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Short Communication

HISTOPATHOLOGICAL ALTERATIONS IN THE GILLS OF FRESH WATER FISH, *LABEO ROHITA* (HAMILTON-BUCHANAN) EXPOSED TO HEAVY METAL MIXTURE (Cd, Cr & Pb)

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ABSTRACT

Histopathological alterations are the changes arising in the tissues of the organisms after exposure to the certain pollutants. Histopathology has been widely used as biomarkers in the analysis of the health of fish exposed to pollutants. Thus they are recommended as useful biomarkers in eco-toxicological research, risk assessment and monitoring programs. The heavy metals like cadmium (Cd), chromium (Cr) and lead (Pb) are very toxic to animals which enter surface water from various sources. Fishes carry on in near contact with the water through their gills and thus liable to heavy metals exhausted from various sources. In this study, an effort has been made to assess the effect of heavy metal mixture on the gill tissues of *Labeo rohita*. The fresh water fish, *labeo rohita* were exposed to heavy metal mixture for short term exposure period (24, 48, 72 & 96 hours). After the stipulated period of exposure fishes were sacrificed and gills were isolated and used for histopathological studies. The important alterations in the tissues were noticed. Gill tissue found that degeneration of epithelial lining and fusion of primary lamellae.

Keywords: Histopathology, *Labeorohita*, Gill tissues, Heavy metal mixture (Cd, Cr & Pb)

1. INTRODUCTION

There are many types of industrial waste water based on different industries and contaminants, each sector produces its own particular combination of pollutants. One of the most important types of pollution is heavy metal water pollution. The most common heavy metal pollutants are cadmium, chromium, lead, copper, and nickel etc. Histopathological changes have been widely used as biomarkers in the assessment of the health of fish exposed to pollutants, both in the laboratory and field studies. One of the great benefits of using histo-pathological biomarkers in environmental monitoring and this group of biomarkers allows examining specific target organs, containing gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [1]. In fishes, the gills are situated on each side of the head underneath a gill-covering operculum and are composed of finger-like filaments attached to a cartilaginous gill bar. Abundant, faint, leaf-like structures, the lamellae, project from each filament and these consist of tiny capillaries covered by a single layer of tinny epithelial cells. The epithelium forms a barrier between the fish's blood and the surrounding water. Gills are commonly considered a good tissue indicator of the water quality and are suitable for the assessment of environmental impact [2]. Histological alterations are the undesirable changes found in the tissues of animals after exposure towards certain heavy metals. Due to their toxicity, accumulation and bio magnification in water, residue, and in aquatic food chain [3] along with their association with various diseases [4], these heavy metals leads to significant environmental hazards for water bodies. Gills of fishes are the body parts for gaseous exchange and complete osmoregulation, acid-base equilibrium and nitrogenous waste excretion [5, 6]. The permanent increase of toxic materials more specifically heavy metals in water due to run off from industries and agriculture have severe impact on the aquatic animals [7]. Histological variations provide a rapid method to detect effects of irritants, especially chronic ones in several tissues and organs [8]. In the present study an effort has been made to assess the effect of heavy metal mixture on the gill tissues of *Labeo rohita*.

2. MATERIAL AND METHODS

2.1. Test organisms

Bulk of sample fishes, *Labeorohita* ranging in weight from 4-5 gms and measuring 6-7 cm in length were procured from Aliyar fish farm, Tamil Nadu, India. The procured

bulk samples of *Labeo rohita* were transported to the laboratory and were acclimatized in the laboratory conditions for two weeks in a large syntax tank. The tank was washed with potassium permanganate to prevent fungal infection prior to stocking. The water was changed daily to maintain the oxygen content and to remove the excreta of the fishes. The fishes were fed with rice bran and oil cake in the ration 1:1. Feeding was stopped two days prior to the experiment in order to keep the animal more or less in the same state of metabolic requirement. The tap water free from contaminants was used as dilution water for the study. Fishes were not fed during the toxicity tests. Fishes of same size irrespective of sexes were selected for the experiment. Continuous artificial aeration was main-tained throughout the acclimatization and exposure periods.

2.2. Acute toxicity tests

In acute bioassay studies, fishes of even size were taken and static bioassay methods are implemented. To find out the LC_{50} value the fishes were exposed to heavy metal mixture (Cadmium, Chromium, & Lead). The determination of LC_{50}, the lethal concentration at which 50% of fish dies. The LC_{50} values were determined for 96 hours by Probit Analysis [9]. The LC_{50} value is 0.500µg/l.

