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Short Communication

Teratological Effect of Various Sublethal Concentrations of Chromium Hexavalent [Cr(VI)] on the Gills of *Cyprinus carpio*

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ABSTRACT

The objective of the present study was to identify the degree and damage to the histological structure of gills in *Cyprinus carpio* exposed to various sublethal concentrations of chromium hexavalent [Cr(VI)]. For this investigation 168 *C. carpio* breeders (W=500±9.5 g; L=25.60±2.6 cm) were divided at random in to control (n=24) and experimental (n=144) @ 8 breeders/tank (8.84 m^3). Control was kept in normal water, while experimental were monthly exposed to various sublethal concentrations of Cr(VI) (25, 50, 75, 100, 125 & 150 mg/L) separately in triplicate for six months. Actual chromium in water was measured on atomic absorption on monthly basis. In exposed group gill epithelium was found to be severely damaged, necrosed and peeled off with hyperplasia at all the Cr(VI) concentrations in temporal dose pattern. Absconding secondary lamellae and talengectases were noted at 125 and 150 mg/L Cr(VI) concentration. Actual chromium concentration varied from 36 to 118 µg/mL in tanks exposed to Cr(VI) from 25 to 150 mg/L. However, gills had normal histological structure in control. Present results showed that Cr(VI) is highly teratogenic metal and its deteriorating affect increase with the increase in concentration of metal. © 2012 Friends Science Publishers

Key Words: *Cyprinus carpio*; Hyperplasia; Necrosis; Lamellae; Gills

INTRODUCTION

Indiscriminate introduction of hexavalent chromium [Cr(VI)] from various industries into the aquatic ecosystem has a major threat for the survival and growth of fish (WHO, 2000). In fish, Cr tends to accumulate in tissues, through the gill surfaces and gut tract wall, at higher concentrations than those found in the environment (Pedro & Alicia, 2008; Mishra & Mohanti, 2009). Prolonged exposure of sub lethal and least lethal Cr(VI) doses exceeded the maximum safe limit for metal accumulation in tissues (Taman *et al.,* 1998; Aslam *et al*., 2011).

Accumulation of Cr(VI) in the tissues of organisms resulted in chronic illness and caused potential damage to the living organisms (Atli *et al*., 2006). The effect of Cr(VI) at sublethal concentration of 48 mg/kg on the gill architecture of the *Salmo giardneri* was investigated for 96 h. Cr(VI) intoxication resulted into histopathological abnormalities in the gills with hyperplasia and hypertrophy of the respiratory epithelium, fusion of lamellae, and hypertrophy of mucous cells and necrosis of epithelial cells (Koca *et al*., 2005). Cr(VI) caused skin lesion along with the irregular movement in fish due to heavy metals, which produced stress on gills epithelia (Jaffri *et al*., 2003). Heavy metals completely deteriorated gill architecture and induce hypoxia, anoxia, hypertrophy and talengectiensis in gills (Ahmet, 2005; Atli *et al*., 2006; Jiraungkoorskul *et al.,* 2007; Pedro & Alicia, 2008; Suwarna *et al.,* 2008; Costa *et al*., 2009; Rauf *et al*., 2009). Naeem *et al.* (2011) suggested that most of the metals (Na, K, Ca, Mg, Cu, Zn, Cr, Cd & Pb) showed an isometric increase while Mn, Fe and Co showed an allometric increase with increasing body weight.

Present study was designed to check the effect of prolonged exposure of various sublethal Cr(VI) concentrations on *C. carpio* gills. This will not only show the level of deterioration by Cr(VI) at different sublethal concentrations but also will confirm the teratogenic effect of Cr(VI) on fish.

MATERIALS AND METHODS

In this experiment 168 *Cyprinus carpio* breeders (W=500±9.5 g; L= 25.60±2.6 cm) were maintained in cemented tanks (8.84 m^3) of Fisheries Research and Training Institute, Lahore @ 8 breeders/tank. They were divided in to control (n=24) and treated (n=144). Control

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were kept in normal water, while treated were exposed to 25, 50, 75, 100, 125 and 150 mg/L Cr(VI) concentrations separately in triplicate for each concentration for six months on monthly basis, basing on LC_{50} of 191mg/L (Tayybah *et al*., 2005). Actual chromium in water was measured on monthly basis through atomic absorption. Breeders were provided with feed (30% protein & 8% carbohydrate rich) @ 7% of wet body weight per day spread over two feeding each day (Jhingran, 1995). Aeration was provided with automatic compressor. The water was removed on every alternate day to siphon off the extra food. After six months five pairs were sacrificed on random basis from control as well as treated group (from each dose). Tissue samples of gills were collected with sharp razor blade for histological evaluation. Tissues were preserved in Bouin's for 6-24 h washed with water and ethanol and then processed routinely in to hours, sectioned at 3 µm and stained with hematoxilin and eosin (Dacie & Lewis, 1991).

