Bioaccumulation And Influence Of Cadmium Chloride On Histology Of Muscles And Gills In Nile Tilapia (*Oreochromis Niloticus*)

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Abstract
Heavy metals Show a heterogeneous pattern of accumulation in different organs. Cadmium is an essential inorganic toxicant widely distributed in the environment. The current study evaluates the histopathological changes in Nile tilapia (*Oreochromis niloticus)* treated with different concentration of cadmium chloride. Fish was stocked in triplicate (control group, T1, T2). The two groups T1 and T2 are exposed to sub-lethal doses $1/5^{th(2.96mg/L)}$ and $1/10^{th(1.48mg/L)}$ of $LC_{50(14.8mg/L)}$ of $CdCl_2$ for 21 days. Fish was dissected, gills and muscle tissues were removed. Histological examination of gills specimen revealed that $CdCl_2$ exposure substantially damages histopathological profile as evident from primary lamellae dilation, secondary lamellae splitting, detached sub lamellae, and inter lamellar cells hyperplasia. It leads to mild edema, acute inflammation, and blood vessels congestion was observed in CdC12 induced group as compared to control group. However, no necrosis and hemorrhage of gills tissues were seen. The histopathology of treated fish muscle was revealed distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration observed in the $CdCl_2$ intoxicated group as compared to control group. It is concluded that exposure with high concentration of $CdCl_2$ causes histopathological alterations in *Oreochromis niloticus.*

Keywords*: Oreochromis niloticus,* toxicology, histopathology

Introduction

Heavy metals toxicants effects of chemical exposure disturb the homeostasis and changes in physiological process of fish (Fidan *et al.*, 2008). It also results in histopathological variations abnormalities in fish reproduction; and public health risk effects for human consumption (Matos *et al.,* 2017). Heavy metals are involved in the rise of many diseases such as diabetes, Alzheimer sickness, and cancers (Sonone *et al.,* 2020). Cadmium, Mercury, Lead and Arsenic are caused dangerous effects to the nervous system of fish (Mahino and Nazura, 2013). Particularly in those regions which can be impacted by agricultural, industrial, and domestic activities. Tilapia are potentially exposed to affect the toxic substances (i.e., cadmium) (Adeyemi *et al.,* 2012). Cadmium other chemicals enter the environment from anthropogenic and natural sources. In natural water Cadmium can rise at concentrations < 0.1 mg/L. However, in closely polluted creeks the concentration is 2 to 16 mg/L (Cao *et al.,* 2012). Cadmium is inorganic toxicant extensively distributed in the environment due to its numerous commercial uses (Besirovic*et al.,* 2010). Cadmium accumulates in aquatic organisms through dietary and aqueous exposure (Kalman *et al.*,2010). Humans are exposed to heavy metals through ingestion of aquatic food. Heavy metallic cadmium is considered an important chemical and environmental pollutant because it may cause harmful effects on organisms (Patnaik *et al.,* 2011). Particularly, Cadmium is accumulated in gills and lower extent in muscles (Sara *et al.,* 2017). The uptake of water-borne Cd in freshwater fish takes place via the gills, which plays important role in homeostasis (MeGeer *et al.,* 2000). In gills and muscles tissues of fish all the heavy metals absorbed in different quantities according to the metal concentration (Muley *et al*.,2000 ; Chi *et al.,* 2007).

Muscles are the primary edible parts of fish through human and consequently preferred tool for the assessment of health risk related with metal pollution in fish (Yi *et al.,* 2011). Muscles are the main eatable part of fish and can directly influenced the human health (Pintaeva *et al.,* 2011). Governments have set up toxicological control for heavy metals in seafood's (Agah *et al.,* 2009). The gills of fish are important sites for absorption of heavy metals that provokes lesions and gill damage (Bols *et al.,* 2001; Yousafzai, 2006). Gill tissues not only do respiration but also do osmoregulation process (Javid *et al.,* 2017). Therefore, gills tissues as close to aquatic environment and affected directly by poisonous chemical compounds (Raju, 2013).

Fish histology used as bio-monitoring tool of aquatic pollutants (Munoz *et al.,* 2015). Histopathological process is used to describe the health status of fish from polluted sites. (Vinodhini and Narayan, 2009). Using histopathological biomarkers in which the advantages of environmental monitoring are achieved and examine the specific target organs including kidney, brain gills, heart and liver (Gemhofer *et al.,* 2001).

