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Lead Nitrate Induced Histopathological Alterations in the Liver and Intestine of African Catfish *Clarias gariepinus* Exposed to Sublethal Concentrations

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Studies were carried out on the liver and intestine tissue of *Clarias gariepinus* after exposure to sublethal concentrations of LC_{50} lead nitrate for 20 days. Fish were distributed into four groups, control (0 mg/L, LC_{50} of $Pb(NO_3)_2$) and three groups exposed to 20, 40 and 60% of the LC_{50} of $Pb(NO_3)_2$ (16.12, 32.24 and 48.37 mg/L respectively). Section of hepatic and intestine tissues showed the normal structure for control group. However, treated hepatic tissues of fish exhibited altered its characteristic architecture, with remarkably high vacuolation in hepatocytes and hepatoctolysis. Also, central vein was dilated and increased hemorrhage in the sections of almost samples from treated fish. Increased number of fibroblasts and Kuppffer cells were observed in the examined sections from treated fish when compared to the control. Therefore, fish treated with 60% LD_{50} of $Pb(NO_3)_2$ observed the proliferation of fibrous in connective tissue particularly near sinusoids and substituting liver parenchyma. Therefore, intestinal sections from the different treated fish groups revealed an evident harmful to intestinal tissue, especially in enterocytes and structures of villi. Also, disturbed longitudinal and circular muscularis were observed, to abnormally dilated, lamina propria, was infiltrated with a huge numbers of inflammatory leukocytes. Mucous secreting goblet cells proliferated and multiplied in all exposed fish.

Keywords: Histological examinations, Liver, Intestine, Lead nitrate

Introduction

Distribution of heavy metals can increase by anthropogenic activities into natural aquatic environment.[1] However, aquatic organisms might be exposed to unnaturally high concentrations of these metals, one of these aquatic organisms is very important the fish that are comparatively sensitive to change in their aquatic ecosystem.[2] Therefore, fish health may reverse and give information evident to understanding a good indicator of the health status of a particular aquatic environment. However, initial effect of toxic exposure from some contaminants may only be apparent on cells or tissue level before significant alternates can be specified in behavior of fish or external and morphological appearance.[3–6] So that, histological investigation seems to be most sensitive parameter and is important to estimating the cellular alternates when fish exposed to pollutants that may be occur in target organs for example, the Nile tilapia liver[7], gills, liver and gut[8] and gills of *labeo rohita* exposed to lead nitrate.[9] Moreover, lead precipitation in different fish organs such as liver, digestive tract, kidneys, spleen and gills.[10]

Lead (Pb) is one of the heavy metal classified as dangerous substance in aquatic ecosystem.[2] However, Pb is a naturally occurring component, its environmental concentrations are highly increased by manufacture sources like metal mining basis, battery industrialization, Pb-based paints and leaded gasoline.[11,12] Sources of accumulates of Pb in aquatic organisms come from both food and water, although there are many evidences that Pb deposition in fish, might be originated from pollution of aquatic environment rather than diet.[13] Therefore, the increased levels of Pb in the water can increase mortality rate and may cause many biochemical and histopathological alterations in survived fish.[14,15] Studies of histopathology have been recognized to be reliable bio-indicators of compression in fish.[16] Therefore, the uptake of heavy metals occurs basically via the gills and epithelium layer of intestine.[17,15]

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The aim of the current study was to evaluate the damage impacts on the hepatic and intestinal tissues of *C. gariepinus* exposed to sublethal concentrations of lead nitrate for 20 days.

Materials and Methods

Experimental fish

Samples of African catfish *C. gariepinus* were brought to laboratory at Zoology department from the hatcheries of fish, King Abdulaziz City for Sciences and Technology, Mozahmiya, Saudi Arabia. Fish were acclimated for two weeks to laboratory procedures before beginning the experimental treatments.

