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Toxic effects of trivalent and hexavalent chromium on histopathology of gills in freshwater carp fish *Catla Catla*

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Abstract
Chromium, the heavy metal is an industrial effluent; a water pollutant enters into the food chain of aquatic ecosystems responsible for changes in the structural and morphology of the organs of the biota such as the fresh water fishes. Catla catla, a fresh water teleost fish, fingerlings when exposed to different sublethal concentrations of chromium for a different periods 8, 16, 32 days of time brought changes in the structure and morphology of the osmatic organs such as gills. In trivalent chromium exposed fish, a few pathological changes were seen during the initial days of exposure and then structural reorganization observed in later days of exposure, whereas in hexavalent chromium exposed fish the gills exhibited conformational degenerative changes such as swellings at the base of secondary gill lamellae, hypertrophy, hyperplasia and necrosis etc., all these changes confirmed structural disruption by hexavalent chromium ions on prolonged exposure.

Keywords: chromium, catla catla, hexavalent chromium, gills

Introduction
Metal toxicity or metal poisoning is the toxic effect of certain metals in certain forms and doses on life. The disorders in the metabolism, of the fish were confirmed by the histological changes observed in the tissues like gills, kidney, liver and muscle. Amongst the aquatic pollutants, heavy metals are of great concern. Excessive mining and anthropogenic activities has polluted our entire ecosystem (Vincent, S., *et al*., 2002) [10]. The concentration of heavy metals is especially high in water bodies that deteriorate the life sustaining quality of water and damage both flora and fauna (Kasherwani, D., *et al*., 2009; Zyadah, M.A *et al*., 2000; Lliopoulou - Georguadaki, J.*et al*., 2001; Verma, *et al*., 2005; Sharma, *et al*., 2005) [8]. Under normal circumstances, metal ions such as copper, zinc, nickel, ferrous, manganese and chromium etc. are beneficial in traces and commonly known as trace elements. However, they become toxic to all organisms above a certain concentration (Javed, M, 2004) [5]. Due to this reason, heavy metals toxicity received considerable attention in aquatic organism especially in fish (Javed, M, 2002) [4]. The metals enter fish bodies through body surface, gill or the digestive tract (Vincent, S., 2002) [10].

Catla catla (Hamilton) is the prime cultured species in India that occupies a prominent position in aquatic system. It is for this reason that the present work was carried out on this animal. In fish the gills are the first target of waterborne pollutants due to their direct and constant contact with surrounding water. Gills are responsible for gas exchange, ion regulation, acid-base balance and excretion of nitrogenous wastes, therefore, highly vascular organ as compared to other tissues.

Histology, is the study of micro anatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary science, first cellular investigation were carried out in the mid nineteenth century (Virchow, 1858) [11]. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical or heavy metal.

The literature clearly documents that different toxicants can cause different structural changes in various organs of aquatic animals. As this line of information provides support for the biochemical alterations, a histopathological study is included in this investigation and studied light microscopic changes in the organs such as gills of fresh water fish *Catla catla* exposed to both trivalent and hexavalent chromium stress. As one day of exposure to the sublethal concentration of either trivalent or hexavalent chromium is too short to find out any deleterious changes in the histology of any tissue, the study was made at three exposure periods i.e., immediately after 8, 16 and 32 days in order to observe the short-term and long-term effects of exposure in the organs such as the gills, were taken for the present study**.**

Materials and Methods
Catla catla (Hamilton, 1822), the Indian major carp is an economically important edible fish, having a great commercial value, occurs abundantly in fresh water tanks and ponds, collected from the department of fisheries, Anantapur, Andra Pradesh, and were immediately transported in big fish containers in the laboratory Then they were released into large cement tanks contained of chlorinated tap water. The fish were fed with commercial fish pellets having around 40% protein content, and allowed to acclimatize for 15 days. Then the fish were isolated into batches having weight of 10±2gms were maintained in static water without any flow. Water was renewed every day to provide fresh water rich in oxygen. The quality of dechlorinated tap water used for the experiment was analysed and various parameters such as dissolved oxygen - 6.8mg/l, alkalinity-130mg/l, hardness-125mg/l and pH-7.3 were measured and maintained. Water temperature was maintained between 22 ± 3ºC as recommended by APHA during experiment. During experimentation water was aerated once a day to prevent hypoxic conditions. As the level of toxicity reported to vary with the interference of extrinsic and intrinsic factors like temperature, salinity, P^H, hardness of water, exposure period,