Present work aim is to investigate the effects of cadmium chloride on fish and observed histopathological test of different organs of *Oreochromis niloticus* e.g., gills and muscle tissues. Cadmium chloride impacts the physiology of fish, if human beings consume cadmium chloride ($CdCl_2$) treated fish how this chemical also effect on the physiology of human health.

Material and Methods

Fish Collection and Rearing

The fish collected from the ponds of Manawa fish hatchery Lahore with the help of fish catching nets. Then these fish samples were shifted to the collection ponds and observed carefully. The dead and defeated pieces were separated. The healthy samples were treated with $KMno_4$ solution and then shifted to the acclimatization ponds. Here the fish was acclimatized for 24 hours at normal environmental conditions. After that fish were packed in plastic bags containing enough oxygen. Finally, these samples were ready for transportation to Lahore.

Placement of Fish in Aquariums

Fish were stock in triplicate for each treatment. Fish of T1 group were exposed to the $1/5^{th}$ (2.96mg/L) and T2 group were exposed to $1/10^{th}$ (1.48mg/l) of LC_{50} of cadmium chloride (CdCl2). Total of nine aquaria were used (three for each group). Fish samples were placed in glass aquariums already filled with 60 L of fresh water. The water was regularly changed after 2 days. The fish were subjected to variable concentrations of cadmium chloride mixture with treated produced water. Then the fish were kept in those aquariums for 21 days. LC_{50} value of $CdCl_2$ for tilapia already calculated by (Garcia-Santos *et al*., 2006) determine $CdCl_2$ concentration.

Fish Groups Division and Preparation of Stock Solution

Fish were divided into three groups control group, Treated group (T1) and Treated group (T2). Both treated groups were exposed to $CdCl_2$Salt for 21 days. After acclimatization, fish were treated with Cadmium chloride ($CdCl_2$) salt. For this purpose, a stock solution of Cadmium Chloride ($CdCl_2$) was prepared by dissolving the salt in 1000 ml distilled water and was stored in reagent bottles.

Cadmium Chloride Exposure and Dissection of Fish

The treated group (T1 and T2) were exposed to Cadmium chloride ($CdCl_2$). The first treated group T1 was exposed 2.96mg/l of Cadmium Chloride ($CdCl_2$) and second treated group T2 was exposed to 1.48mg/l solution. After the completion of experiment, fish from each tank were accumulated and dissected.

Histopathological Examination

Tissue specimens from fresh Nile tilapia were taken (gills, muscles) and fixed in 10 % buffered neutral formalin. They were processed to obtain five microns thick paraffin sections then stained with Hematoxylin and Eosin (Bancroft et al., 1996, Nida *et al*., 2022) and examined under light microscope.

Ethical Statement

The investigations do not involve any unnecessary pain or stress to live animals and human subjects and there is no objection from ethical board of Institute of Molecular Biology and Biotechnology, The University of Lahore, 54590, Punjab, Pakistan.

Results

Histopathological Analysis of Fish Gills in Control Group

The results of histopathology analysis of fish gills in control group, as shown in Figure 1. There is no primary lamellae dilation, and secondary lamellae splitting along with intact sub lamellae with no inter lamellar cells hyperplasia, edema, necrosis, inflammation either acute and chronic as well as no hemorrhage, and no blood vessels congestion

The seven days' toxicity of$CdCl_2$ toxicity on fish gills of T2 group is depicted in Figure 2**.** Outcomes of the investigation revealed that $CdCl_2$ exposure significantly damages histopathological profile as evident from primary lamellae dilation,

secondary lamellae splitting, detached sub lamellae, and inter lamellar cells hyperplasia. It leads to mild edema, acute inflammation, and blood vessels congestion in $CdCl_2$ induced group as compared to the control group. However, no necrosis and hemorrhage of gills tissues were seen at this stage, which is comparable to the control group.

