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Pendimethalin Toxicity: Histopathological Insights into Liver and Renal Damage in Cyprinus carpio

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Abstract

The extensive use of herbicides creates worries about their effects on nontarget aquatic creatures like fish. The ultimate purpose of this study was to assess the toxicity of a widely accessible herbicide pendimethalin on the histology of the kidney and liver of *Cyprinus carpio*, an economically beneficial fish. Acute toxicity was determined with a 96-hour LC50 value of 2.20µL/L. Fishes were divided into 3 groups: The primary group was designated the control group. The second group received a 1/10th concentration of the estimated 96-hour LC50 (0.22µl/L), whereas the third group received a 1/15th concentration of the 96-hour LC50 (0.146µl/L). Control fish kidneys and liver do not show any morphological alterations in structure. The second group exposed to pendimethalin showed severe damage to the kidney and liver such as glomerular degeneration, rupturing of proximal convoluted tubules, damaged distal convoluted tubules and internal bleeding in the kidney and vacuolization, internal bleeding, binucleate formation and degeneration of hepatocytes in the liver. The third group showed mild to moderate changes with an increase in the concentration of pendimethalin in kidney and liver tissue such as low to mild glomerular degeneration, ruptured proximal tubules and vacuolization in some areas in the kidney and damaged hepatocytes, mild to high necrosis of nuclei and damaged cytoplasm in the liver. These findings highlight the potential dangers of pendimethalin contamination in aquatic environments, underscoring the need for stringent regulatory measures.

Keywords: Pendimethalin, *C. carpio*, Liver, Kidney, Histopathology.

INTRODUCTION

Pesticides belong to the group of strong chemicals that pollute aquatic environments. Numerous kinds of anthropogenic and natural processes take these toxicants into the aquatic ecosystem which will ultimately have extremely detrimental effects on aquatic ecosystems and living things (Rohani Md. Fazle., 2023). Pesticide-contaminated water bodies can harm fish either directly or indirectly by elevating the quantity of pollutants in fish tissues and lowering the growth, pace of development, reproduction, and survivability. Because of their widespread use in agriculture and homes, herbicides are frequently found in aquatic habitats where they can damage aquatic life once they find their way into water systems. They reach water bodies by several channels, including agricultural field water drainage, groundwater leaching, spraying, and direct application. Fish often absorb it by their skin or gills during respiration (Ihsan *et al.,* 2018).

Pendimethalin is a chemical compound that belongs to the dinitroaniline family and is used on crops such as corn, rice, potatoes, soybeans, peanuts, carrots, onion, apple, citrus fruits, wheat, cabbage and maize (Ivantsova and Martyniuk, 2024). According to the Herbicide Resistance Action Committee (HRAC), pendimethalin falls under the K1 group of herbicides based on its mechanism of action. The major form of pendimethalin action is to limit both cell division and elongation in sensitive weeds, hence affecting root and shoot development (USEPA, 2009).

Fish are commonly used as observatory organisms to detect harmful chemicals due to their ability to respond to small amounts of hazardous toxicants and quantify the biological effects of toxins and the quality of the environment (Ayas *et al.,* 2007). *Cyprinus carpio*, a member of the Cyprinidae family, order: Cypriniformes and class: Osteichthyes, is one of the world's extensively disseminated and commercially important freshwater fish. Because of its adaptability for aquaculture, such as rapid growth, durable nature, the market's needs, and so on, it has become a crucial species for freshwater aquaculture in the nation at large (Mahanta, 2010). Histology investigates the organization and arrangement of cells and tissues within an organ or structure, which aids in understanding their role in overall organ function. The liver and kidneys are the major organs in the fish body that perform several functions. Hepatocytes, the most prevalent cell type of liver, perform the majority of the body's vital tasks, including metabolism, removal of toxins, storage, and expulsion of xenobiotics, lipid regulation and amino acid deamination. whereas the kidney helps remove excess water and toxins through osmoregulation and a counter-current system (Kobayashi *et al.,* 2019). The current study examined pendimethalin's toxic effect in *C. carpio* by histopathological investigation of the liver and kidney.

