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Research Article

Zinc-Induced Histopathological and Enzymatic Alterations in Kidney and Liver of Common Carp *Cyprinus carpio* var. Communis: A Comprehensive Analysis

Ibraq Khurshid, Irfan Ahmed Noorani*, Towfeeq Bilal, Shafia Mushtaq, Muzafar Ahmad Mir

Department of Zoology, Central University of Kashmir, Ganderbal, Jammu and Kashmir, INDIA.

ABSTRACT

In aquatic environments, heavy metals have been a significant threat to aquatic fauna, including fish, and are considered the most potent contaminant in the aquatic environment. The severity of heavy metal contamination and its potential to alter many physiological and biochemical mechanisms of fish metabolism has attracted the world's attention. **Aim:** The current study was conducted to investigate the impact of Zinc Sulfate ($ZnSO_4$) exposure on serum enzyme activities and histopathological alterations in the liver and kidney of *Cyprinus carpio*. The study was conducted in the Research Laboratory of the Department of Zoology, Central University of Kashmir, between October 2021 and May 2022. The experiment was carried out by obtaining 30 healthy individuals of *Cyprinus carpio* from the manasbal fish farm of both sexes without any prejudice. Length-weigh parameters were recorded. The specimens were transported from the farm to the laboratory in well-oxygenated containers, and the water was changed three times during transportation. These specimens were placed in a glass tank with 60 L of pure dechlorinated tap water after being treated with a 0.05% KMnO4 solution for 2 min. Before starting the experiment, the fish were acclimatized to laboratory settings for 7 days. **Materials and Methods:** Fish were exposed to sublethal concentrations of $ZnSO_4$ for 21 days, and levels of Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) in serum were measured. Histopathological examinations of the liver and kidney were conducted for 21 days to assess tissue damage. **Results:** Elevated serum enzyme activities in the exposed group indicated liver and kidney damage. Histopathological analysis revealed hepatocyte degradation, blood vessel congestion, hyalinization, vacuolation, and fatty changes in the liver, while kidney sections exhibited glomerular lesions, dilations of Bowman's capsule, haemorrhage, and tubular disruptions. **Conclusion:** The alterations in the liver and kidney were attributed to oxidative stress induced by excess Zn^{2+} ions, leading to lipid peroxidation and inflammatory response and suggesting that $ZnSO_4$ exposure adversely affects the liver and kidney of *Cyprinus carpio*, disrupting normal tissue architecture and function.

Keywords: *Cyprinus carpio*, Histopathology, Zinc, Alkaline phosphate, Kidney, Liver.

Correspondence:
Mr. Irfan Ahmed Noorani
Department of Zoology, School of Life Sciences, Central University of Kashmir, Ganderbal-191201, Jammu and Kashmir, INDIA.
Email: Irfan.noorani29@gmail.com
ORCID: 0009-0001-2591-1594

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INTRODUCTION

Fish, being natural and living components of the aquatic ecosystem, interact with and rely on water quality, and any changes to their habitat or water quality significantly impact their health.[1] Common heavy metals include chromium, cobalt, zinc, arsenic, cadmium, mercury and lead. Sources of these heavy metals include mining, industrial effluents, domestic and urban wastes, metal input from rural areas, pigments, batteries, paints, use of fertilizers, textiles, dental and cosmetic products, petroleum industries and activities like smelting of copper.[2] Heavy metals typically exhibit greater detrimental effects due to their tendency to bioaccumulate from the aquatic environment into the fish body and finally in the food chain.[3,4] Fishes tend to accumulate heavy metals in their tissues because they are absorbed and stored more quickly than excreted or removed.[5] The capacity of different species to withstand elevated metal concentrations and their vulnerability to the deleterious consequences of metal exposure vary.[6] Metal content, metal absorption mechanism, exposure duration, environmental conditions, and fish age are all variables that influence the buildup of metals in tissues.[7]

Zinc, which activates over 100 enzymes and functions as a structural and catalytic component of various proteins, is the trace element with the greatest abundance after iron. When taken in excess, however, it can be toxic due to its disruption of acid-base and ion homeostasis, resulting in hypoxia and disruption of

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tissues and organs.[8] Fish growth depends on zinc at a certain quantity,[9] yet excessive zinc buildup is toxic to exposed species. According to,[10] zinc is released into aquatic environments through the corrosion of galvanized goods, the decomposition of automobile tyre rubber, and urban runoff. If subjected to higher levels of trace metals in their surroundings, fish may gather them in their bodies. Additionally, when their exposure to zinc exceeds what their liver can handle, the circulation transports the highly toxic form of Zinc (Zn^{2+}) to other organs. Furthermore, zinc cannot be eliminated biologically, as it can only be changed from one state to another, such as an organic complex or an oxidised state. Thus, it persists in the environment and accumulates in the organs of the fish, causing damage to organs such as the liver, gills, and kidneys.[8] Like other heavy metals, extra zinc restricts the sites of activity of enzymes, co-enzymes, and membrane receptors, binding to free thiol groups on macromolecules to have physiologically detrimental consequences.[11] High zinc levels also have an impact on protein metabolism and lipid profiles. Moreover, the metal species may release Reactive Oxygen Species (ROS) after accumulating. Oxidative stress, brought on by ROS, can result in lipid peroxidation and dysregulation of the osmoregulatory system. In cases of excessive ROS, the mitochondrial pathway can induce apoptosis.[12] Fish organs that accumulate heavy metals experience structural damage and functional abnormalities.[7]

The activity of several enzymes is considered a sensitive biochemical marker for assessing the impact of toxicants before harmful effects manifest in fish. Enzymes found in serum, including ALP, AST, and ALT, are considered sensitive indicators for observing effects caused by exposure to sublethal doses of pollutants.[13] The presence of excess Zn (zinc) in aquatic environments can lead to alterations in fish biochemical parameters by promoting oxidative stress. This happens because zinc ions provide electrons to oxygen, which causes the creation of free radicals that can inflict oxidative damage and contribute to the generation of Reactive Oxygen Species (ROS).[10] Consequently, fish can be an indicator species, signalling potential aquatic pollution and metal-related stress.[14,15]