After 14 days of exposure the effect of $CdCl_2$on fish gills in T1 group is described that $CdCl_2$ intoxication substantially disrupted the histological profile by causing dilation of primary lamellae, as well as secondary lamellae splitting with detached sub lamellae and inter lamellar cells dilation or hyperplasia. Despite all of these, it leads to moderate edema, necrosis, acute inflammation, and blood vessels congestion in $CdCl_2$ exposed group as compared to the $CdCl_2$ induced group for seven days. There was no hemorrhage of gills tissues at this stage, which is comparable to the control group (Figure 3)**.**

After 21 days of exposure the effect of $CdCl_2$ on fish gills of T1 group, the histopathological profile of fish was substantially disrupted as it dilated primary lamellae, along with secondary lamellae splitting, detached sub lamellae, and inter lamellar cells dilation or hyperplasia. Additionally, it leads to moderate edema, necrosis, inflammation, and severe blood vessels congestion in $CdCl_2$ exposed group as compared to the $CdCl_2$ induced group for fourteen days. However, gills tissues hemorrhage was not observed as compared with the control group (Figure 4).

The effect of $CdCl_2$ on fish gills of T2 group was observed. The seven days' toxicity of $CdCl_2$ toxicity on fish gills is depicted in Table 3 and (Figure 5). Outcomes of the research revealed that $CdCl_2$ exposure substantially damages histopathological profile as evident from primary lamellae dilation, secondary lamellae splitting, detached sub lamellae, and inter lamellar cells hyperplasia. It also leads to mild edema, acute inflammation, and blood vessels congestion in $CdCl_2$ induced group as compared to the control group. However, no necrosis and hemorrhage of gills tissues were seen at this stage, which is comparable to the control group.

After 14 days of exposure the effect of CdCl2 on T2 group showed that the $CdCl_2$ intoxication substantially disrupted the histological profile by causing dilation of primary lamellae, as well as splitting of secondary lamellae with detached sub lamellae and interlamellar cells hyperplasia. Despite all of these, it leads to moderate edema, necrosis, acute inflammation, and blood vessels congestion in $CdCl_2$ exposed group as compared to the $CdCl_2$ induced group for seven days. There was no hemorrhage and necrosis of gills tissues were observed at this stage, which is comparable to the control group **(**Figure 6).

After 21 days of exposure the effect of $CdCl_2$on fish gills of T2 group, the histopathological profile of fish was substantially disrupted as it dilated primary lamellae, along with secondary lamellae splitting, detached sub lamellae, and inter lamellar cells hyperplasia. Additionally, it leads to moderate edema, necrosis, inflammation, and blood vessels congestion in $CdCl_2$ exposed group as contrasted with the $CdCl_2$ induced group for fourteen days. However, gills tissues hemorrhage was not examined as matched with the control group (Figure 7).

Histopathological Analysis of Fish Muscles in Control Group

Present study showed that the control group exhibited a typical histopathological profile as it has shown intact muscles orientation and hypertrophy of muscle volume without splitting muscle fibers (Figure 4).

Outcomes of the current study showed that $CdCl_2$ intoxication considerably damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration was observed in the $CdCl_2$ intoxicated group as compared to the control group (Figure 5). After 14 days of exposurethe effect of $CdCl_2$on fish muscles of T2 group in which$CdCl_2$ intoxication considerably damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscledeposition, and muscle degeneration were observed in the $CdCl_2$ intoxicated group as compared to the control group (Figure 6).

Histopathological analysis of fish muscles after 21 days of exposure to $CdCl_2$ showed that $CdCl_2$ intoxication considerably damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration was observed in the $CdCl_2$ intoxicated group as compared to the control group (Figure 4).

Demonstrate the histopathological analysis of fish muscles after seven days of exposure to $CdCl_2$. Is demonstrated (Figure 5). Outcomes of the current study showed that $CdCl_2$ intoxication considerably damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration was observed in the $CdCl_2$ intoxicated group as compared to the control group

After 14 days of exposure effect of $CdCl_2$ on fish muscles of T2 group showed damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally,

edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration were observed in the $CdCl_2$ intoxicated group as compared to the control group (Figure 6).

After 21 days of exposure effect of $CdCl_2$ on fish muscles of T2 groupshowed that $CdCl_2$ intoxication considerably damaged the histopathological profile as it has shown distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition, and muscle degeneration were observed in the $CdCl_2$ intoxicated group as compared to the control group. (Figure 7).