Experimental design

Ninety six healthy acclimatized fish weighing 119.14± 5.21 g were distributed to four groups. After acclimatization period, 24 fish were placed in each glass aquaria containing 80 L of dechlorinated tap water as a replicate for each treatment. First group of these four was unexposed and served as control and other three groups were exposed to 20, 40 or 60% of the LC_{50} of lead nitrate $Pb(NO_3)_2$[18] which is 80.61 mg/L for *Clarias gariepinus* and calculated for this study to be 16.12, 32.24 and 48.37 mg/L of $Pb(NO_3)_2$[18] respectively then dissolved in deionized water, the experiment was carried out for 20 days. *C. gariepinus* were fed two times a day using commercial diet from Maram Feed Factory containing approximately 32% crude protein at the rate of 2% of the biomass. Water temperature was thermostatically controlled and maintained at 29±1°C, also, parameters of water quality were arranged and maintained in the suitable range for example, ammonia-N from 0.08 to 0.20 mg/L, pH from 7.0 to 8.0, nitrite-N from 0.15 to 0.35 mg/L, nitrate-N from 4.35 to 5.77 mg/L, and dissolved oxygen adjusted between 5.3 to 6.7 mg/L.

Histological studies

Five fish of each treatments were sacrificed for histopathological studies, sections of livers and intestines tissues of the control and the exposed fish were fixed in 10% neutral buffered formalin (NBF) solution, After suitable dehydration by graded concentrate of alcohols, paraffin blocks were prepared an 5µm thick bar were cut in the Rotator Microtome then stained with Haematoxylin and Eosin.[19] Histopathological alterations were scored −: a lack of structural alterations; +: slight structural alterations; ++: mild structural alterations; +++: severe structural alterations.[20]

Results and Discussion

Histopathological alterations in the hepatic tissues

The normal structure of the hepatic tissue from the control fish group is shown in Fig. 1A. The histopathological examination of the hepatic sections from the different treated fish groups revealed an obvious damage to this tissue ranged from mild to severe alterations. The treated liver sections examined observed that it had lost its characteristic architecture, with remarkably increased ratio of vacuolation in hepatocytes and hepatoctolysis (Fig. 1A). Moreover, the treated groups showing cytoplasm of the hepatocytes were characterized by rough, pink and darkly stained vacuoles and granules with hyperchromatic nuclei. The central vein was dilated in the sections of almost all samples from treated fish. In addition, increased hemorrhage in the central vein was observed. An increased number of fibroblasts and

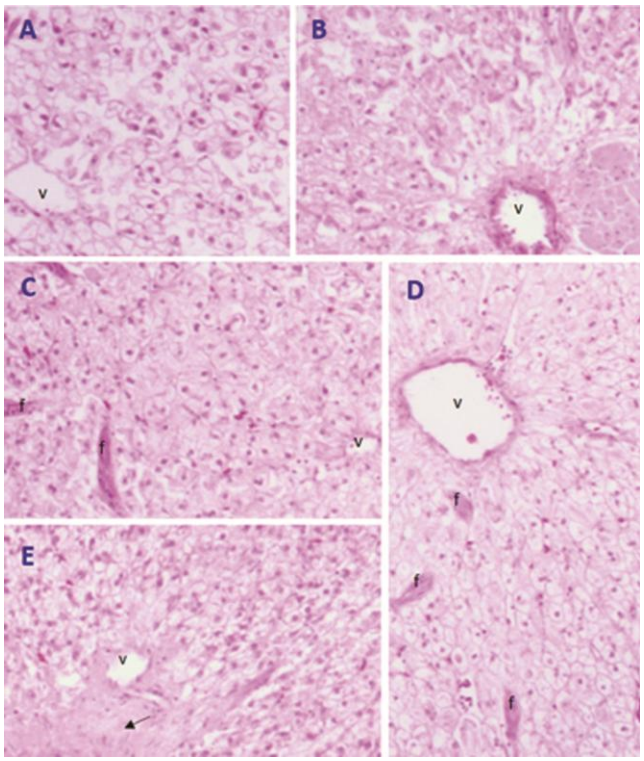

Fig. 1 — Representative photos of the hepatic sections (100X; H & E) (Control fish (A) observed the normal hepatic tissue structure, 20% of LC_{50} lead Nitrate (B, C) showing the thickness in the wall of the central vein (v) and increasing of the fibroblasts (f), 40% of LC_{50} lead Nitrate (D) showing the dilated central vein (v) and fibroblasts and 60% of LC_{50} of Lead Nitrate (E) showing the fibrosis (arrow))

Kuppffer cells were observed in the examined sections from exposed fish when compared to the control sections (Fig. 1; Table 1). After treatment with 60% of LD_{50} of Lead Nitrate, the proliferation of fibrous connective tissue especially near sinusoids observed, substituting liver parenchyma (Fig. 1E).