Studies are frequently undertaken to examine the effects of heavy metals on specific organs, including the kidneys, gills, and liver.[13] As an essential detoxifying organ, the liver is vital to the body's metabolism and removal of toxic compounds. The liver's extensive blood supply is a target for heavy metal exposure, leading to notable accumulation and subsequent histological changes.[16] The bioaccumulation of zinc in fish is contingent upon zinc concentrations and the duration of exposure to these concentrations.[17] In the liver, zinc binds to Metallothioneins (MTs), proteins that enhance the donation of zinc to metalloenzymes, causing their inhibition, which results in toxicity.[18] Zinc also results in histopathological changes in kidneys as they are the main organs for the elimination of ions and ion regulation. These alterations demonstrate the sensitivity of fish to zinc metal. Several histopathological changes in the liver and kidneys, like necrosis, hyalinization, haemorrhage and infiltration of neutrophils, act as reliable biomarkers of toxic damage.[19]

To evaluate *Cyprinus carpio*'s health, the current study looked at enzyme levels and histopathological alterations. Specifically, the analysis focused on ALP, AST, and ALT to gauge the physiological condition. These parameters were chosen as indicators of stress biomarkers, providing insights into the well-being of the fish. The evaluation encompassed histopathological examinations of the liver and kidneys, as these organs are integral in reflecting the overall health status of *Cyprinus carpio*.

MATERIALS AND METHODS

Fish Procurement and Acclimatization

Thirty healthy *Cyprinus carpio* fish samples were collected from the Manasbal fish farm. The fish of both sexes were collected without bias, and their weight was taken using an electronic balance. The weight of fish varied between 100-140 g, and the length ranged from 15-25 cm. The fish were kept in well-aerated plastic containers and transported to the laboratory.

The fish were relocated to aquariums filled with 60 L of de-chlorinated tap water, characterized by a pH level of 7.6, Dissolved Oxygen (D.O.) content of 6.4 mg/L, Carbon Dioxide (CO_2) concentration of 5.79 mg/L, and a temperature maintained at 20±2°C. Before being introduced into the glass tanks, the fish underwent treatment with a 0.05% $KMnO_4$ solution for 2 min (Figure 1). This precautionary measure was implemented to prevent any potential microbial infections, as outlined in the study by.[20] The aquaria were equipped with aerator for maintaining proper oxygen level. Acclimatization process for a period of 7 days to the aquarium conditions was undertaken for the fish while exposing the fish to 12-hr light and 12-hr dark using fluorescent lamp.[21] During the acclimatization period, the fish underwent a feeding regimen of two times a day, with rate of feeding fixed at 2% of their body weight. The diet comprised bran and oil feed, as specified by.[13] Any residual material, whether it be leftover feed or faecal matter, was promptly removed through siphoning, and regular water changes were conducted. Each day, settled fish waste was siphoned out along with one half of the volume of water in each aquarium, following the procedure outlined by.[22] Subsequently, de-chlorinated tap water was poured into each aquarium to restore the initial volume.

In vivo Exposure to Zinc Sulphate

To prepare the stock solution for the test metal compound, 1 g of analytical-grade Emplura ($ZnSO_4.7H_2O$, Merck Life Science Pvt. Ltd., Mumbai, India) was measured using a weighing balance and then dissolved in 1000 mL volume of distilled water. Thus,

resulting in a concentration of 1000 mg/L, as outlined in the methodology by.[8]

The exposure procedure followed the methodology outlined by[23] with certain modifications. After a period of acclimatization, a total of 30 fish were subjected to a 24-hr fasting period before the commencement of exposure. The fish population was then divided into three groups: a control group designated as ZS_C, and two test groups denoted as ZS_{T1} and ZS_{T2}. The control group was kept unexposed while both the test groups were exposed to the sublethal concentration of 10mg/L $ZnSO_4$.[8] To ensure that the desired exposure concentration was reached, a determined amount of the stock solution prepared earlier was mixed in the water of each tank to create the desirable test medium[24] which corresponds to the 25% of 96 hr LC_{50} of $ZnSO_4$.[25] determined in a prior investigation carried out by[26] and determined to be 41.10 mg\L. Bath exposure for a continuous period of 21 days was given to the test fish, during which the fish were fed twice a week to prevent accumulation of nitrogenous wastes in the aquaria[25] and the test media was renewed every week to prevent drastic changes in its concentration.[27]

Blood Collection and Enzyme Quantification

The ALP, AST and ALT levels show changes due to stress and can act as indicators for detecting stressful conditions for fish.[17] During the experiment, the blood samples were taken from fish using 3 mL sterilized syringes through cardiac puncture on the 7th, 14th and 21st day, and blood samples were collected in vials without anticoagulant or non-EDTA vials (Figure 1). The samples were centrifuged for 10 min at room temperature at 5000 RPM to separate the supernatant (serum).[17]

The supernatant is used for enzyme analysis. ALP, AST and ALT levels were determined through UV-Kinetic method[28] using commercial reagent kits manufactured by Coral Clinical Systems, a unit of Tulip Diagnostics (P) Ltd., by a semi-automatic analyzer.[29] According to this method, AST and ALT measurements were taken by adding 50 µL of serum to the working solution prepared by mixing enzyme reagent and starter reagent in 4:1 ratio and aspirating the same mixture through the analyzer which measures the absorbance at 340 nm and for measuring ALP concentration 10 µL of serum were added to the working solution prepared by mixing reagent A and reagent B in 4:1 ratio and aspirating the same mixture through the analyzer which measures the absorbance at 405 nm, the concentration of enzymes in the sample was measured from the absorbance shown by the sample.

