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Toxico-histological Effects of Sublethal Concentrations of Lead Nitrate on the Gills of the African Catfish, *Clarias gariepinus*

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This study aimed to investigate the effects of subleathal lead doses on the histological architecture of gills in the freshwater African catfish, *Clarias gariepinus*. Results revealed three stages of changes after exposure to three doses of lead nitrate. The changes were mild in fish exposed to 16.12 mg/L of Pb $(NO_3)_2$ (stage I 20% of LC_{50}) and severe in case of 40% of LC_{50} (stage II, 32.24 mg/L) and 60% of LC_{50} (stage III 48.37% mg/L) in comparison to control. The histological examination revealed normal architecture of gills in control fish. Normal gills were characterized with primary gill lamellae with a central hyaline cartilage. However, three-stage changes were observed after exposure to the three doses of lead nitrate. In stage I, a cartilaginous matrix appeared along with loosening of the epithelial lining of the cartilaginous core and abundant vascular spaces were observed on the secondary gill lamellae. An extra cartilaginous matrix, loosening of the epithelial lining of the cartilaginous core, de-shaped gill lamellae, and degeneration of the secondary gill lamellae were noted in stage II. Lesions in the gill pattern in stage III were represented by shortening of the tips of secondary gill lamellae, damaged gill lamellae, a cartilaginous matrix, and hyperplasia. This indicated that lead exposure resulted in severe histopathological changes in the gills in a dose-dependent manner.

Keywords: lead, African catfish, Gills, Histological architecture

Introduction

In developing countries, many raw chemicals are discharged into the water without treatment[1]. Despite the large body of research showing its harmful effects, pollution of water with toxic chemicals is still one of the biggest challenges facing the aquatic ecosystem. Furthermore, pollution of the water with toxic chemicals is increasing because of anthropogenic activities, i.e. mining, coal and cement industries[2]. Hence, aquatic organisms, especially fish, are at severe risk due to the disposal of heavy metals in both fresh and marine waters[3]. Fishes are of economic importance for many countries, because they are a major source of protein and a good source of omega-3 fatty acids for humans[4]. Because they are sensitive to a wide range of chemicals, fish have been suggested as environmental biomarkers, to measure the heavy metal levels affecting organisms and ecosystem health[5]. Most heavy metals accumulate in fish, primarily in gills, liver, kidney, and bones, leading to functional disorders and structural lesions[6]. The toxic effects may hinder growth, reproduction, and physiological functions, thereby increasing mortality in fish[7].

Lead is a heavy metal that causes detrimental effects on aquatic organisms, especially fish, where its sublethal levels can produce hematological and neurological effects[2]. In fish, the gills contain a large surface area in contact with water and play a major role in diffusion between capillaries and dissolved O_2 for gas exchange[8]. Because of their delicate structure, gills are sensitive organs that can be easily damaged by minimum concentration levels of aquatic pollution[9]. Gills have been widely used for analyses of several pollutants and especially to detect lead toxicity[10]. The present study investigated the effects of lead on histological architecture of the gills in the freshwater fish, the African catfish, *C. gariepinus* a common fish species for the assessment of pollutant effects. We examined gill histology exposed to three sublethal lead doses.

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Materials and Methods

Experimental fish

The African catfish samples were collected from hatcheries in the King Abdulaziz City for Sciences and Technology, Mozahmiya, Saudi Arabia. Fish were transported to the lab in tanks supported by aeration units then acclimatized for 2 weeks to laboratory conditions prior to the start of the experimental treatments.

Experimental design

Ninety-six healthy acclimatized fish weighing 119.14 ± 5.21 g were divided into four groups of 24 fish each. Each group was placed in a glass aquarium (80 L, 100 × 50 × 40 cm^3) containing dechlorinated tap water. The first group of the four served as a control and was unexposed. The remaining three groups were exposed to 20, 40, and 60% of the LC_{50} of lead nitrate, $Pb(NO_3)_2$, according to a previous study[11], which reported that the LC_{50} was 80.61 mg/L for *C. gariepinus*. Thus, the calculated values for this study were 16.12, 32.24, and 48.37 mg/L of $Pb(NO_3)_2$, respectively, dissolved in deionized water. The experiment lasted 20 days. The *C. gariepinus* were fed twice a day with a pellet diet. The temperature was thermostatically controlled and ranged between 28 to 30°C. Furthermore, other parameters were adjusted and maintained in a suitable range for the *C. gariepinus*. For example, ammonia-N ranged 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 from 5.3 to 6.7 mg/L.