2. Methods and Protocols

2.1 Test Chemical

The test compound pendimethalin of analytical grade (30%EC) was purchased from the local market of Shimla under the brand name pendimol. To prepare the test chemical's stock solution, 1 ml of pendimethalin was dissolved in 100 mL of distilled water. The experiments were carried out at Himachal Pradesh University Shimla, Himachal Pradesh.

2.2 Dietary Formulation

During the acclimation phase, the fish were provided with a commercial pellet feed that consisted of wheat flour, soybean, corn, yeast and vitamins. This feed had a nutritional profile with protein content of at least 30%, fat content of approximately 4%, crude fiber of at least 4%, and crude ash not exceeding 15%.

2.3 Test Organism

Fish (*C. carpio*) were transported to the laboratory in aerated polythene bags from the Fish and Breeding Farm at Deoli, Ghaggus. Then they were disinfected with 0.1% potassium permanganate for two to three minutes. Following that, the fish were placed in 1000 L plastic pools for 15 days to acclimate to laboratory conditions. The tank water was refreshed regularly, and any dead fish discovered were removed. The experiment was conducted under natural photoperiod circumstances at 24 ± 3ºC temperature range, 7.1 ± 0.3 pH, 7.4 ± 0.6mgL-1 dissolved oxygen, and total hardness ($CaCO_3$) of 130 ± 3.5 mgL-1.

2.4 Median and Sublethal Concentrations

96 h acute toxicity value of pendimethalin toxicity for *C. carpio* was determined to be 2.20µl/L. According to this value, two concentrations, 1/10th and 1/15th of LC50, were chosen to investigate the sublethal effects of pendimethalin on the liver and kidney of the freshwater fish *C. carpio*.

2.5 Histopathology Technique

Before starting the experiment, common carp individuals measuring 14–17 cm long and weighing 60-70 g were isolated and starved for 24 hours. A sublethal dose was administered to five specimens at 0, 7, 14, and 21 days, while a separate control group was kept under constant observation. The fish were anesthetized with clove oil and then euthanized at 0, 7, 14, and 21 days of exposure. Their brains were excised and fixed in Bouin's solution for 24 hours. After fixation, the tissues were thoroughly rinsed under running water to remove residual yellow color. The tissues were then serially dehydrated in graded alcohols (30%, 50%, 70%, 90%, 100%) and cleared in xylene. They were subsequently embedded in paraffin wax at 58–60°C. Sections approximately 5 µm thick were cut using a rotating microtome, stained with hematoxylin and eosin, and mounted on permanent slides. These slides were examined, and significant histopathological changes were documented through photography.

3. RESULTS

During the study of sublethal effects of pendimethalin, no mortality of fish (*C. carpio*) was observed. The liver and kidney of the control fish do not show any changes.

KIDNEY

The control fish kidney shows normal glomeruli (G), proximal convoluted tubules (PCT), and distal convoluted tubules (DCT) **(Fig 1)**.

After pendimethalin exposure severe changes were observed with an increase in exposure duration. After treatment with a sublethal dose of pendimethalin i.e., $1/10^{th}$ of LC50 i.e., 0.22µl/L kidney of *C. carpio* demonstrates glomerular contraction (GC) **(Fig 2)**. After 14 days of treatment, glomerular contraction (GC) and proximal tubule damage (RPCT) were observed **(Fig 3)**. Similarly, the kidney shows glomerular degeneration (GD), rupturing of proximal convoluted tubules (RPCT), damaged distal convoluted tubules (DDCT) and internal hemorrhage (IH) in some areas after 21 days of treatment with pendimethalin **(Fig 4)**.

Sublethal exposure to $1/15^{th}$ concentration of LC50 (i.e., 0.146µl/L) carp kidney illustrates little contraction of glomeruli (GC) after 7 days **(Fig 5)**. After 14 days of pendimethalin treatment kidney possesses more glomerular contractions and shrinkage (GC) and mild proximal tubules degeneration (RPCT) **(Fig 6)**. The kidney of *C. carpio* shows damaged glomeruli (DG), more ruptured proximal tubules (RPCT) and vacuolization in some areas (V) after 21 days of exposure to pendimethalin.