Histopathological bio-indicators have been firstly used in aquatic organisms such as fish to specify and estimate the toxic effects when exposed to heavy metals.[21] In addition, histology of fish liver might be used as a pattern for research in the reactions between ecological factors and liver structures and functions.[22] There are two factors revealing the deleterious effect of contaminants on liver histology of some fish species, the exposure period and the concentration levels of the identified heavy metals.

Current study demonstrated the pathological changes in fish exposed to three different doses of sub-leathal concentration of $Pb(NO_3)_2$. The examination of liver tissues showed loss its characteristic architecture, with remarkably excessive vacuolation in hepatocytes and hepatoctolysis. Exposure of *Oreochromis niloticus* to lead acetate showed histological alterations in the hepatic tissues which include degeneration and vacuolation of hepatocytes. In addition, Vacuolation of hepatocytes may cause inhibition of structure of protein, reduction of energy and assemblage of microtubules.[23] This might be attributed to an imbalance among the rate of structure of materials in the cellular of parenchyma and their releasing rate into the circulatory system.[24] This also, might be related to the event that when fish get exposed to heavy metal the damage to hepatopancreas occurred and then cause loss of contact between hepatocytes and pancreocytes and atrophy.

Also, the current study showed that the hepatic tissue of control groups displayed a normal structure and there were no histopathological changes. The hepatocytic cytoplasm of the exposed fish was characterized by rough, pink and darkly stained vacuoles and granules with hyperchromatic nuclei as presented in Fig. 1(B,C,D and E) and Table 1. The histopathology of liver for *C. gariepinus* exposed to various sublethal concentrations (20, 40 and 60% of LC_{50}) of lead nitrate observed significant changes including hypertrophy of hepatocytes, blood congestion and dilatation of central veins. Fibroblasts/fibrosis increased in the parenchymal tissues of liver with increased concentration of $Pb(NO_3)_2$ especially when fish is exposed to 60% of LC_{50}. It's demonstrated that the increase of lead levels in the environment as a pollutant causes cytoplasmic vacuolation and cellular degeneration. It is also harmful to nuclei and cause congestion in the blood sinusoids. Also, *Tilapia zillii*, exposed to aluminum showed that increase of aluminum concentration made histopathological alterations in hepatocytes.[25] The cells that are more related with the detoxifying and biotransformation process are the hepatocytes, and this was attributed to its position, function, and blood supply.[16] Moreover, liver histopathology of fresh water fish *Cirrhinus mrigala* exposed to lead nitrate at 4 mg/L concentration showed characteristics of congestion and lower degeneration of hepatocytes in the liver parenchyma. Other treated groups exposed to 6 mg/L showed that hepatocytes were decrease in size and liver parenchyma with feature of congestion and increased cytoplasmic vacoulation were observed. However, the histopathological alterations of liver fish exposed with 8 mg/L lead nitrate observed severe harmful and remarkable proliferation. Liver parenchyma showed cytoplasmic vacuolation of hepatocytes[26], this is in agreement with the above discussed studies.

Pollution by heavy metals can either decrease or increase the activities of hepatic enzyme[14] and can progress to histopathological changes in hepatic tissues, depending on the metals type, concentration, species of fish, period of exposure and other environmental factors.[7] The observation of histological changes in hepatocytes of fish is a highly sensitive and delicate way to assess the impacts of xenobiotic complex in the field and treatment studies.[27]

Table 1 — Histopathological alterations in the liver tissues. Sections of the liver from the control and from the different fish treatments exposed to 20%, 40% and 60% of the LC_{50} of Lead Nitrate

	Lead nitrate dose (mg/L)			
Parameters	Co.	20% of LC_{50}	40% of LC_{50}	60% of LC_{50}
Hepatocytolysis	--	++	++	--
Hepatic cytoplasm vacoulation	--	++	++	++
Kuppffer cells	--	+	++	++
Fibroblasts/fibrosis	--	++	++	+++
Dilatation of Central vein	--	++	++	+
Congested blood	--	+++	++	+
Hemolyzed blood	--	--	++	--
Melano-macrophage	--	--	--	--
The general architecture	--	+	+	++

−: a lack of structural alterations; +: slight structural alterations; ++: mild structural alterations;+++: severe structural alterations.