Histological studies

Sections of tissues from gills of the control and exposed fish were fixed in 10% neutral buffered formalin solution. After suitable dehydration using a graded concentration of alcohol, paraffin blocks were prepared and 5 μm thick bars were cut using a Rotator Microtome and stained with hematoxylin and Eosin. Histopathological alterations were evaluated for each section. They were scored between no change (−) and severe structural changes (+++). Sections from at least five fish from each treatment were carefully examined based on the methods of Dommels *et al*.[12].

Results and Discussion

Table 1 summarizes the histopathological alterations to the gill structure resulting from exposure to the three percentages of the lead nitrate LC_{50} in comparison to the control group of African catfish. The histological examination represented in Figure 1 revealed the normal architecture of gills in the control fish. Normal gills were characterized with primary gill lamellae with a central hyaline cartilage, and a secondary gill lamellae perpendicular to the primary lamellae with vascular spaces. However, three-stage changes were observed after exposure to the three doses of lead nitrate. Alterations in the gill pattern in stage I (20% of LC_{50} lead nitrate) were considered mild (Fig. 2) changes in comparison to that of the control group. These pathological alterations could be reversible with improvement in environmental conditions. The effects of 20% of LC_{50} lead nitrate on gills of African catfish included the appearance of a cartilaginous matrix, loosening of epithelial lining of the cartilaginous core, and abundant vascular spaces on the second gill lamellae. The alterations in the gill pattern in stage II (40% of the LC_{50} of lead nitrate) were considered to be severe lesions in comparison to that of the control group of African catfish. The representative photomicrographs (Fig. 3) for the effects of 40% of the lead nitrate LC_{50} on gills of

Table 1 — Histopathological changes for control and treatment groups (20%, 40%, and 80% of the lead nitrate LC50) in gills of African catfish

Parameters	Control group	20% LC_{50}	40% LC_{50}	80% LC_{50}
Central hyaline cartilage	+	+	+	+
Vascular spaces	+	++	--	--
Cartilaginous matrix	--	++	+	+++
Loosening of epithelial lining of cartilaginous core	--	+++	++	+
Distortion of the gill lamellae	--	--	+++	--
Degeneration of secondary gill lamellae	--	--	+++	--
Shortening of tips	--	--	--	+++
Damaged gill lamellae	--	--	--	++
Hyperplasia	--	--	--	+

--: Lack of structural changes; +: slight structural changes; ++: moderate structural changes; +++: severe structural changes