Histopathological Analysis

The impact of varying Zinc concentrations on the liver and kidneys of fish was examined following a 21-day exposure period. Subsequently, both control and test fish were euthanized to facilitate the examination of the liver and kidneys. The liver and kidneys were then extracted and rinsed thoroughly with sterile water. The processing of tissues for micro-sectioning was done using paraffin embedding technique. The extracted organs were preserved by immersing them in 10% formalin for a duration of 24 hr. Following fixation, a process of dehydration was carried out using ascending grades of alcohol, followed by clearing using xylene. Subsequently, the organs were embedded in paraffin wax, following the methodology outlined by.[30] Upon completion of the tissue block preparation, thin sections were sliced with the help of a microtome while maintaining a uniform thickness of 5 µm for the sections being sliced (Figure 2). Mayer's fixative was used to attach sections to the slide. The sections were then processed, hydrated through a descending series of alcohol grades and stained utilizing the Hematoxylin and Eosin (H & E) stain, cleared using xylene and finally mounting was done using DPX.[31] Photomicrographs were taken with an Olympus IX 71 inverted Olympus microscope.

RESULTS

Enzyme Activities Serum ALP, AST and ALT Levels

The test groups exhibited elevated serum levels of the enzymes under investigation (ALP, AST, and ALT) after a 7-day exposure to a concentration of 10 g/L $ZnSO_4$ compared to the control group (Table 1). The serum ALP, AST, and ALT values were lowest in the control group following the 7-day exposure period (42.6±3.27, 42.1±1.45, and 23.7±2.496 IU/L, respectively). Conversely, the test fish displayed the highest values after the 7-day exposure, with levels of (115.25±3.93, 418.8±15.20, and 109.7±5.06 IU/L, respectively). After 14 days' exposure, the serum ALP, AST and ALT levels decreased to 93.5±3.50, 309.5±5.50 and 93.5±3.50 IU/L, respectively, but were higher than the control (40.4±1.26, 41.9±1.28 and 23.0±2.10 IU/L respectively). After 21 days of exposure, the serum ALP, AST and ALT levels showed a further decrease to 42.72±1.64, 204.46±2.785 and 54.4±2.76 IU/L, respectively, but the values were still higher than the control (39.4±1.57, 40.8± 1.13 and 22.4±1.64 IU/L respectively).

The serum ALP levels of the control group was 42.6±3.27 IU/L,40.4±1.26 IU/L and 39.4±1.57 IU/L after 7, 14 and 21 days respectively. The ALP levels in the experimental group showed an increase to 115.25±3.93 IU/L after 7-day exposure to 10 mg/L zinc concentration, this decreased to 93.5±3.50 IU/L after 14-day exposure and to 42.72±1.64 IU/L after 21-day exposure, as illustrated in Figure 3.

The AST level of the control group was 42.1±1.45 IU/L, 41.9±1.28 IU/L and 40.8± 1.13 IU/L for 7, 14 and 21 days respectively (Table 1). The serum AST showed a significant increase to 418.8±15.20 IU/L after 7-day exposure to 10 mg/L zinc concentration in the experimental group. Following a 14-day exposure, the serum AST level decreased to 309.5±5.50 IU/L, and it further decreased to 204.46±2.785 IU/L after a 21-day exposure. However, it remained higher than the control group. Consequently, the AST

demonstrated a decrease with an increase in exposure time, as illustrated in Figure 4.

The serum ALT levels of the control group was 23.7±2.496 IU/L, 23.0±2.10 IU/L and 22.4±1.64 IU/L after 7, 14 and 21 days respectively (Table 1). The assessment of ALT levels in the experimental group showed an increase to 109.7±5.06 IU/L after a 7-day exposure to a 10 mg/L zinc concentration. This value subsequently decreased to 93.5±3.50 IU/L after a 14-day exposure and further declined to 54.4±2.76 IU/L after a 21-day exposure. However, it remained higher than the levels observed in the control group, as depicted in Figure 5.

Histopathological Examination

The test fish subjected to 10 mg/L $ZnSO_4$ and the control group both had their liver and kidney histopathologies assessed after the completion of the 21-day exposure period.

Histopathology of Liver

The photomicrographs of the liver sections of *Cyprinus carpio*, of the test fish and the control fish are illustrated in Figure 6. Hepatocytes in the control fish's liver sections seem normal and densely arranged without any abnormalities. The sections exhibit characteristic sinusoids, and the cytoplasm surrounding a central spherical nucleus is homogeneous (6.a). Fish liver treated with zinc sulphate showed apparent histopathological changes compared to the control samples. Hyalinization is one of the main histopathological alterations found in the exposed specimens (6.d) in which cells appear as a translucent, pink and homogenous mass under the microscope; cytoplasmic vacuolation in the cell (6.d) which forces the nuclei to the periphery causing the formation of clear spaces in tissue, necrosis of hepatic tissue (6.e) sinusoidal congestion (6.b) due to cellular swelling of hepatocytes resulting in narrowing of sinusoidal spaces; cellular swelling (6.c) haemorrhage (6.f) due to damage to the blood vessels; heterogeneous parenchyma of hepatic tissue (6.d) due to cellular swelling and fatty changes; fatty changes in which clear vacuoles of different size appear in cytoplasm as lipid globules (6.b) infiltration of neutrophils (6.e) by extravasation.

Histopathology of Kidney

The photomicrographs of kidney sections of the *Cyprinus carpio* (control fish and test fish) are shown in Figure 7. The kidney of the control shows the normal structure (7.a) without any alteration while the histological sections of the kidney of experimental fish show histopathological changes like dilation of Bowman's Capsule (7.b), hyalinization (7.c) in which cells appear as a translucent, pink and homogenous mass under the microscope, cytoplasmic vacuolation (7.e), necrosis due to degeneration of renal tubules (7.d), cellular swelling in renal tubular cells (7.c), and haemorrhage seen as infiltration of blood cells (7.f).