The fishes were exposed to lethal concentration of the heavy metal mixture for 24, 48, 72 and 96 hours. Another group was maintained as control. At the end of exposure period, fish were randomly selected for histopathological examination.

2.3. Histopathological examines

Tissues of gills were isolated from control and experimental fishes and the tissues were fixed in 10 percent formalin solution. After proper dehydration by graded alcohols, paraffin blocks were prepared and 4-5µ thick ribbons were cut in rotary microtome and were stained with Eosin and Haematoxlin. The histopathological changes observed were photographed.

3. RESULTS AND DISCUSSION

Gill histology of control (Fig. 1) fish showed the complete nature of both primary and secondary gill lamellae. The secondary lamellar surface was enclosed with simple squamous epithelial cells and capillaries divided by mucous cells. Each primary gill lamellae was flat leaf like in structure. It consisted of double rows of secondary lamellae with the central supporting axis. They were situated laterally on either side of the interbranchial septum. The secondary lamellae on both sides were highly vascularized and protected by a layer of cells with uniform interlamellar spaces. When the fish was exposed for 24 hours (fig. 2) to the short term exposure of heavy metal mixture, there was changes occurred like, lamellar fusion and degeneration of epithelial lining. After 48 hours (fig. 3) of exposure, a prominent degeneration was noted in the secondary lamellar of gill. After 72 hours (fig. 4) of exposure, there was fusion of secondary lamellae with irregular lamellar spaces. After 96 hours (fig. 5) of exposure, structural alterations such as epithelial proliferation, lamellar fusion and necrosis were detected. Edematous changes, described by epithelial detachment were perceived in gill filaments and secondary lamellae, Moreover, aggregations of inflammatory cells were observed in gill filaments also dilation and congestion in blood vessels of gill filament were detected. Atrophy of secondary lamellae was noted. The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion. Dilation and congestion in blood vessels of gill filament and atrophy of secondary lamellae also observed [10]. One of the main observations in the present study was the fusion of secondary lamella. This could be attributed to counter stress and transformation of electrically charged properties of the epithelial cells which favor adhesion between the cells of two neighboring secondary lamellae [11]. The fusion of secondary lamellae causes a extreme reduction in the respiratory surface area. Heavy metal could have prompted fusion of secondary lamella of gills. Hence it could be supposed that copper sulphate intoxication caused severe aerobic stress in *Anabastestudineus* leading to wear and tear in the gill epithelium. The other variations in the gill epithelium were the separation of respiratory epithelium from basement membrane leading to increasing thickness of secondary lamella thereby declined diffusion capacity and forming a barrier to inhibit entering of dissolved heavy metals [12]. Cell proliferation with thickening of gill filament epithelium may lead to the lamellar fusion [13]. The fusion and hyperplasia of gill lamellae may be induced by the effect of the toxin which alters glycoprotein in the mucus covering of the cells, thereby affecting the negative charge of the epithelium and favoring adhesion to adjacent lamellae [14]. Lamellar axis vasodilatation was also found in tilapia exposed to aluminum. Garcia-Santos, Fontaínhas Fernandes, and Wilson [15] mentioned that this lesion can encourage changes in pillar cell normal structure, with subsequent loss of their support function and probably, and was liable for the emergence of

lamellar aneurysms in fish. The pillar cell nucleus showed necrosis and vacuolation in the secondary gill epithelium. The disorganized fusion in secondary gill epithelium was obviously observed. Similar histological alterations in the gills were remarked by Velmurugan and coworkers [16] after exposure to organophosphates leading to epithelial proliferation, congestion of blood vessel and hyperplasia of mucus cells. In the present experiment, the gills of *Labeorohita* exposed to heavy metal mixture revealed the thickening of the primary lamellar epithelium and clubbing of secondary lamellae. Similar to those reported in *Latescalcarifer* exposed to 10 and 0.8 mg/L of cadmium for 96 h and 90 d, respectively, gill alterations included edema of epithelial cells with the breakdown of pillar cell system, aneurysms, hypertrophy and hyperplasia of chloride and mucous cells [17].