Discussion

In this study, the exposure of Cadmium Chloride ($CdCl_2$) on Nile tilapia (*Oreochromis niloticus*) resulted significant histopathological variations in treated groups (T_1 and T_2) as compared to control group. Heavy metals accumulation in fishes in which the primary parts are muscle tissue and gills. Nile tilapia have ability for the bioaccumulation of metals due to a low sensitivity of heavy metals (Mokhtar *et al.,* 2009; Jia *et al.,* 2017). Fish mortality increased with higher concentration and exposure of heavy metals (Chen *et al.,* 2012). Heavy metals can accumulate in different body organs of fish and then disturb the physiology and proper functioning of these organs. In present study, histopathological changes were analyzed in different organs like muscles and gills after $CdCl_2$ exposure.

These histopathological changes could not be traced in organs of control group. The control group presents the typical histopathological profile of gill tissues. There is no primary lamellae splitting along with intact sub lamellae with no inter lamellar cells hyperplasia as well as no hemorrhage, and no blood vessels blocking. But in treated groups (T_1 and T_2) the $CdCl_2$ exposure significantly damages histopathological profile as evident from primary lamellae dilation, secondary lamellae splitting, detached sub lamellae, and inter lamellar cells hyperplasia. Additionally, moderate edema, necrosis inflammation, and severe blood vessels congestion were also seen.

Histopathological changes in gills inclusive of edema, hyperplasia and necrosis of the second filaments, hyperemia, jerky movements and fusion of second filaments, $CdCl_2$ exposure caused changing in gill morphology of fish and damaged the gill shape (Jourdehi, 2013). These damages as a result of $CdCl_2$ are lamella fusion, hyperemia cell necrosis and hyperplasia (Rostami *et al.,* 2000). Therefore, Hypertrophy and hyperplasia disturbed the epithelial cells of gills (De-Giorgi *et al.,* 2009).The hypertrophy observed and secondary lamella fusion of gills due to increase chloride cells of gills caused thickness in epithelial cells (Alvarado *et al.,* (2006). The gills are the entrance site of heavy metals that causes lesions and distruct gills morphology (Bols *et al*., 2001).

The histopathological changes in which gills showed structured primary filaments and secondary lamellae with flat chloride cells and epithelial cells that locate on the bases of the secondary lamellae. Gill hyperplasia and detached the lamellar Epithelium are compensatory mechanisms that increase the depth of the epithelial cell layer, which form protective barriers between the external and internal environments (Bhagwant and Elahee, 2002). The metals absorption in gills can reveals the level of metals in water where the fish live, whereas the concentration in kidney and liver characterizes the storage of metals (Rao *et al.,* 2000). In environmental bio monitoring programs in which fish muscles are very important, because this tissue is the main tissue of fish consumed by the people (Yousafzai and Shakoori, 2007)

The accumulation of Cadmium chloride in the muscles of the common carp (*Cyprious carpio)* have been enhanced under increased the concentration and duration of Cd exposure (Kim *et al.,* 2004; Malekpouri *et al.,* 2011). Similar increasing the pattern of Cadmium accumulation in olive flounder muscle. Exposure of cadmium decreased glucose uptake and glycogen content in white muscles of Nile tilapia which affected the body weight gain. Increased accumulation also enhanced the toxicity level and can lead to muscle degradation by protein depletion (Haredi *et al.,* 2020).

The maximum levels of the heavy metals found in the gills, liver and kidney. While the lowest levels found in the muscle (Sultana *et al*., 2016). The lowest bioaccumulation in muscle tissues are associated to the absence of metallothione metal binding proteins and to the lower metabolic activity of muscles (Uluturhan and Kucuksezgin, 2007). The bioaccumulation of metals and histopathological alterations in tissues of *Oreochromis niloticus* heart muscles (myocardial fibers) of Tilapia nilotica showed hemorrhage and hemolysis with the aggregation of inflammatory cells and vacuolar degeneration (Kaoud and EI-Dahrshan, 2010).