DISCUSSION

Heavy metals are very severe in their action of toxicity to aquatic life,[4] because heavy metals are taken up and accumulated rather more quickly than they are eliminated or degraded. Thus, these tend to accumulate in tissues.[32] In this study, 30 specimens were

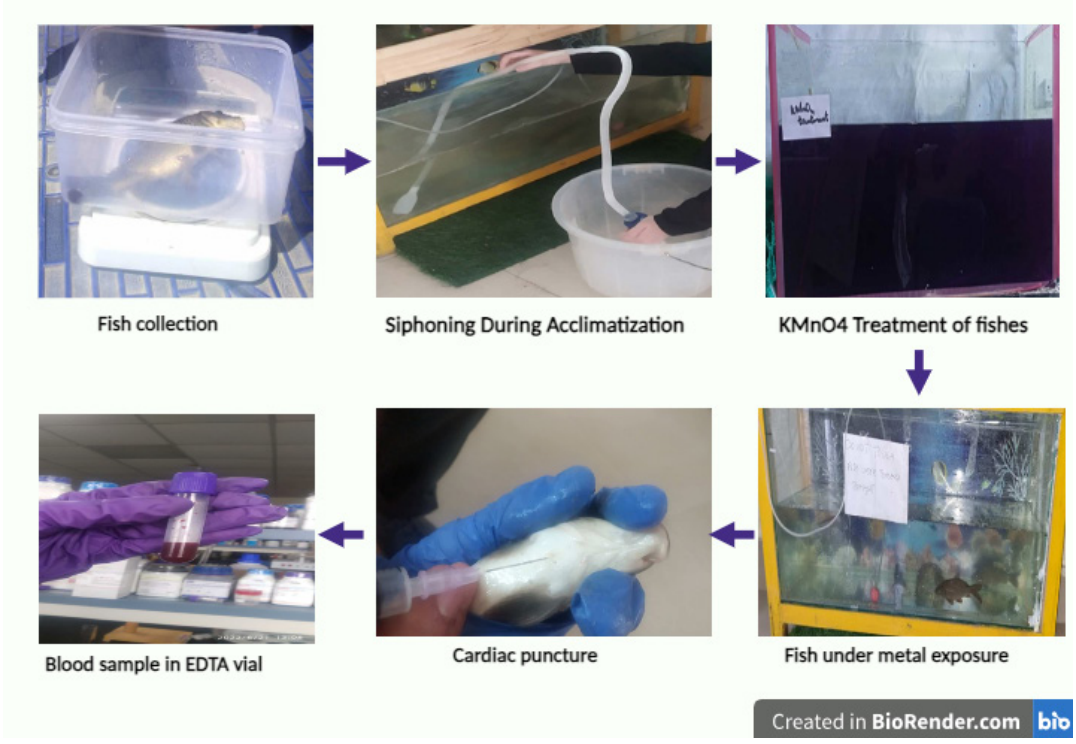

Figure 1: Fish acclimatization and Blood sample collection.

taken, 10 as control and 20 as experimental, and the effect of 10 mg/L Zn concentration on the serum enzyme activities and histology of the liver and kidney was investigated.

Transamination is a vital biochemical process involving two enzymes known as transaminases, specifically Alanine and Aspartate aminotransferases. This process is critical in sustaining the free amino acid pool required for protein synthesis. Additionally, Alkaline Phosphatase (ALP) is an enzyme in the bloodstream that contributes to the breakdown of proteins within the body. It plays an important role in membrane transport and, at alkaline pH, acts as transphosphorylase and plays a pivotal part in carbohydrate metabolism.[33] These enzymes are involved in the conversion of protein to glycogen, which serves as a key reserve fuel during stress or at times of high energy demand. In our study, AST, ALT, and ALP activities were significantly elevated in the test group compared with that of the control group. This notable increase in enzyme activities serves as an indicator of heightened energy demands and an accelerated metabolic rate, revealing the organism's reaction to stress brought on by metals.[19] According to research by[30,34] releasing these enzymes from injured hepatocytes into the circulation is responsible for the increase in enzyme concentrations. Additionally, it suggests potential damage to the renal ducts as indicated in[35] work. Preliminary elevation in AST and ALT levels in some treatment of metal mixture may reflect the initial toxic effect of metals on these enzymes, and with the increase in the exposure time, the decrease in these enzyme levels may be due to liver necrosis induced by metal toxicants. These outcomes concur with the findings of,[36] who elucidated that due to lead toxicity in albino rats, ALT and AST levels increased on the 5th day and then decreased significantly on the 20th day.[37] reported hepatocellular damage and liver necrosis in liver of *Cyprinus carpio* and *Barbus conchonius* due to cadmium exposure

Table 1: Representing Enzyme levels at different exposure levels and time intervals.

Exposure time (days)	No. of fish used for sample collection	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
7	**Control** (*n*=10)	42.6±3.27	42.1±1.45	23.7±2.496
	Test (*n*=20)	115.25±3.93	418.8±15.20	109.7±5.06
14	**Control** (*n*=10)	40.4±1.26	41.9±1.28	23.0±2.10
	Test (*n*=18)	93.5±3.50	309.5±5.50	93.5±3.50
21	**Control** (*n*=10)	39.4±1.57	40.8±1.13	22.4±1.64
	Test (*n*=18)	42.72±1.64	204.46±2.785	54.4±2.76