Alterations in the size of the nucleus in *Brachydaniorerio* exposed to sublethal concentrations of copper sulphate were studied by [18] Paris-Palacios *et al*. Das and Mukherjee, [19] reported on the dilation of tubules, necrotic variations characterized by karyorrhexis and karyolysis in the nuclei of affected cells of *Labeorohita* exposed to hexachlorocyclohexane. The lifting of lamellar epithelium is other histological change perceived, probably induced by the incidence of severe edema [20].

HISTOPATHOLOGY OF THE GILL OF *LABEO ROHITA* ON SHORT TERM EXPOSURE TO HEAVY METAL MIXTURE (Cd, Cr &Pb)

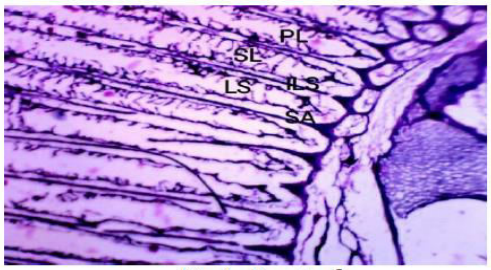

Fig 1: Control

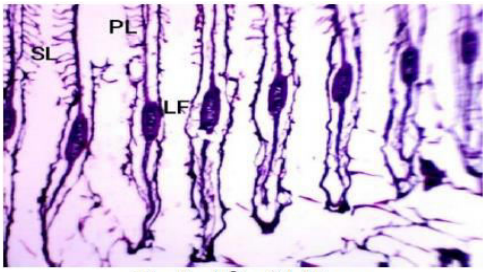

Fig 2: After24 Hours

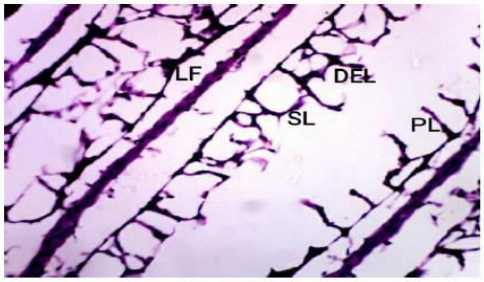

Fig3: After 48 Hours

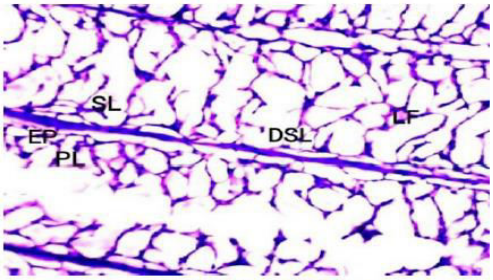

Fig 4: After 72 Hours

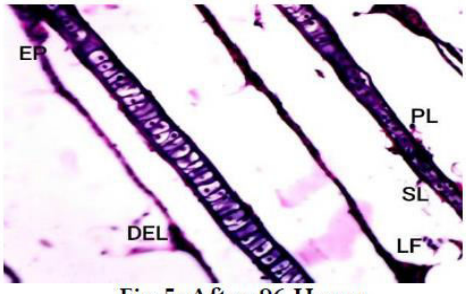

Fig 5: After 96 Hours

Fig 5: After 96 Hours

PL - Primary Lamellae
SL - Secondary Lamellae
LS - Lamellar Space
ILS - Inter Lamellar Space
SA - Supporting Axis
LF - Lamellar Fusion
DEL -Degeneration of epithelial lining
EP - Epithelial proliferation
DS - Degenerated Secondary Lamellae

4. CONCLUSION

The present study showed that the toxic effect of heavy metal mixture caused destructive effect in the gills of the fish, *Labeo rohita*. The results have confirmed histopathological alterations like cellular hypertrophy, hyperplasia, vacuolation, epithelial lifting, shortening, curling and abnormal elongation of the secondary lamellae, fusion of adjacent lamellae, telangiectasis, blood congestion, interstitial edema, necrosis, architectural distortion and degeneration of gills. Histopathological studies on acute exposure of fish to heavy metal mixture helps to diagnosis the impact of heavy metal in the fish tissues. The extent of damage of the gill tissue was proportionate to the dose and duration of exposure of the fish to heavy metal mixture. The pollution in natural aquatic ecosystems is one of the main causes for fast depletion of fish diversity. It will not only interrupt the natural ecology but will also seriously disturb the commercially important fish fauna.

5. ACKNOWLEDGEMENT

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