density of the animals, size, sex etc., (Sivaramakrishna *et al*., 1991), and precautions were taken throughout this investigation. Lethal concentration (LC50) of chromium chloride (trivalent and hexavalent) to fish *Catla catla* was determined by "Probit method" of Finney (1971). Based on the fact that the effect of a metal on fish becomes consistent within 96 hour of exposure (Eisler, 1977), LC_{50S}/96 hours of trivalent and hexavalent chromium are considered as lethal concentrations. So, about 1/10 th of the 96 h LC_{50} lethal concentration was taken as sublethal concentration i.e., 59.68mg/l, 100 mg/l(Cr as 35.40mg/lit) were the lethal concentrations, 5.96 mg / l of trivalent chromium and 10 mg /l(Cr as 3.54 mg/lit) of hexavalent chromium respectively was taken as the sublethal concentration for further studies. For experimentation, 200 healthy fishes were taken and divided into two batches, each batch again divided into 5 groups and each group contains 20 fishes. Batch-1 was exposed for sublethal concentration (1/10 of LC50) of trivalent chromium and Batch-2 was exposed for sublethal concentration of hexavalent chromium. The I-group fishes of two batches was controlled unexposed, II-Group exposed for 1 day, III-Group for 8 days, IV-Group for 16 days and V-Group for 32 days. After the completion of stipulated exposure period, the fish were sacrificed and isolated tissues such as gills under laboratory conditions for biochemical analysis and histopathological studies. These tissues were removed and washed with saline then fixed in buffer formalin (10%) processed for sectioning (5-6um) and staining with haematoxyline and eosin. Photographs were taken.

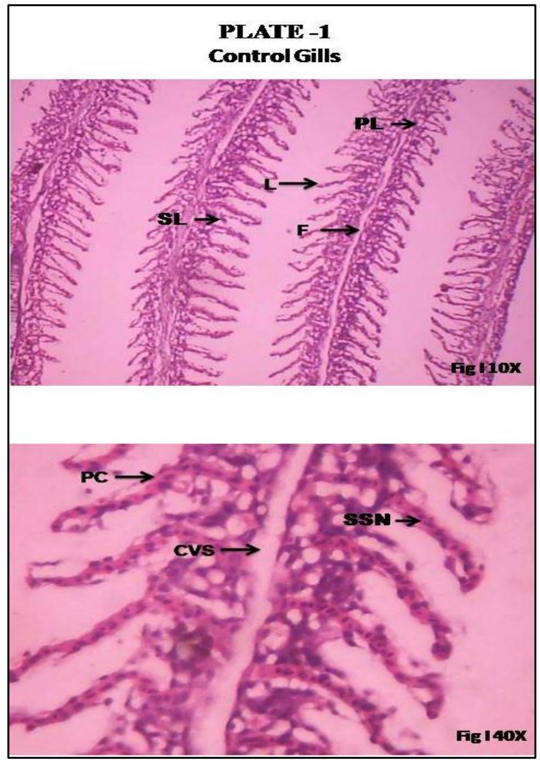

Fig 1: Control Gill with lower magnification (10X); and Higher magnification (40X).