The histopathology of fish *Cyprinus carpio* exposed to sub lethal concentrations of lead and cadmium. The fish showed distinct thickening and separation of muscle bundles with intracellular edema. The destruction and vacuolation of the muscle cells in *Oreochromis* species were observed in fish exposed to chromium. (Ayoola and Alajabo, 2012). Outcomes of the current study showed that the control groups of muscle tissue exhibited a typical histopathological profile as intact muscles orientation and hypertrophy of muscle volume without splitting muscle fibers. In control group fishes were perceived no edema, no necrosis, no focal muscles and no inflammation. The histopathology of experiment fish muscles treated with sub-lethal concentrations cadmium chloride exposure showed distorted muscle orientation and hypotrophy of muscle volume with splitting muscle fibers. Additionally, edema, necrosis, focal muscles, inflammation, muscle deposition and muscle degeneration were observed. In the mild of all these observations and additionally the consequences of the prevailing look at, it may be inferred that heavy metals can accumulate in exclusive organs of fish and damage the proper function of these organs. Heavy metals can cause the histopathological regulations in a specific organ of fish and disturb the fish health.

Conclusion

The present study shows significant histopathological changes in (*Oreochromis niloticus*). The high concentration of cadmium chloride caused more harmful effects on the different body organs of fish.

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Author Contribution

Corresponding Author: Supervision, Conceptualization, Project designing, Methodology, Formal Analysis
First Authors: Conceptualization, Formal Analysis, Methodology writing
Second Author: Formal Analysis, Methodology Writing, Writing original draft
Third Author: Supervision, Formal Analysis, Methodology, Writing - review and editing, Data Curation
Fourth Author: Formal Analysis, Writing -review and editing, Data formatting

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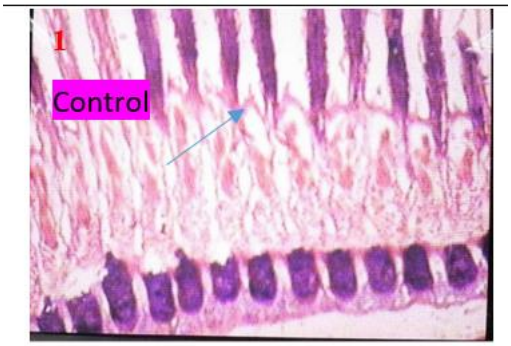


Figure 1: Histological analysis of fish gills of control group.

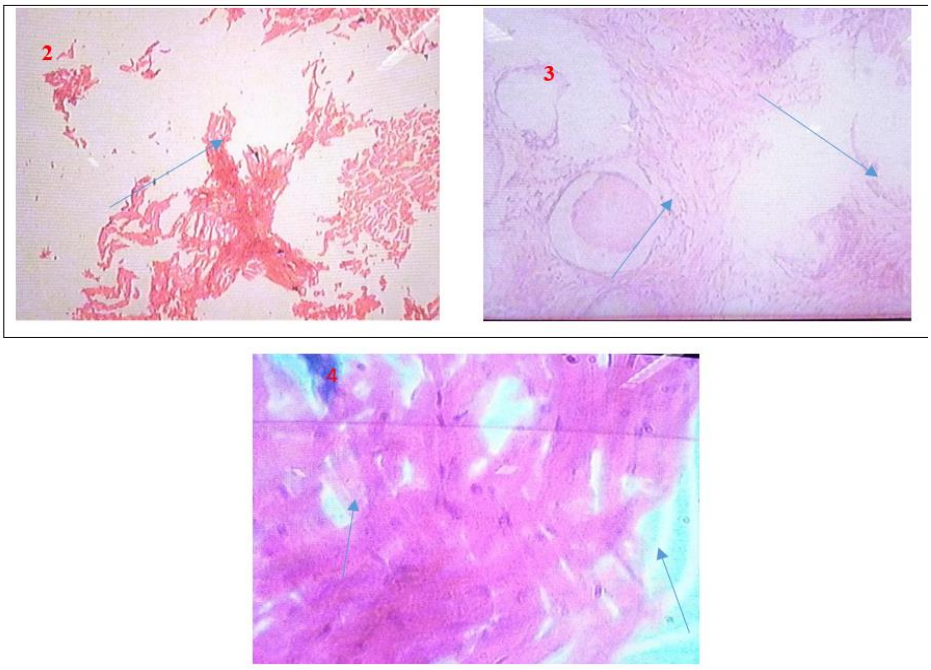
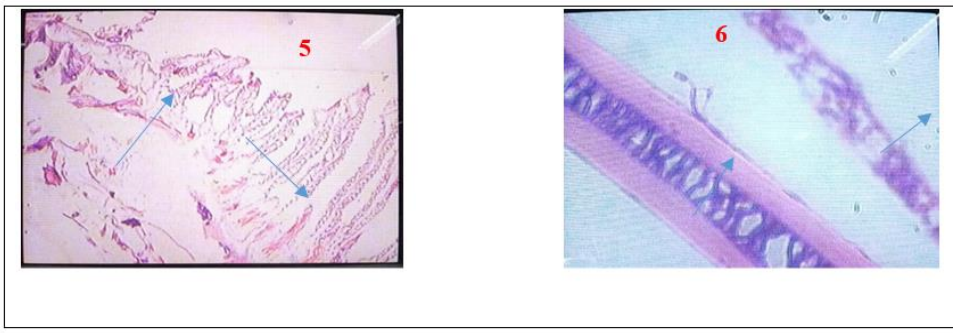