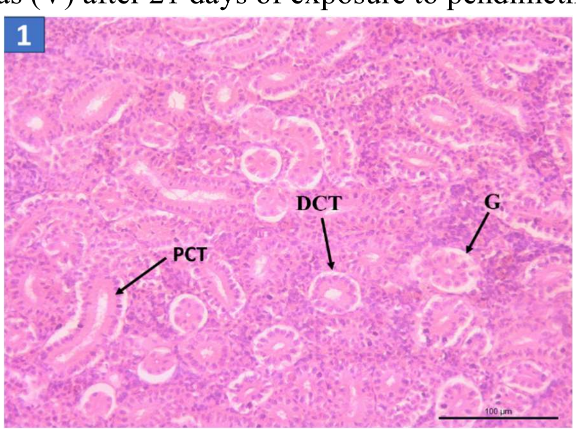
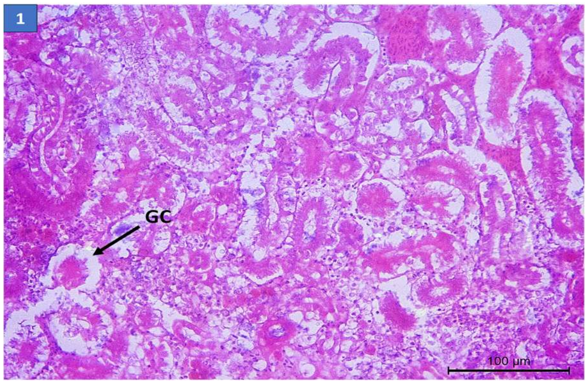
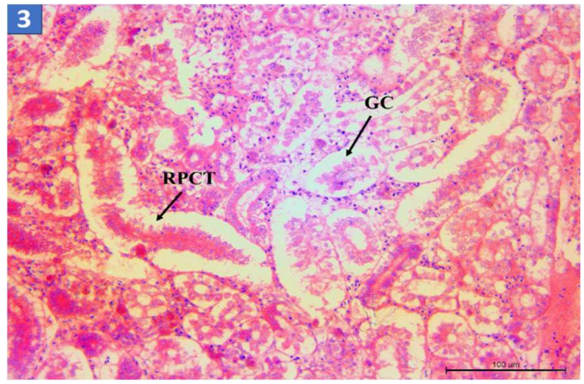
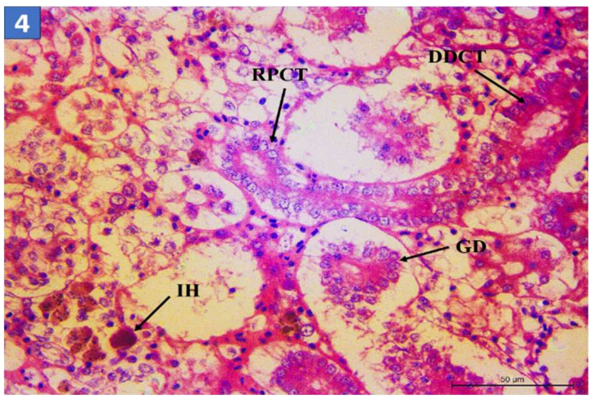


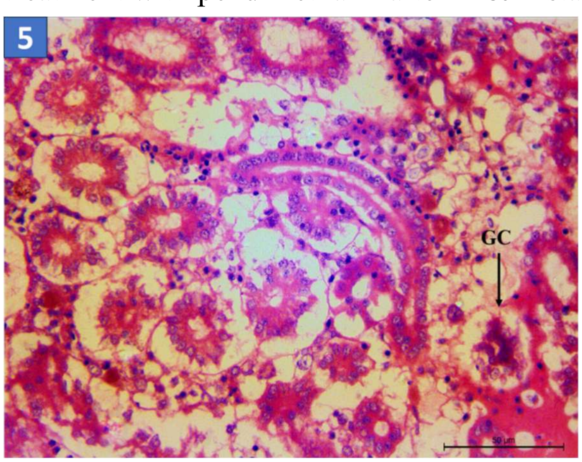
Figure 1: Kidney of control *C. carpio* demonstrating normal glomeruli (G), proximal convoluted tubules (PCT), and distal convoluted tubules (DCT) after H & E staining at 20X.