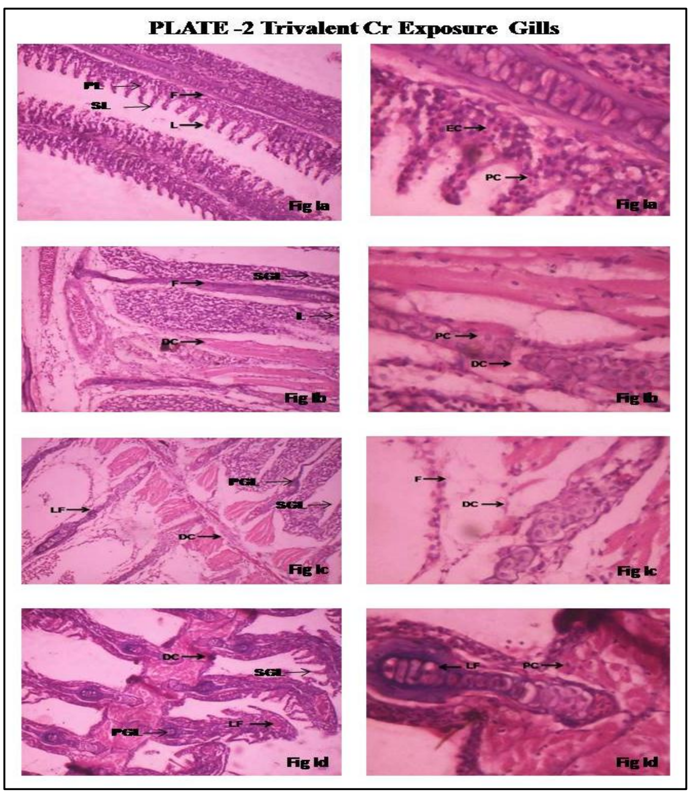

Fig 2: Fish exposed to sublethal conc. of trivalent chromium for day1, with lower magnification (10X); and Higher magnification (40X).Fig:Ib. Fish exposed to day 8, with lower magnification (10X); and Higher magnification (40X).Fig:Ic. Fish exposed to day 16, with lower magnification (10X); and Higher magnification (40X).Fig:Id.Fish exposed to day 32, with lower magnification (10X); and Higher magnification (40X).

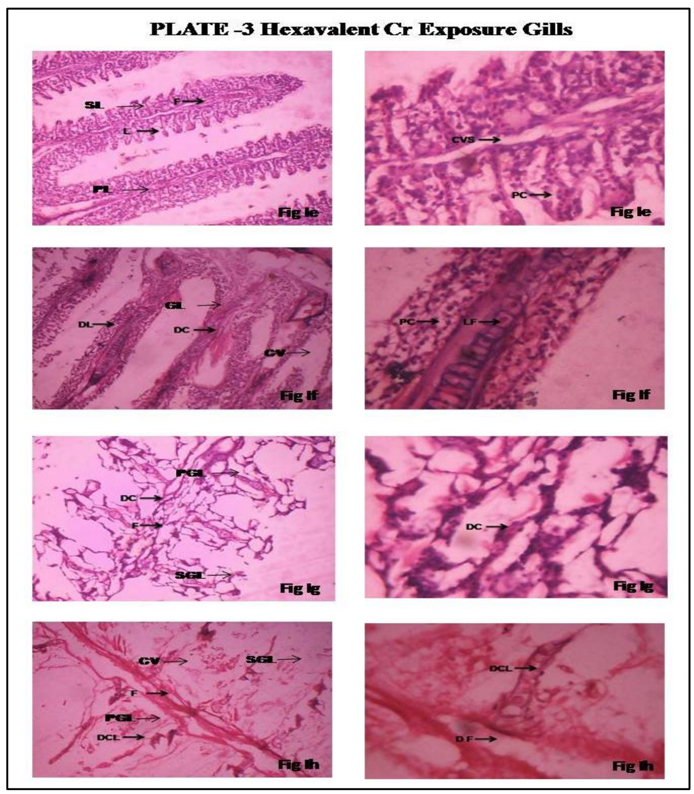

Fig 3: Fish exposed to sublethal conc. of hexavalent chromium at day 1, with lower magnification (10X); and higher magnification (40X).Fig: If. Fish exposed to day 8, with lower magnification (10X); and higher magnification (40X).Fig: Ig. Fish exposed to day 16 with lower magnification (10X); and higher magnification (40X).Fig: Ih. Fish exposed to day 32, with lower magnification (10X); and Higher magnification (40X).

Results and Discussion

The structure of the gill of normal control fish consists of primary and secondary lamellae, with well-marked inter lamellar spaces. The gill lamella consists of respiratory epithelial cells and pilaster cells situated in between the blood capillaries (Fig I).