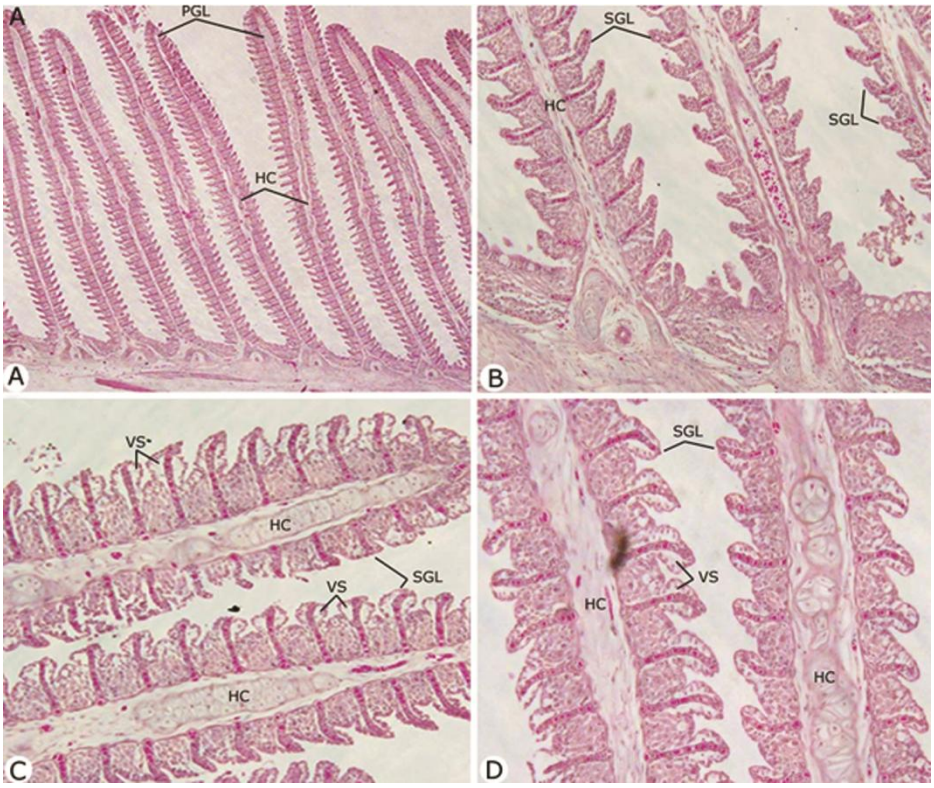

Fig. 1 — Photomicrographs for the control group with a normal gill architecture having a primary gill lamellae (PGL), a central hyaline cartilage (HC), and a secondary gill lamellae (SGL) perpendicular to the primary lamellae with vascular spaces (VS) (100×, 400×).

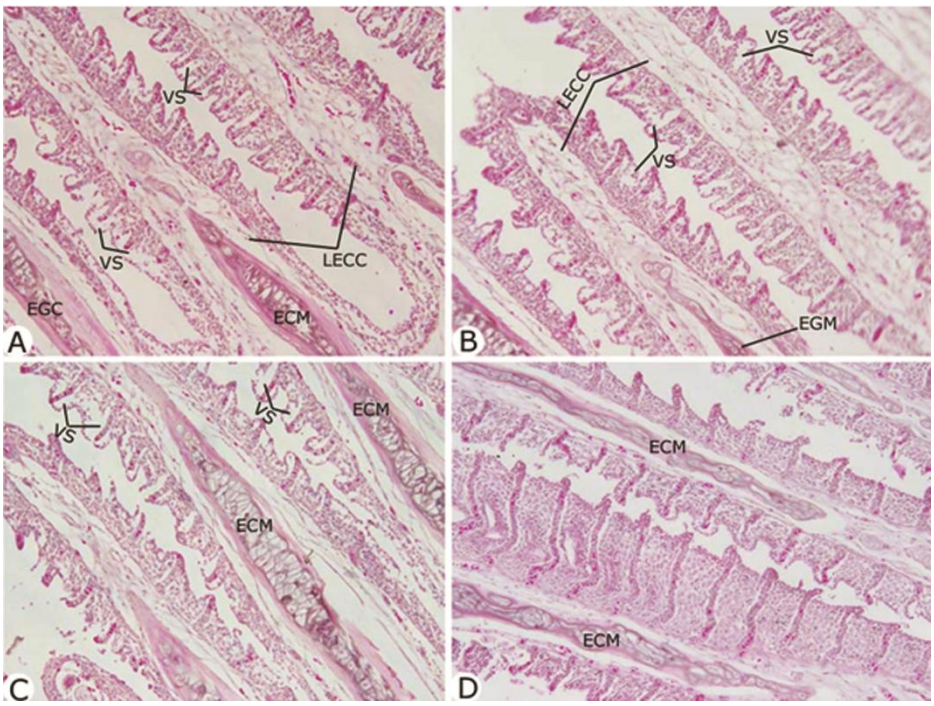

Fig. 2 — Photomicrographs for the effect of 20% of the lead nitrate LC_{50} on gills of African catfish showing the appearance of a cartilaginous matrix (ECM), loosening of the epithelial lining of the cartilaginous core (LECC), and abundant vascular spaces (VS) on the secondary gill lamellae (400×).