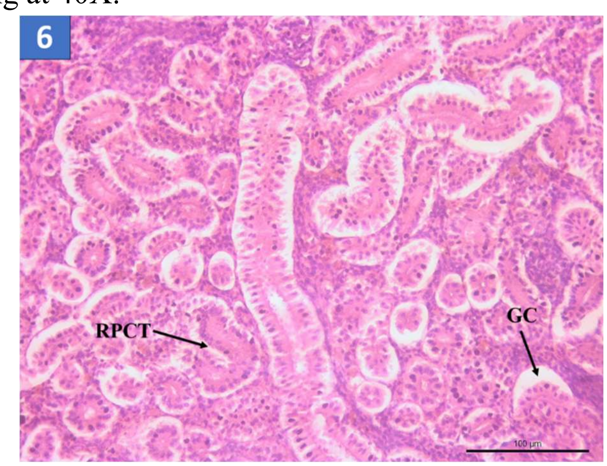


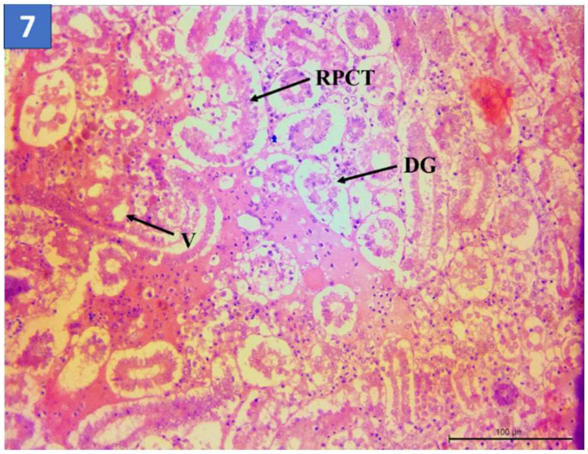




Figures 2,3 and 4 Show the kidney of carp treated with 1/10th concentration of LC50 value for pendimethalin toxicity in carp **Fig 2:** Shows glomerular contraction (GC) after H & E staining at 40X. **Fig 3:** After 14 days of treatment, glomerular contraction (GC) and proximal tubule damage (RPCT) were seen after H & E staining at 20X. **Fig 4:** Shows glomerular degeneration (GD), rupturing of proximal convoluted tubules (RPCT), damaged distal convoluted tubules (DDCT) and internal hemorrhage (IH) in some areas after 21 days of treatment with pendimethalin after H & E staining at 40X.







Figures 5, 6 and 7 Show kidney of carp *C. carpio* treated with 1/15th concentration of LC50 of pendimethalin showing little contraction of glomeruli (GC) **(Fig. 5)** after 7 days H&E staining at 40X, after 14 days of treatment more glomerular contractions and shrinkage (GC) and mild proximal tubules degeneration (RPCT) were seen **(Fig 6)** after H & E staining at 20X and **Fig. 7** shows damaged glomeruli (DG), more ruptured proximal tubules (RPCT) and vacuolization in some areas (V) after 21 days of exposure to pendimethalin after H & E staining at 20X.

LIVER

Light microscopy revealed unevenly formed lobules in the liver, isolated by hepatocytes. The liver of the control fish shows normal nuclei (N), normal hepatocytes (HP) and uninucleate hepatocytes (UN) (Fig 1). After sublethal administration with pendimethalin carp liver showed an increase in abnormalities with the increase in dose.

After treatment with a sublethal dose i.e., 1/10th of LC50 (0.22µl/L) liver of *C. carpio* showed vacuolization in between hepatocytes (HV) and damaged cytoplasm (DC) (Fig 2). After 14 days of treatment, vacuolization (V), nuclear infiltration (IN) and pyknosis in nuclei (PN) were observed (Fig 3). Similarly, the liver demonstrates vacuolization (V), internal hemorrhage (IH), binucleate formation (BN) and hepatocytic degeneration (HD) after 21 days of treatment with pendimethalin. (Fig 4).

Exposure to 1/15th value of acute toxicity of pendimethalin (i.e., 0.146µl/L) carp liver showed little vacuolization in some areas (V) and normal nuclei (N) after 7 days (Fig 5). After 14 days of pendimethalin exposure liver possesses more vacuolization (V), necrotic areas (NA), and binucleate hepatocytes (BH) (Fig 6). The liver of common carp showed damaged hepatocytes (DH), more necrosis of nuclei (NN), damaged cytoplasm (DC) and thrombosis in some areas (T) after 21 days of treatment with pendimethalin.