On exposure of the fish for 1 day to sublethal concentration of trivalent chromium no significant pathological changes were observed in the gills of the fish (Fig Ia). On exposure of 8 days certain changes were observed in the gill of the fish. There were hypertrophy and hyperplasia in the secondary gill lamellae, erosion of surface epithelial cells, hollow spheres due to the loss of mucus from mucus cells, secondary gill lamellae became thin and wavy appearance of pillar cells and capillaries. At some regions loss of lamellar structures in the gill was seen due to atrophy of the gill lamellae (Fig Ib). For a period of 16 days a further damage was occurred in the gill structure. The primary and secondary gill lamellae showed heavy hypertrophy. In the secondary gill lamellae there was atrophy and swelling at the base with desquamation of the hyperplasic epithelia, significant hemorrhagic conditions are also noticed in the primary gill lamellae (Fig Ic). for a period of 32 days very less degenerative changes were observed in the structure of the gills appeared with mild degree of hypertrophy in the lamellar cells with a moderate degree of necrosis. But, the primary and secondary gill lamellae were distinct with interlamellar spaces. The cells of the gill lamellae seemed to have undergone a mild degree of hyperplasia (Fig Id).

On exposure for a period of 1 day to the sublethal concentration of hexavalent chromium, no significant changes were observed in the structure of normal control gill (Fig Ie). On exposure of 8 days certain degenerative changes were noticed in the structure of the gill, swellings at the distal and basal portions of the gill lamellae, greater loss of mucus from the mucus cells followed by the atrophy of the gill lamellae. There appeared nuclear pycnosis, moderate hemorrhagic condition, distinction of gill filaments and cytoplasmic vacuolization (Fig If). On exposure of 16 days a further damage is noticed in the gill structure. Heavy swellings in the primary and secondary gill lamellae and the secondary gill lamellae exhibited atrophy at the base with desquamation of the hyperplasic epithelia, significant hemorrhagic conditions are also noticed in the primary gill lamellae (Fig Ig). On exposure of 32 days, further degeneration of the gill lamellae observed with eventual fibrosis and telangiectasis was noticed. Hemorrhagic condition in the primary gill lamellae with cytoplasmic vacuolization was seen. The secondary gill lamellae have undergone a clear increased hyperplasia with desquamation of epithelial cells. The lamellar structure was totally loss with cytoplasmic vacuolization and erosion of gill epithelial cells (Fig Ih).

Conclusions

1. The disorders in the metabolism, of the fish were confirmed by the histological changes observed in gills of the fish. In trivalent chromium exposed fish, a few pathological changes were seen, during the initial days of exposure a structural reorganization observed on further exposure.
2. The increase in oxidative and glycolytic cycles might have facilitated the protein synthetic abilities for the structural reorganization in the trivalent chromium exposed fish, whereas in hexavalent chromium exposed fish the gills exhibited conformational degenerative changes such as swellings at the base of secondary gill lamellae, hypertrophy, hyperplasia and necrosis
3. On the whole, based on the biochemical and histological responses it can be inferred that the adaptive ability of the fish to heavy metals is not only dependent on the concentration of the metal but also the nature of the metal and the length of exposure.
4. Even though the sublethal concentration of trivalent chromium initially suppressed some of the biochemical activities, disturbed their structural integrity, but on prolonged exposure these animals could resist the trivalent chromium stress by bringing the necessary biochemical changes leading to the repair of the loss of structural integrity.
5. So, the *Catla catla* can tolerate the lower concentrations of trivalent chromium to a considerable degree and can adapt to such concentrations without suffering lasting effects. It could be due to the rapid protein synthetic ability at the organ level and metabolic reorganization at cellular level.
6. But the same fish could not resist the sublethal concentrations of hexavalent chromium for a longer period due to the suppression of its biochemical activities by hexavalent chromium ions. The loss of biochemical integrity of the cell due to the interaction of hexavalent chromium ions might be the reason for its incapability to adapt to the hexavalent chromium.

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