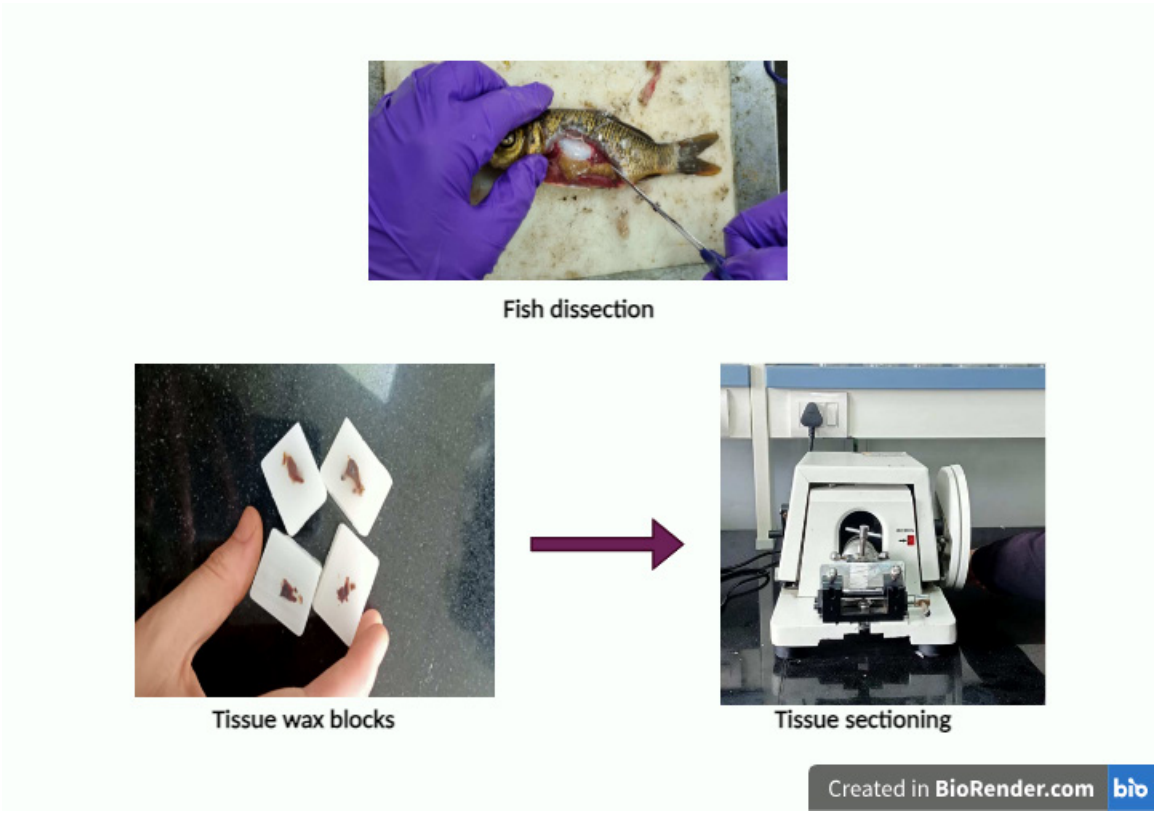

Figure 2: Preparation of tissue wax blocks for histopathological studies.

leading to reduction in AST and ALT levels after longer exposure time and associated the reduction of these enzyme levels with tissue damage leading to reduced enzyme turnover as well as to the slow and constant inhibition of enzymes by heavy metals as their protein binding tendency is high and bind to the thiol groups in these enzymes causing oxidative modification in these enzymes.[38] The fish's tolerance to the toxin may be the cause of the decrease in enzyme activity seen with longer exposure times, a phenomenon previously documented in *Nile tilapia* exposed to zinc sulfate by.[39] These findings align with the results reported by.[40] The observation of a decrease in ALP activity to disruptions in the membrane transmission system caused by tissue damage over the course of the exposure period was linked by.[41] The present findings are consistent with the results reported by.[13] where an initial increase in the activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) due to zinc exposure was observed. At 21-days exposure, a decline in ALT, AST and ALP levels was observed in comparison to the control group. This decrease in enzyme values may be attributed due to the adaptation of fish to the toxicant over time, leading to a return of the enzyme levels to baseline values.[42]

The findings from the biochemical indices were validated through histopathological examinations of crucial organs, including the liver and kidney. Elevated levels of hepatic biomarkers, including AST and ALT, confirmed the histopathological changes in the liver that were seen. The increased synthesis of Reactive Oxygen Species (ROS) brought on by an excess of Zn^{2+} ions is thought to be the cause of oxidative stress, which is responsible for these alterations. Hepatocyte degradation, blood vessel congestion and swelling were seen in the liver tissue of *Clarias gariepinus* by.[43]

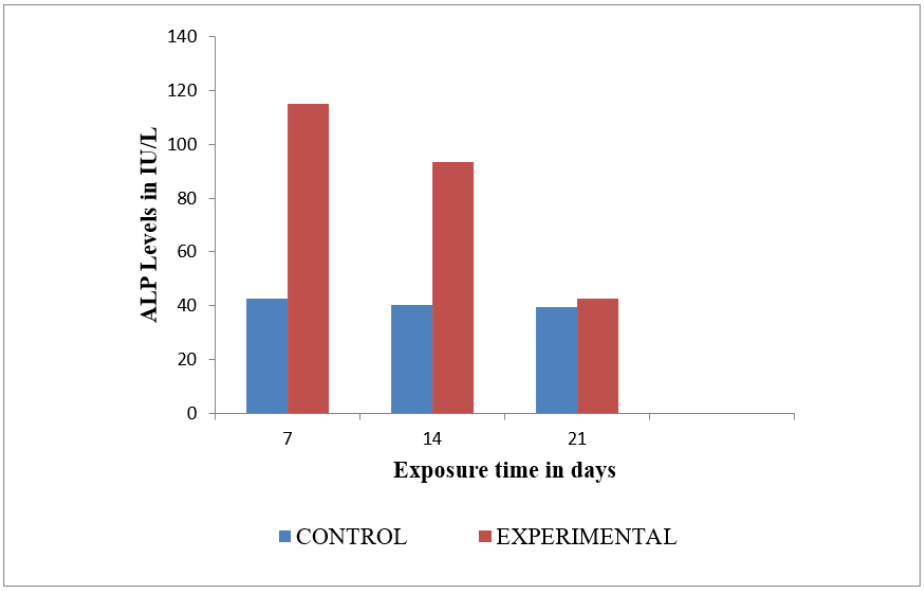

Figure 3: ALP levels of Control and Experimental Group.