In addition, histopathological alterations in hepatic tissues has resulted from disturbances of different biochemical processes when *Oreochromis niloticus* exposed to various concentrations of cadmium chloride.[15] Moreover, current study observed high number of fibroblasts and Kuppffer cells in the examined hepatic sections from exposed fish groups when compared to control group (Fig. 1; Table 1) especially when *C. gariepinus* is exposed to high concentration of $Pb(NO_3)_2$ (60% of LC_{50}), the proliferation of fibrous connective tissue particularly near sinusoids are observed, substituting parenchyma liver tissues (Fig. 1E). Therefore, increase in cellular size occur when hypertrophy is generally characterized, observed by the increasing pollution of heavy metals in the aquatic ecosystem. Fish exposed to some components causes hypertrophy indicated by induced proliferation of the endoplasmic reticulum membranes.[28] These might be related to vacuoles in the hepatocytes, cytoplasm may contain glycogen and lipids, which are attributed to the normal liver metabolic function.[25] This is in accordance with the above discussed researches. Therefore, histopathological alterations of the gill epithelium and liver increased when *O. niloticus* get exposed to waterborne copper. It also affect the size of hepatocytes that might be attributed to the high amount of the lipids[27].

Histopathological alterations in the intestinal tissues

The normal structure of the intestinal tissue from the unexposed fish which serve as control group is shown in Figs 2A and 3A. The histopathological study on sections of the intestinal tissues from the various treated fish groups revealed an obvious harm to intestinal tissue, especially in the enterocytes and structures of villi (Table 2), as was histologically detected in repeated samples. The disturbed longitudinal and circular muscularis were also observed (Fig. 2, Table 2). Furthermore, to abnormally expanded, lamina propria, was infiltrated with an increased number of inflamed leukocytes (Fig. 3, Table 2). Mucous found to be secreting goblet cells proliferated and multiplied in all exposed fish as a defense mechanism against the damage (Fig. 3, Table 2). The dependent manner of the dose observed the damage of the tissues.

The first organs those can uptake metals are gills which can mainly affect directly from aquatic environment, thus may also be absorbed in the gut epithelium.[4] Therefore, toxicity of contaminations

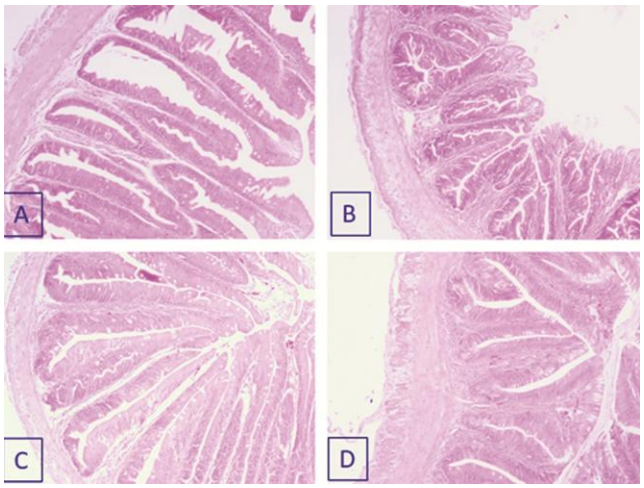

Fig. 2 — Histopathological alterations in the intestinal tissues (100X; H&E) (Sections of the intestinal tissues from the unexposed fish served as a control (A) and from the different fish groups exposed to 20% (B), 40% (C) and 60% (D) of the LC_{50} of Lead Nitrate; The representative photos from the control and the exposed fish groups show the different intestinal regions, muscularis, lamina propria and mucous layers)

