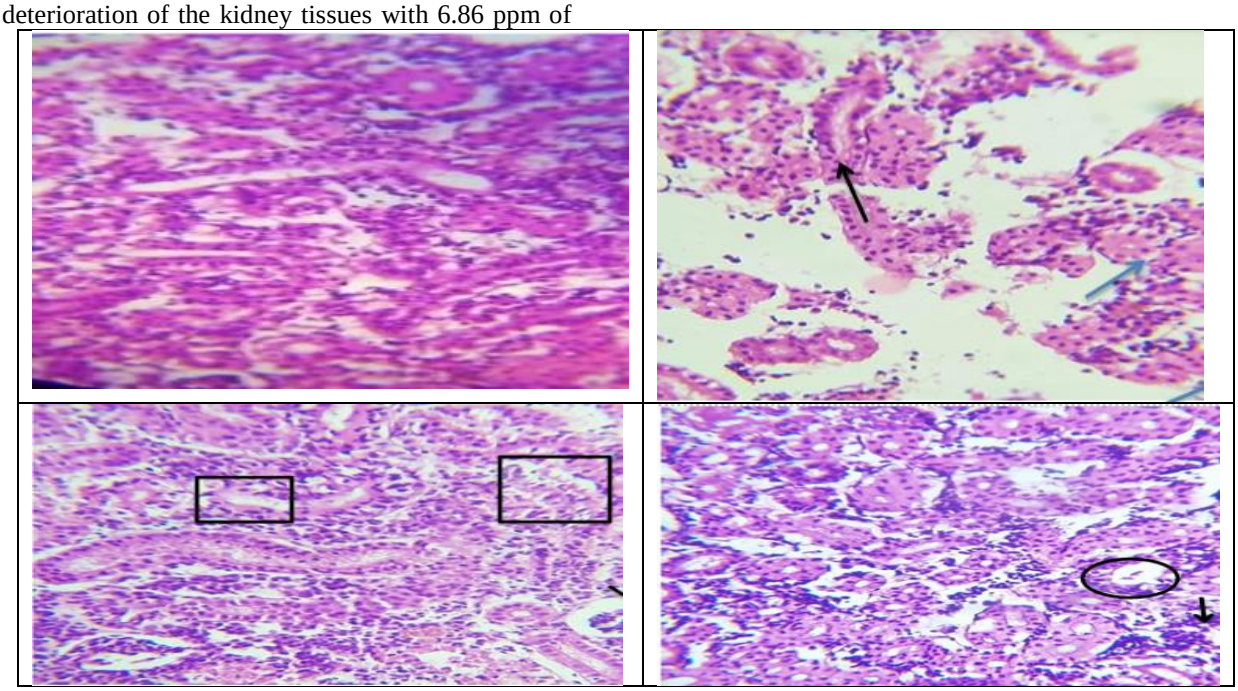

Figure 7 photomicrograph of kidney sections of L. rohita after 30 days with 0,6.83ppm, 3.433ppm, 1.71ppm lead acetate effect respectively