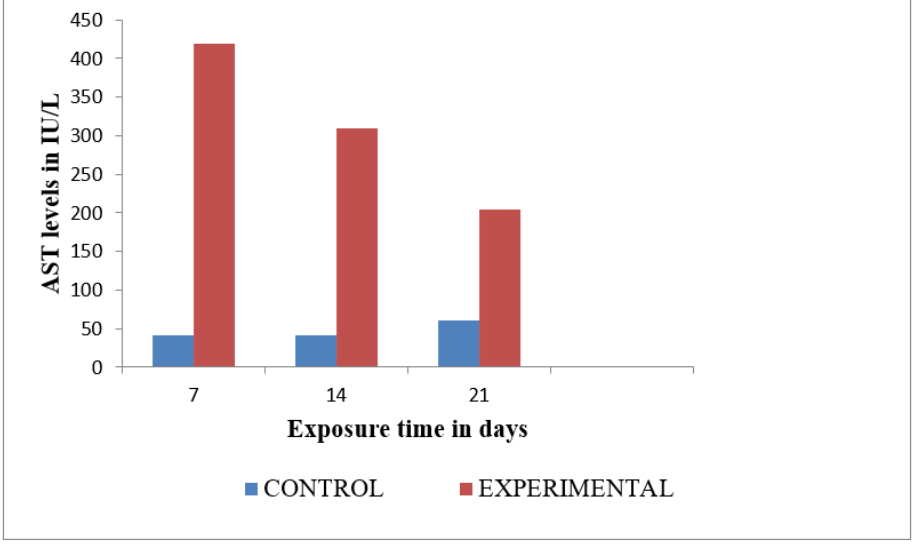

Figure 4: AST levels of Control and Experimental Group.