Figure 2,3,4: Histological analysis of fish muscles dose of T1 group after 7th (figure 4), 14th (figure3) and 21(figure 4) days of exposure.



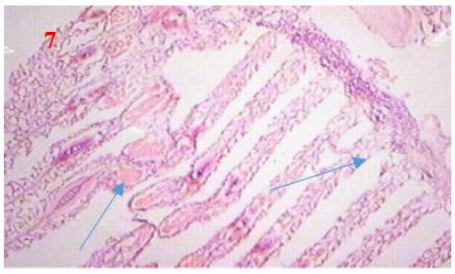


Figure 5,6,7: Histological analysis of fish gills dose of T2 group after 7th (figure 5), 14th (figure 6) and 21(figure 7) days of exposure.

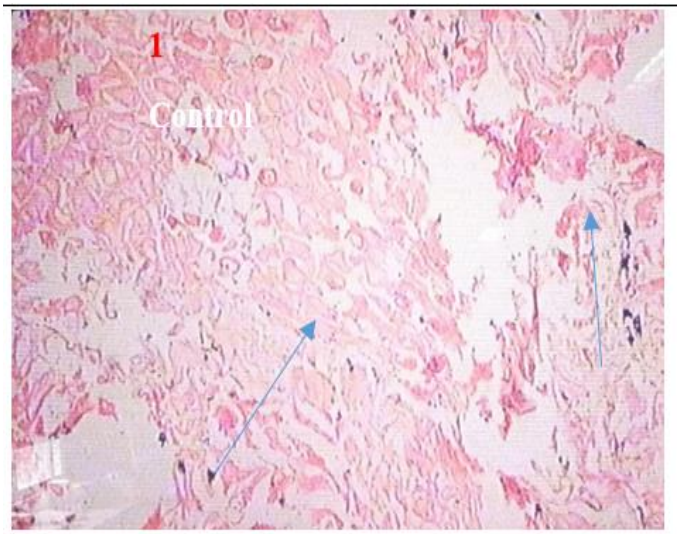
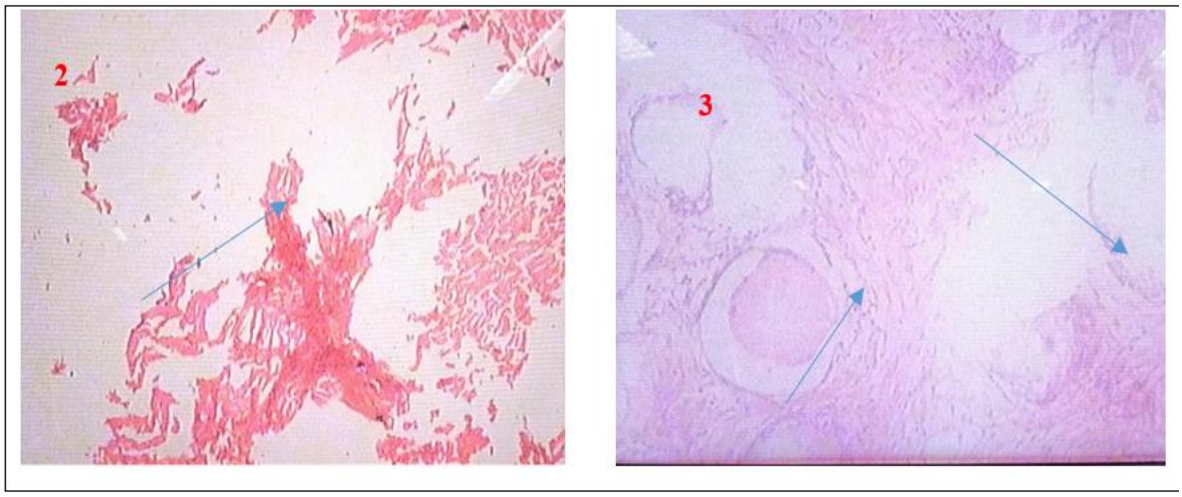


Figure 1: Histological analysis of fish muscles of control group.