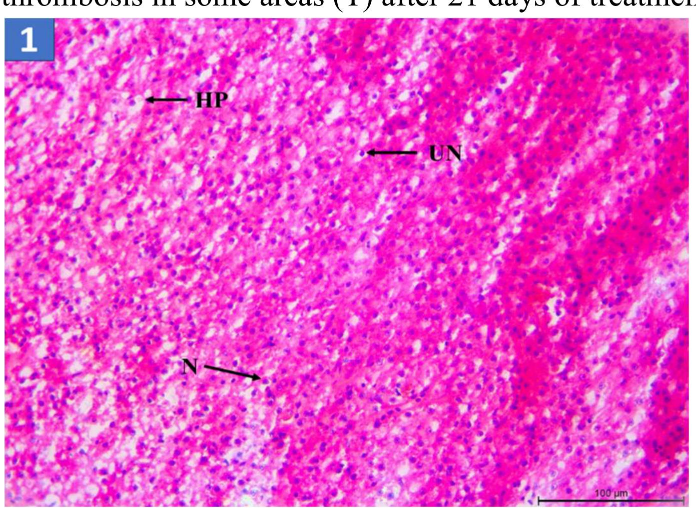
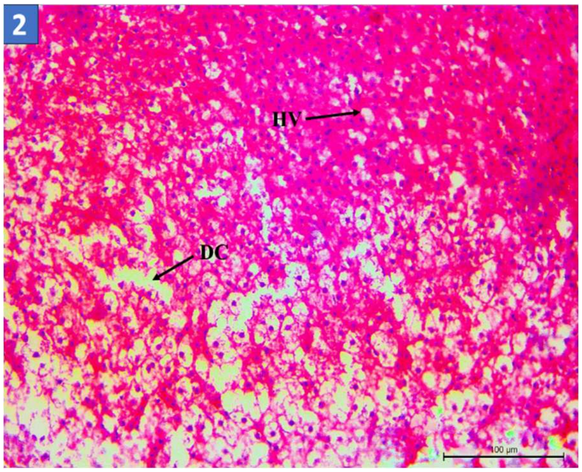
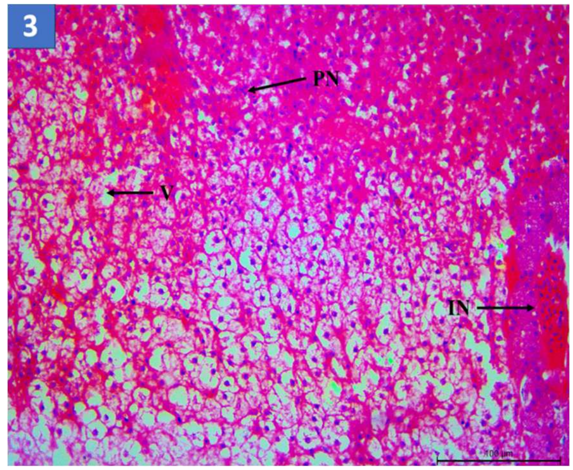
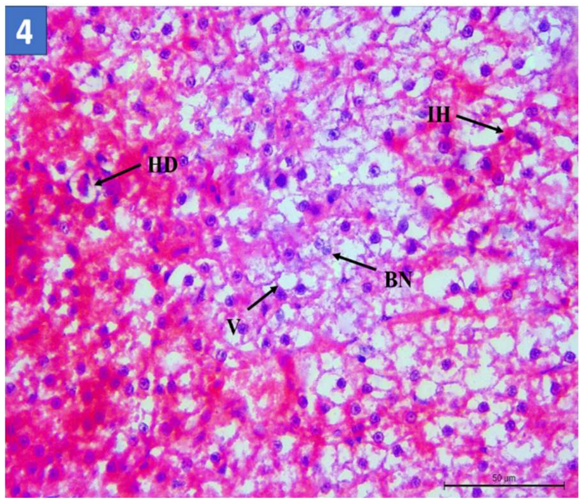