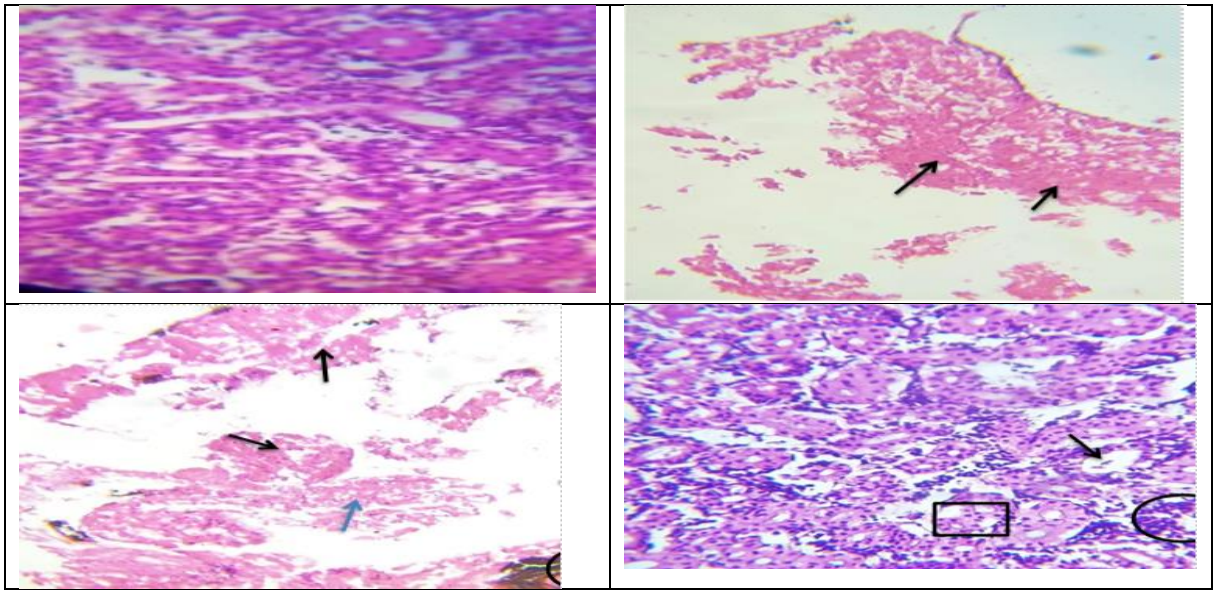

Figure-8 photomicrograph of kidney sections of L. *rohita* after 40 days with 0,6.83ppm, 3.433ppm, 1.71ppm lead acetate effect respectively

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Biochemical Changes (protein) in Liver

Table-3 Shows the variations in level of protein activity in liver sections of fish *L.rohita* on the effect of the lead acetate

Groups	Concentrations of Lead Acetate (LC50)	Moisture (%)	Crude Protein (%)
Group-1 (Control)	No lead acetate	0.085	2.20
Group-2	$1/5^{th}$ LC_{50} (6.86 ppm) of the lead Acetate	0.035	1.75
Group-3	$1/10^{th}$ LC_{50} (3.43 ppm) of the lead Acetate	0.055	1.98
Group-4	$1/20^{th}$ LC_{50} (1.71 ppm) of the lead Acetate	0.075	2.20

Biochemical Changes (protein) in the Kidney

Table-4 Shows the variations in level of protein activity in kidney sections of fish *L.rohita* on the effect of the lead acetate

Groups	Concentrations of Lead Acetate (LC50)	Moisture (%)	Crude Protein (%)
Group-1 (Control)	No lead Acetate	1.000	2.20
Group-2	$1/5^{th}$ LC_{50} (6.86 ppm) of the lead Acetate	0.300	1.75
Group-3	$1/10^{th}$ LC_{50} (3.43 ppm) of the lead Acetate	0.800	1.98
Group-4	$1/20^{th}$ LC_{50} (1.71 ppm) of the lead Acetate	0.900	2.20

The carp species known as Rohu Labeo, rohu, or rui (Labeo rohita), are found in rivers in South Asia. The most significant carp among the three primary carp found in the Indus River is the Rohu (Labeo rohita). It belongs to the family Cyprinidae. It is a large omnivore that is considered an important fish in ecology and is widely used in aquaculture. The rohu fish is the most popular in South Asia due to its high-quality meat and resilience in harsh environments. (Awoyemi et al., 2014).

The study examined histological alterations in the liver and kidneys at short periods of 10, 20, 30, and 40 days. Findings on these changes were found to be comparable to those of Mustafa, et al., (2017). They looked into histopathological alterations in the kidney and liver tissues of Cyprinus carpio fish subjected to varying lead acetate concentrations for sixty days. The primary histological changes brought on by lead acetate in the liver include hepatic necrosis with nuclear piknosis and cytoplasmic vacuolation. More tissue deterioration was seen in the kidneys, including renal tubule necrosis with piknotic nucleus and hydrobic dystrophy.