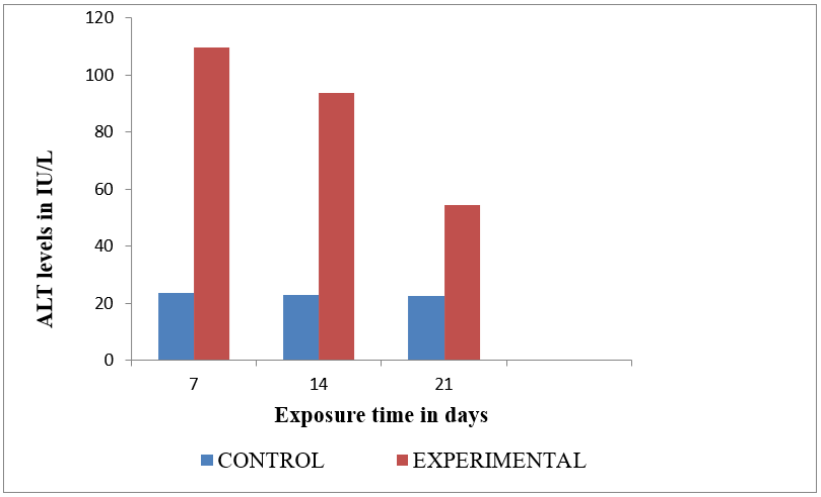

Figure 5: ALT levels of Control and Experimental Group

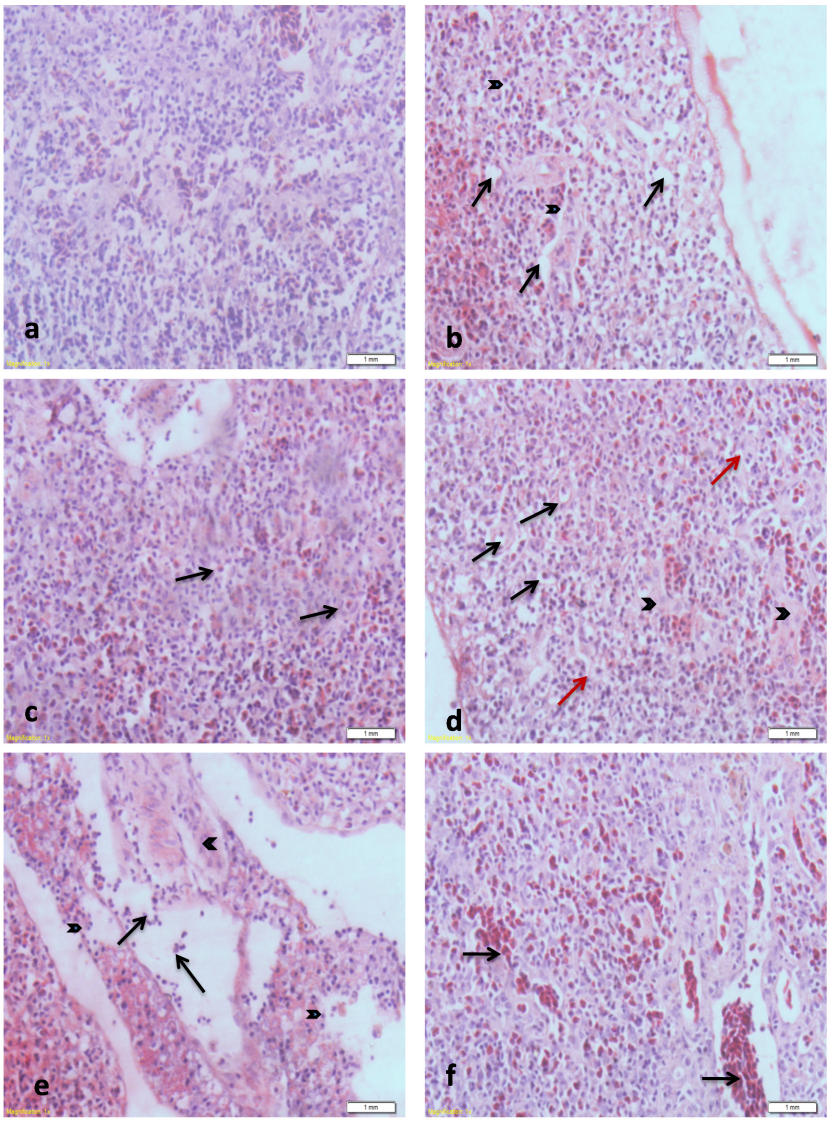

Figure 6: The Photomicrographs of Liver Showing; 6.a) Normal liver tissue of control, 6.b) Fatty changes (black arrows) and sinusoidal congestion (arrow heads) 6.c) cellular swelling 6.d) cytoplasmic vacuolation (black arrows), heterogeneous parenchyma (Red arrows) Hyalinization (arrow heads) 6.e) Necrosis (black arrows), infiltration (arrow heads) 6.f) Hemorrhage.

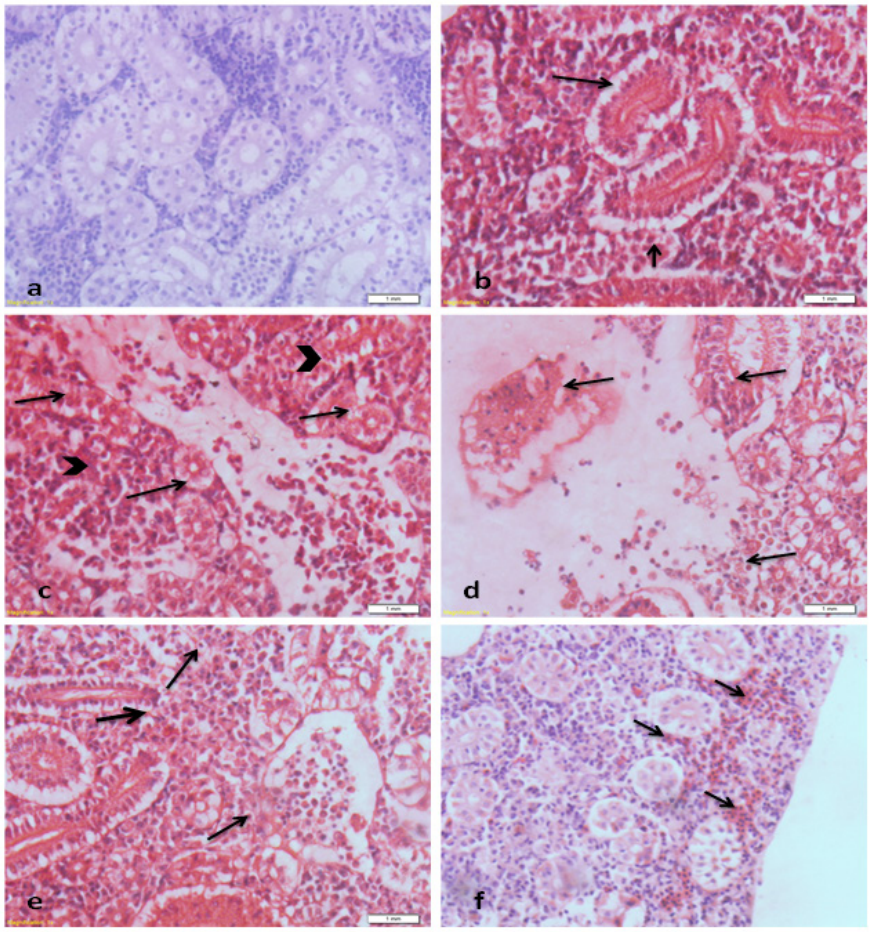

Figure 7: The Micrographs of Kidney Showing 7.a) Normal Kidney tissue of control, 7.b) Dilation of Bowman's capsule 7.c) cellular swelling (arrow heads), Hyalinization (black arrows) 7.d) Necrosis 7.e) cytoplasmic vacuolation 7.f) Hemorrhage.

They also saw hepatocyte inflammation, which resulted in blood vessels and sinusoidal congestion, linking these observations to the detrimental effects of pollutants on the liver.[44] observed hepatic sinusoidal haemorrhage and congestion associated with vascular congestion in *Oreochromis niloticus* liver that had been subjected to exposure of heavy metal-contaminated water, including metal salts. Together with the shrinkage of the pancreatic acini, they also noticed hepatic cell vacuolization and degeneration along with related fatty changes. During oxidative stress, the leucocytes migrate in large numbers in trans endothelial way for clearing ROS (detoxification); this leads to the opening of endothelial protein junctions to increase vessel permeability for leucocytes and result in damage to blood vessels, causing the release of blood cells in the tissue.[45] Hyalinization refers to the process of conversion of stromal connective tissue, collagen and other proteins into homogenous translucent mass and is caused by the disturbances in protein synthesis, by zinc ions and ROS, like reduction of the sulfhydryl groups and oxidation of proteins which induce stable protein-lipid adducts which affect protein metabolism.[46] Vacuolation is connected to the hindrance of protein synthesis, depletion of energy, disintegration of microtubules, and modifications in substrate utilization induced by Zn^{2+} ions.[45,47] reported that metal toxicities usually show the presence of protein inclusion bodies. Denaturation of volume-regulating ATPases causes cellular swelling, as does interruption of the cell's energy transfer mechanisms, which are essential for ionic regulation, cellular swelling also results from the release of inflammatory mediators released by macrophages fighting ROS.[46] The oxidative damage products, such as malondialdehyde, which is created when free radicals attack PUFA double bonds and accumulates in tissue, are what cause the fatty alterations observed in test fish.[44,45] Additionally, this causes hepatocytes to produce vacuolar structures.[48] Furthermore, the accumulation of heavy metals and the rise in their concentrations in the liver may be connected to hepatocyte necrosis. Reactive Oxygen Species (ROS) contribute to lipid peroxidation, damaging the membrane. Additionally, they cause the release of cytochrome C into the cytoplasm due to mitochondrial membrane damage, activating caspases and initiating apoptosis. This sequence leads to cell damage and releases various inflammatory mediators from migrating macrophages and neutrophils, causing further cellular harm, as documented by.[48] According to,[49] liver inflammation, sinusoidal congestion, and lipid alterations upset the normal architecture of the liver, resulting in heterogeneous parenchyma.