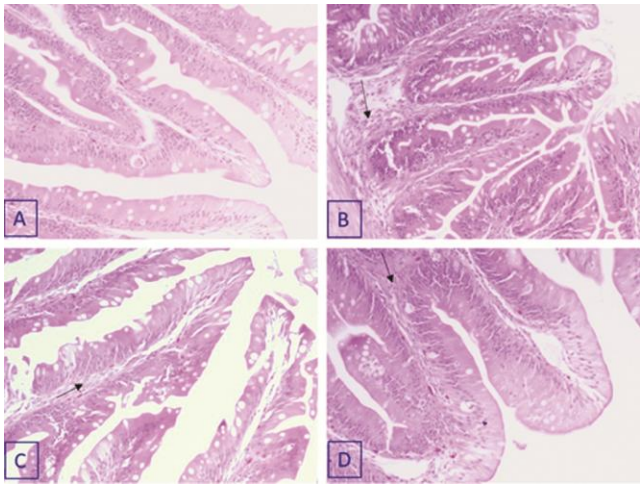

Fig. 3 — Alterations of histopathological in the intestinal tissues (400X; H&E) (Sections of the intestinal tissues from the control (A) and from the different fish groups exposed to 20% (B), 40% (C) and 60% (D) of the LC_{50} of Lead Nitrate; Arrows show the inflammatory infiltration of leukocytes into the lamina propria of the treated fish)

can enter the digestive system of fish through the uptake of the water and food, which might lead to the deterioration in the intestine causing alterations in its structure and function.[29] The current study revealed significant harm to the intestines of *C. gariepinus* fish exposed to various concentrations of sublethal doses of LC_{50} lead nitrate for 20 days. This histological examination was in accordance with Younis *et al*.[15] who found that exposure of

Table 2 — Alterations histopathological in the intestinal tissue. Sections of the intestinal tissues from the control and the different fish groups exposed to 20%, 40% and 60% of the LC_{50} of Lead Nitrate

Parameters	Lead nitrate dose (mg/L) Co.	20% of LC_{50}	40% of LC_{50}	60% of LC_{50}
Circular Muscularis	--	+	++	++
Longitudinal Muscularis	--	++	+++	+++
lamina propria infiltration	--	+	+++	+++
lamina propria connective tissue	--	++	+++	+++
Villi length	--	+	++	+++
Mucous cells	--	--	+++	+

−: a lack of structural alterations; +: slight structural changes; ++: moderate structural alterations; +++: severe structural alterations.

O. niloticus to cadmium chloride caused harm to the intestinal structure and function.[15] Current study demonstrated normal structure of the intestinal tissue from the control fish group. However, the other fish groups which exposed to 20, 40 and 60% of LC_{50} of $Pb(NO_3)_2$ showed evident harm to the tissue of intestine, especially in enterocytes and villi structures (Figs 2A and 3A, and Table 2). A disturbed longitudinal and circular muscularis were also observed. This is in agreements with the above discussed results. Moreover, pathological change in the intestine of *O. niloticus* when exposed to cadmium include atrophy in the muscularis mucosa, degeneration and necrosis in the submucosa and mucosa, and necrotic cells which found to be aggregated in the lumen of the intestine and edema in addition to atrophy in the submucosa.[15]

This study found remarkable changes and dilates in the lamina propria, infiltrated with an increased number of inflamed leukocytes (Fig. 3, Table 2). Also, mucous found secreting, the goblet cells multiplied and proliferated in all histological examinations for *Clarias gariepinus* exposed to $Pb(NO_3)_2$ as a protection mechanism against the damage (Fig. 3, Table 2), these harms to the tissue might be related to dependent manner of the dose. These histological examinations observed that the alterations in the histological sections of the intestine in *O. niloticus* treated by chromium and lead toxicity included severe degradation and necrotic changes in mucosa layer of the intestine.[30] This also, found that the edema observed between the submucosa and mucosa might be caused by the uptake of the toxic components. However, epithelial deterioration, inflammatory cell infiltration into the submucosa, and submucosal edema have also been observed in the intestine when mono-sex tilapia was treated with carbofuran pesticides for long time of exposure.[31]

Conclusion

In conclusion, this study was conducted to determine the health of fish influenced by the pollutants of aquatic ecosystem and pathological toxicity. The *C. gariepinus* is known as a good biological model for studying possibility of acclimations to contaminations. This investigation has confirmed the effect of toxicity of lead nitrate (as an aquatic environmental pollutant) to the *C. gariepinus*. Therefore, all sublethal tested concentrations of lead nitrate that can enter the digestive tract through the absorption of the food and water can have effect on histological sections causing harm and degeneration of structures in the intestine and hepatic tissues. Investigations using pollutants in this species are still very scarce thus it needs more considerations for hematological and histopathological studies on other organs such as kidney, gills and gonads.

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