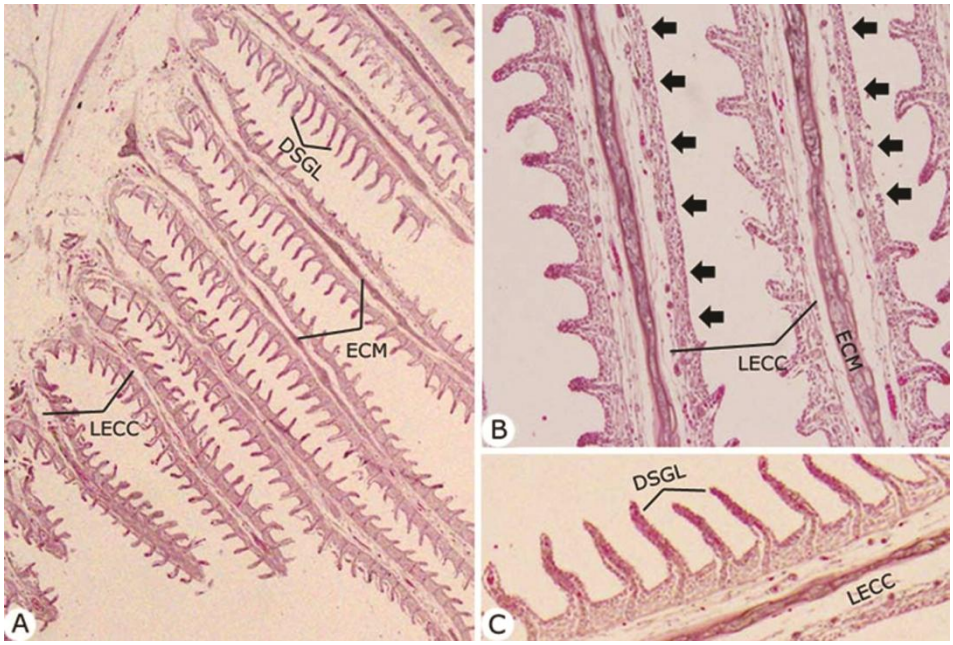

Fig. 3 — Photomicrographs for the effects of 40% of the lead nitrate LC_{50} on gills of African catfish showing an extra cartilaginous matrix (ECM), loosening of the epithelial lining of the cartilaginous core (LECC), distortion of the gill lamellae (DSGL), and degeneration of the secondary gill lamellae (arrows) (400×)

African catfish show an extra cartilaginous matrix, loosening of the epithelial lining of the cartilaginous core, de-shaped gill lamellae, and degeneration of the secondary gill lamellae. Furthermore, the lesions in the gill pattern in stage III (80% of the lead nitrate LC_{50}) could be considered severe changes in comparison to that of the control group (Fig. 4). The representative photomicrographs from gill sections for the effect of 80% of the lead nitrate LC_{50} on gills of African catfish showed the shortening of the tips of the secondary gill lamellae, damaged gill lamellae, the appearance of a cartilaginous matrix, and hyperplasia. It is clear that the exposure of various species of fishes to heavy metals in the environment is associated with obvious structural damage to various organs, including the gill epithelium. The role of the gills in respiration and ionic regulation in fish has encouraged many researchers to investigate the effects on this organ based on changes in the aquatic ecosystem[13]. The gills are indicators of aquatic pollution[3]. Lead is an element of industrial importance. However, it is the most damaging to aquatic fauna because it accumulates in the body of the organism, especially in fishes[2]. The current results are supported by Sharma *et al*.[14], who reported that lead nitrate has many effects on the gills of fishes, including structural and degenerative changes. The gills participate in gaseous exchange and also perform osmo regulation and excretion functions. Histopathological changes in the gills observed in this study are similar to those of previous observations of the results of the exposure of other freshwater fish to lead[14] and calcium[15]. It has been reported that gills accumulate the highest lead concentration. This may disrupt gaseous exchange and ion regulation[16]. Here, we found a wide range of histological changes in the gill tissue of the African catfish after lead nitrate exposure, which depended on the concentration of the exposure. For 20% of the LC_{50} of lead nitrate, the gills showed the appearance of a cartilaginous matrix, loosening of the epithelial lining of the cartilaginous core, and abundant vascular spaces in the secondary lamellae. Changes in the fish gills slowed the oxygen supply entering the blood, which, in turn, reduced gaseous exchange. The current results are supported by Sharma *et al*[14]. Furthermore, 40% of the LC_{50} of lead nitrate caused an extra cartilaginous matrix, loosening of the epithelial lining of the cartilaginous core, de-shaped of the gill lamellae, and degeneration of secondary gill lamellae. These results are in accordance with those of Brraich and Kaur[2]. The degenerative changes may be caused by additional toxicant penetration. Lead accumulates in the gills because of their direct contact with the water, which