Figure 1: The liver of the control fish shows normal nuclei (N), normal hepatocytes (HP) and uninucleate

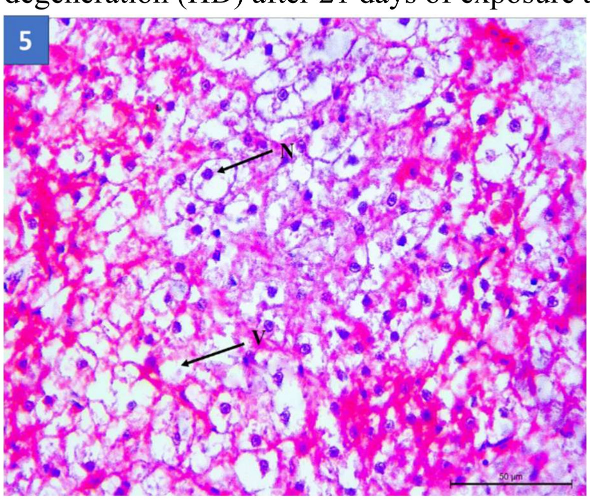
hepatocytes (UN) after H & E staining at 20X.

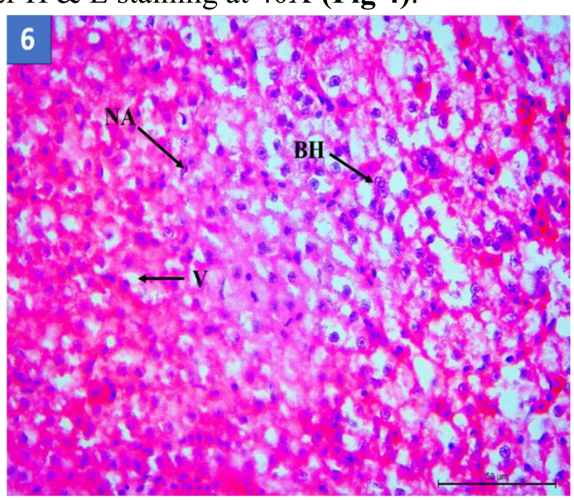


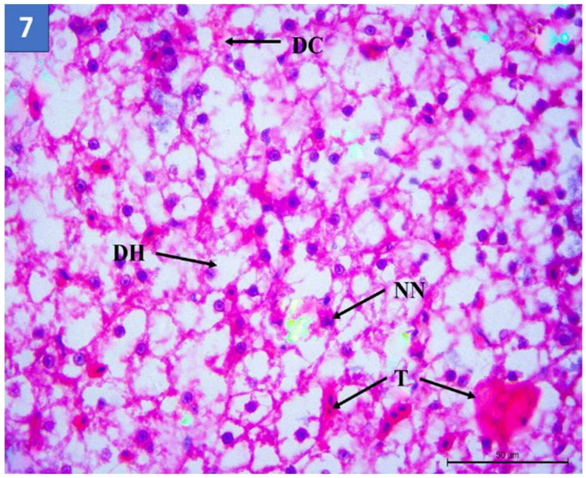




Figures 2, 3 and 4: Show the liver of *C. carpio* administered with 1/10th concentration of LC50 for pendimethalin toxicity in C. carpio demonstrating vacuolization in between hepatocytes (HV) and damaged cytoplasm (DC) after H & E staining at 20X **(Fig 2)**. After 14 days of treatment, liver shows vacuolization (V), nuclear infiltration (IN) and pyknosis in nuclei (PN) after H & E staining at 20X **(Fig 3)**. Similarly, the liver shows vacuolization (V), internal hemorrhage (IH), binucleate formation (BN) and hepatocytic degeneration (HD) after 21 days of exposure after H & E staining at 40X **(Fig 4)**.







Figures 5, 6 and 7 Show liver of C. carpio treated with 1/15th concentration of obtained LC50 of pendimethalin demonstrating little vacuolization (V) and normal nuclei (N) after 7 days after H & E staining at 20X **(Fig 5)**. After 14 days of pendimethalin treatment liver shows more vacuolization (V), necrosis in some areas (NA), and binucleate hepatocytes (BH) after H & E staining at 20X **(Fig 6)**. The liver of common carp illustrates damaged hepatocytes (DH), more necrosis of nuclei (NN), damaged cytoplasm (DC) and thrombosis in some areas (T) after 21 days of treatment with pendimethalin after H & E staining at 40X **(Fig 7)**.