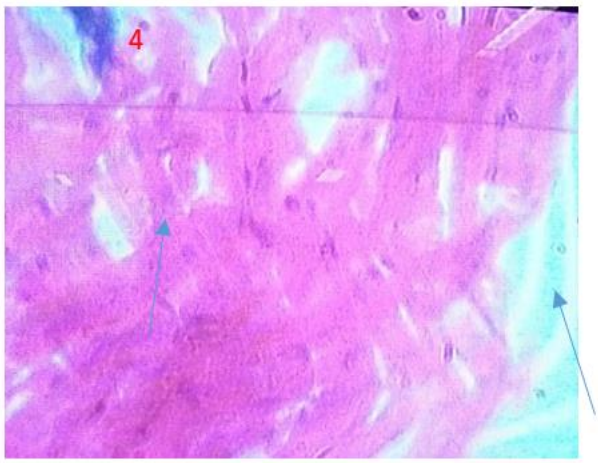


Figure 2,3,4: Histological analysis of fish muscles dose of T1 group after 7th (figure 4), 14th (figure3) and 21(figure 4) days of exposure.

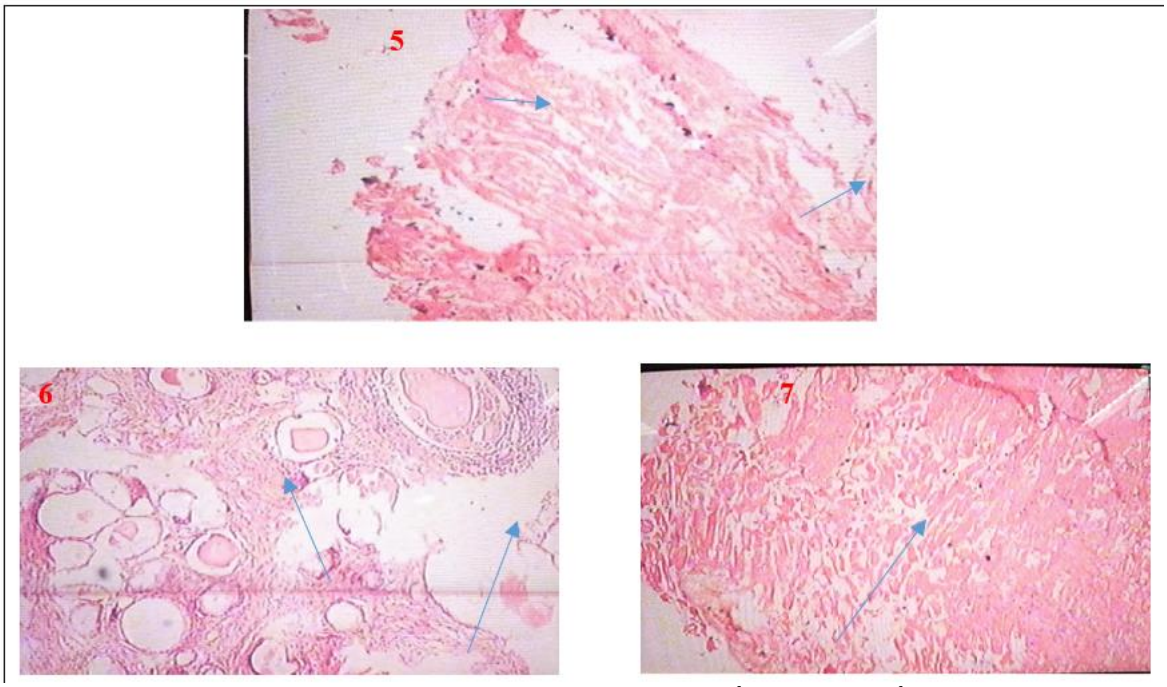


Figure 5,6,7: Histological analysis of fish muscles of T2 group after 7th(figure 7), 14th (figure 6) and 21(figure 7) days of exposure.