This study showed that the single metal lead acetate had harmful effects on the liver of the fish Labeo rohita, causing alterations and degeneration in the liver. The findings of this study also supported those of Doaa and Hanan (2013). They looked at the effects of the sublethal lead acetate concentration on particular tilapia sections that were subjected to 0.4 & 0.7 mg of lead acetate per liter of water. They noticed histological alterations in the liver, such as hepatocyte vacuolar degeneration and hepatic sinusoidal dilatation.

Naz et al., (2021) evaluate the hematological and histological alterations in the major carp (Catla catla) exposed to varying levels of cadmium (Cd) and copper (Cu). They examined histopathology of liver tissues revealed hemorrhages, congestion, hepatic cell degeneration, and karyorrhexis and identified histological changes in the kidney including melanomacrophage aggregates, enlarged urinary spaces, necrosis of renal tubular cells, atrophy of glomeruli, and degeneration of renal tubules. They conclude that longer-term exposure to Cu and Cd results in detrimental hematological and histological alterations in Catla fish. The current study's findings involving liver and kidney tissue lesions at varying lead acetate dosages were remarkably similar.

Sharma et al., (2013) noted that Cu, Ni, and Pb had detrimental effects on the gills, kidney, liver, and muscles of fish species Nile tilapia (Oreochromis niloticus). They looked at cytoplasmic vacuolization, hepatic capillary expansion, and swelling of the Bowman's capsule gap as histological abnormalities in the kidney. The liver and kidney tissues of fish Rahu had nearly identical alterations, according to the study's findings. They saw many changes in the kidney, including necrosis, damage, and distortion of the renal tubules, expansion of the Bowmen capsule space, destruction and loss of hemopoietic structures, and constriction of the glomerulus. The current study's findings involving liver and kidney tissue lesions at varying lead acetate dosages were remarkably similar.

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Bangeppagari and Gooty (2014) investigated the biochemical changes caused by exposure to lead acetate in the liver of Cyprinus carpio fish. Comparing the protein level to the control group, they saw a declining trend. The current study's findings revealed nearly identical findings on the liver of the fish Rohu. Nayak, Sharma, Nayak, Pradhan, Nayak, and Patnaik, (2023) studied sublethal toxicity of the lead acetate on fish species *Anabas testudineus*. They exposed fish to different concentrations of the heavy metal lead acetate which are 1.291, 1.936, and 3.873 mg/L for 96 hour to observe acute toxicity. They also investigated the biochemical parameters such as protein and glycogen for assessment of lead acetate toxicity and observed that protein content decreased in liver tissues of experimental groups in comparison with the control group. Findings of a recent study showed nearby similar observations regarding liver of fish Rohu.

Conclusion

The purpose of this investigation was to examine the histological alterations in the liver and kidneys of the fish Rohu (*Labeo rohita* L). One hundred *L. rohita* fingerlings were distributed into four groups, with twenty-five fingerlings in each group, and were allowed to acclimate in the glass aquarium for seven days. Four lead acetate concentrations as Nil, 1/5th LC50 (6.86 ppm), 1/10th LC50 (3.43 ppm), and 1/20th LC50 of 1.71 ppm were made and distributed at random to groups 1, 2, 3, and 4, in that order. The fingerlings in group 2, which received a greater concentration of lead acetate during the fourth week of the trial, had the greatest number of lesions. As the concentration of lead acetate in the fingerlings of groups 3 and 4 dropped throughout this time, fewer lesions were observed in the liver and kidneys. However, compared to the fourth week of the trial, fewer lesions were seen in the kidneys and liver in the first, second, and third weeks. The fingerlings' protein and moisture contents showed a declining tendency as compared to the control group (1.75 vs. 2.20), and there was no significant ($P<0.05$) difference in the protein contents of the kidneys or liver between treatments. In a similar vein, the liver and kidneys' moisture contents trended downward when compared to the control group. Overall, the study's findings showed that higher concentrations of lead acetate harmed the fish's liver and kidneys; as a result, exposure to this chemical must be avoided to maintain the fishes' overall health.

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

Funding Statement

Not applicable

Conflict of Interest

There is no conflict of interest among the authors regarding this case study.

Authors Contribution

All authors contributed equally.