DISCUSSION

The acute toxicity (96h LC50 value) for pendimethalin was 2.20µl/L. Sublethal effects were found utilizing 1/10th and 1/15th concentrations of the measured 96-hour LC50 values. Histology offers valuable insights into how tissues react to different treatments, drugs, or environmental factors. This information is crucial for developing a deeper understanding of toxicity mechanisms and the level of damage to various organs. A current study was done to find histopathological alterations caused by pendimethalin in the liver and kidney of *C. carpio.*

KIDNEY

Histopathological changes in the kidney of *C. carpio* exposed to sublethal concentration of pendimethalin showed contraction or shrinkage in glomeruli, proximal tubule damage, damaged distal convoluted tubules, vacuolization and internal bleeding in some areas of the kidney Similar results were reported by Hassan *et al.,* 2024 such as glomerulus shrinkage, bowman's space enlargement, renal tubule epithelial damage and epithelial membrane cytolysis in the kidney of Nile Tilapia after exposure to dimethoate herbicide. Similar patterns in histopathology were identified in the kidney of *Channa punctatus* after exposure to pendimethalin (Tabassum *et al.,* 2016). Research conducted by Ramesh *et al.,* 2024 and Shahzadi *et al.,* 2024 on histological changes in the kidneys of *Labeo rohita* fish after exposure to Triazophos and carbofuran showed the same results in line with our findings. The same histological patterns like renal tubule degeneration were reported by Afzal *et al.,* 2024 in the kidneys of *Cirrhinus mrigala* exposed to herbicide 2-methyl-4-chlorophenoxyacetic acid. Parallel histopathological results were observed by Loganathan *et al.,* 2024 on the kidney of *Heteropneustes fossilis* administered with triazophos like narrowing of the tubules, nuclear pyknosis, hypertrophy and degeneration in the kidney.

LIVER

The liver showed vacuolization, cytoplasmic damage, clustering of nuclei, pyknosis in nuclei, binucleate and damaged hepatocytes, damaged cytoplasm and clotting in some areas, binucleate formation and hepatocytic degeneration after exposure to sublethal doses of pendimethalin. Equivalent histopathological findings were documented by Ragab *et al.,* 2024 on the liver of *Clarias gariepinus* after exposure to the herbicide bispyribac-sodium. The fish's liver showed vacuolation, pyknotic nuclei, necrosis, cytolysis, infiltration of inflammatory cells and bleeding in some areas. Similar patterns in histopathology were identified in the liver of *Channa punctatus* such as cytoplasmic degradation, atrophy, and vacuolization after exposure to sub-lethal concentrations of pendimethalin (Tabassum *et al.,* 2016). Similar histological alterations were reported by Afzal *et al.,* 2024 in the liver of *Cirrhinus mrigala* exposed to herbicide 2-methyl-4-chlorophenoxyacetic acid and Loganathan *et al.,* 2024 in the liver of *Heteropneustes fossilis* administered with triazophos like vacuolization, nuclear pyknosis, and necrosis.

Conclusion

This study found that pendimethalin exposure induces severe histopathological damage in the liver and kidney of *C. carpio*. Notable changes include liver necrosis and vacuolation, as well as kidney tubular degeneration and glomerular damage, with the effects worsening with greater dosages and prolonged exposure. These findings emphasize the possible hazards of pendimethalin pollution in aquatic habitats, emphasizing the necessity for strict controls. Future studies should look at the underlying processes and potential preventive measures for aquatic species.

Acknowledgment

I extend my sincere gratitude to the Department of Fisheries, Himachal Pradesh, for their crucial support and resources throughout this project. I also deeply appreciate the Chairman of the Department of Biosciences, Himachal Pradesh University, Shimla, for their invaluable assistance and support in carrying out this research.

Ethical Statement

This study was approved by the Institutional Animal Ethics Committee (IAEC), and all procedures adhered strictly to IAEC guidelines. The research was conducted with a focus on minimizing discomfort and ensuring the humane treatment of animals, in line with ethical standards for responsible animal use.

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