The liver lesions found in this investigation are consistent with findings published by other researchers in cases of acute exposure to varied types of contaminants.[43,48,50]

Histopathological alterations in kidneys like glomerular lesions, dilations of Bowman's capsule, haemorrhage, and swelling of glomerulus were also documented by several workers.[49-52] ROS produced in huge quantities due to oxidative stress brought on by zinc ions, are the source of these necrotic and degenerative changes found in the kidneys. In the renal tissue of tilapia (*Oreochromis mosambicus*) and trout (*Salmo trutta*) treated with mercuric-chloride enlarged Bowman's capsule and renal tubular disruptions were observed as per the findings of[53] and was reported to be the most common alteration found in kidneys. Hyalinization is caused due to disturbances in protein synthesis by zinc ions and ROS.[46] The fat globules appear due to the formation of oxidative damage products like malondialdehyde, which is formed by the attack of free radicals on double bonds of PUFAs, it has a longer life span and is accumulated in tissue.[44,45] resulting in cytoplasmic vacuolation.[48] The outcomes of our study are also supported by the finding of,[50] who reported necrosis, hypertrophy of tubules, hyaline spots, hydropic swelling and vacuole formation in the kidneys of fish subjected to cadmium exposure. The observed necrosis may result from lipid peroxidation brought on by ROS, which damages membranes and, ultimately, cells, as well as the bioaccumulation of heavy metals in the renal tissue.[54] reported that tubular changes are commonly associated with the interference of the normal tubular functions that constitute the re-absorption or secretion of ions and molecules. The increased number and infiltration of neutrophils and lymphocytes could cause tissue deterioration by the release of inflammatory cytokines, causing cellular swelling. Fish are particularly sensitive to heavy metal exposure, as indicated by the histopathological changes in kidney tissues, which point to a sluggish immune response of the cells for heavy metal removal or immobilization.[55]

CONCLUSION

The main aim of this study was to assess the effect of Zinc on organ histology and enzyme activity of *Cyprinus carpio*. The enzyme levels in the test fish were increased compared to the control and showed a decreasing pattern over the exposure period due to adaptation of the *Cyprinus carpio*. The liver showed histopathological alterations like necrosis, hyalinization, fatty changes and swelling. The kidney also showed histopathological alterations like tubular degeneration, dilation of bowman's capsule and haemorrhage. The current study revealed that zinc is a potent toxicant and causes histopathological alterations in the liver and kidney as well as in the enzyme level of *Cyprinus carpio*. These changes might result from oxidative stress induced by excessive zinc ions in the tissues. Furthermore, these changes in enzyme levels and organ histology of *Cyprinus carpio* is a suggestive of zinc toxicity and hence can be used as biomarkers to indicate effect of metallic pollution in aquatic organisms.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALT: Alanine aminotransferase; **AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase; **ROS:** Reactive oxygen species; **EDTA:** Ethylenediamine Tetraacetic Acid; **PUFA:** Polyunsaturated Fatty acids; **DO:** Dissolve Oxygen.

AUTHORS CONTRIBUTION

IK: Conceptualization, final approval of the version to be published and supervision of the entire research work. **IAN:** Critical revision, editing and preparation of the final version of the article and supervision. **TB:** Study design, acquisition, analysis and interpretation of data and writing the original draft. **SM:** Study design, acquisition, analysis and interpretation of data and writing the original draft. **MAM:** Literature review, manuscript revising and editing.

SUMMARY

This study examines the toxic effects of Zn on enzyme activity in serum and hepato-renal tissue histology of common carp, *Cyprinus carpio*. It is well established that heavy metals such as zinc tend to be accumulated in aquatic organisms, resulting in a process of toxicity because of their low excretion rate. 30 specimens were considered, 10 of them as control and 20 treated with 10 mg/L zinc. The levels of the key enzymes Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were determined. Enzyme activities of this group of animals were significantly higher in the test group in comparison to the control group, which led to an indication of increased metabolic stress and to potential damage in the liver and kidneys. This elevation results from the leak of such enzyme or enzymes from damaged cells to the bloodstream, a common response to hepatocellular injury. With continued metal exposure, enzyme levels may fall as a result of liver necrosis, which develops over time of metal exposure, as it has been reported in several studies. The balance of the rapid elevation and subsequent decline of the activities of enzymes might correspond with adaptation or disease progression in the organism.

Histological examinations revealed significant alterations in liver and kidney tissues. In the liver, changes like hepatocyte

degeneration, blood vessel congestion, and swelling, indicating oxidative stress and tissue damage, were observed. The kidneys exhibited glomerular lesions, dilation of Bowman's capsule, haemorrhage, and swelling, indicating renal dysfunction. These histopathological changes are consistent with oxidative damage caused by Reactive Oxygen Species (ROS) generated due to zinc exposure.

The study demonstrates that subchronic exposure to zinc sulfate induces significant biochemical and histopathological alterations in *Cyprinus carpio*. Elevated serum enzyme activities, particularly AST, ALP, and ALT, indicate liver and kidney dysfunction. Corresponding histopathological changes, such as hepatocyte vacuolation, necrosis, and renal tubular damage, further confirm the toxic effects of zinc exposure. These findings underscore the importance of monitoring and regulating zinc concentrations in aquatic environments to protect fish health.

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