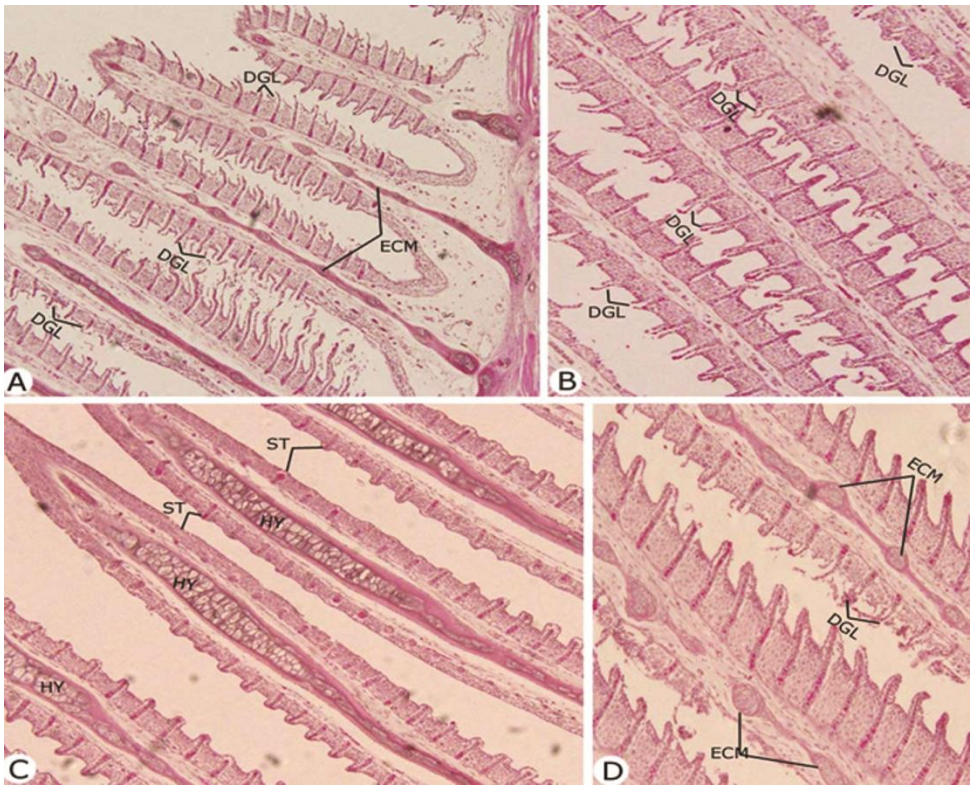

Fig. 4 — Photomicrographs for the effect of 80% of the lead nitrate LC_{50} on gills of African catfish showing the shortening of the tips (ST) of the secondary gill lamellae, damaged gill lamellae (DGL), the appearance of a cartilaginous matrix (ECM), and hyperplasia (HY) (400×).

allows the dissolved materials to be absorbed into the delicate epithelium. The degenerated cartilaginous matrix of the gill indicated that oxygen that reached the cells was significantly decreased, which, in turn, affects the growth rate of the cartilaginous matrix[2].Swelling and clumping of the secondary lamellae in the gills occurred in the marine winter flounder (*Pseudopleuronectes americanus*) exposed to copper. Because this species normally faces water loss due to osmosis, it is clear that copper is not merely increasing the osmotic permeability of the gill epithelium in this case. Given these structural changes, it is clear that heavy metals can produce profound effects on gill solute and water transport. Because it is evoked by many chemicals, oxidative stress in fish is of great importance in environmental and aquatic toxicology. Oxidative stress is defined as the imbalance between oxidants and antioxidant defenses[17]. Excess reactive oxygen species (ROS) can destroy nucleic acids, proteins, lipids, and other cellular compounds. Oxidative stress in fish is mostly studied in relation to environmental contamination by pollutants[18]. Thus, the gill damage in the present study might have been caused by the free radicals that were increased under the stress of lead toxicity. The release of ROS after chemical exposure can be realized via two ways: 1) the uncoupling of the electron transport chain from mono oxygenase activity and 2) altered metabolism. Rotenone can inhibit electron transport in the mitochondria and thereby increase ROS production[19].

Taken together, our data show that lead exposure resulted in severe histopathological changes in the gills of the African catfish. Our investigation also indicated that the histopathological changes observed in the gill tissue structure of African catfish were lead nitrate dose-dependent.

Acknowledgments

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