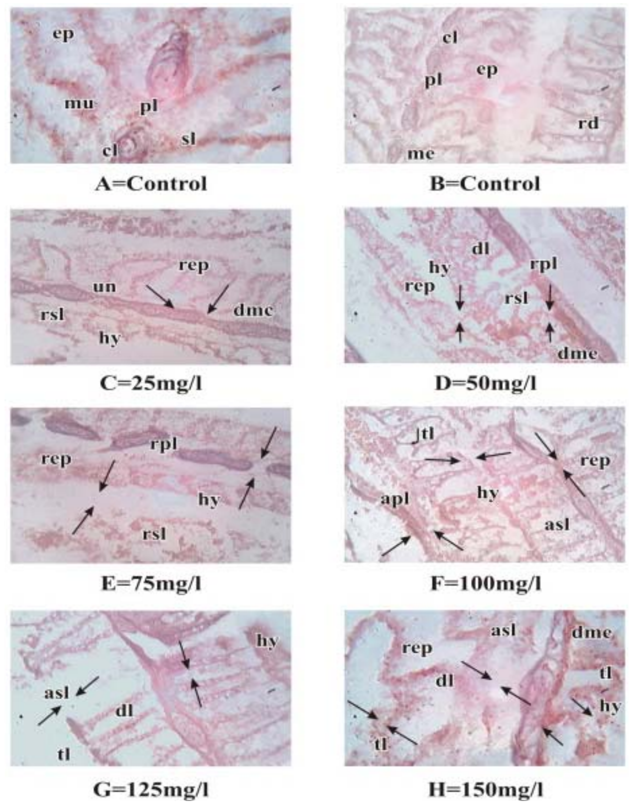
RESULTS AND DISCUSSION

In control water no Cr(VI) was detected, while in exposed 36, 43, 73, 88, 112 and 118 µg/mL chromium was detected at 25, 50, 75, 100, 125 and 150 mg/L. Breeders exposed to Cr(VI) through water has maximum concentration of metal in water. From water it entered in to fish gills. Gills are the respiratory organs, continuously exposed to water and playing important role of gaseous exchange in fish. The diffusion of oxygen in to blood took place at the surface of thin walled gill lamellae that's why any change in fish due to toxicity is first detected through gills (Fig. 1). Gills in control *C. carpio* (Fig. 1A & B) had a proper basal membrane, mucous cells, primary and secondary lamellae. Epithelium of lamellae was 1-2 cells thick. The chloride cells are small in number and located on the sides of lamellae.

However, gill filament of treated had degenerative changes. At 25 mg/L Cr(VI) dosage chloride and mucous cells were shrunken in size (arrows) with necrosed epithelium, hypertrophied and abnormal lamellae (primary & secondary) and hyperplasia (Fig. 1C). Peeling off respiratory epithelium, oedematus (separation of primary lamellae from basal membrane), ruptured lamellae (primary & secondary) with club shaped tips was noted at 50 (Fig. 1D) and 75 (Fig. 1E) mg/L. Shortening of primary lamellae with abundant gaps, vacuolization at the basal region of primary lamellae and lamellar talengectases on the secondary lamellae (arrows) was noticed at 100 mg/L (Fig. 1F). Decrease in chloride, mucous and pillar cells with abundant destroyed lacuna, fusion of secondary lamellae, talengectases, acute ruptured lamellae (primary & secondary) and damaged blood system due to Cr(VI) toxicity was noted at 125 and 150 mg/L (Fig. 1G & H). Fish gills were the first entry point for any pollutant present in water. Gills showed histopathological changes as proliferation of epithelium, fusion of lamellae and

Fig. 1: Microphotograph showing marked histological alterations in *C. carpio* gills due to Cr(VI) toxicity at 25(c), 50(D), 75(E), 100(F), 125(G) and 150(H) mg/L respectively while control (A & B) showed normal tissue architecture

Note: dl=destroyed lacuna, asl=absconding secondary lamellae, hy=hypoplasia, apl=abnormal primary lamellae, cl=chloride cells, dmc=destroyed mucous cells, tl=talengetases, apt=apoptotic tissue, rsl=ruptured secondary lamellae, rd=rodlet, un=undifferentiated basal cells, rpl=ruptured primasry lamellae, rbm=ruptured basal membrane (100X)



necrosis. It damaged the gill tissues and caused coagulation of mucous in the gills region as well as on the skin. This in turn provided respiratory stress and sluggishness in swimming movement of *C. carpio* breeders with the increase in Cr(VI) dosage from 25 to 150 mg/L (Gauthier *et al.,* 2006; Fernandes *et al*., 2007). Hyperplasia in gills due to Cr(VI) initially entered the tip of secondary lamellae and then spread inward, indicating a proliferative bronchitis that caused epithelial hyperplasia. Hyperplasia might also cause obliteration of the entire interlamellar space and in severe cases also caused fusion of adjacent lamellae (Abbas & Ali, 2007). Lamellar hyperplasia is a long term response of the malpighian cells, often indication of low level of irritations. They migrated distally, often in the early stages, resulting in an accumulation of cells at the leading edge of the secondary lamellae, known colloquially as clubbing of the lamellae (Suwarna *et al.,* 2008). Telangiectasis noted on secondary lamellae may be due to the dilation of small blood vessels by Cr(VI) toxicity. Frank necrosis of gill tissues was characterized by the destruction of secondary lamellae and in severe cases the stripping of gill tissues down to the cartilaginous skeleton of the primary lamellae (Ahmad *et al.,* 2006; Lucy *et al.,* 2006; Fernandes *et al.,* 2007; Pedro & Alicia, 2008; Costa *et al*., 2009; Mishra & Mohanty, 2009; Hussain *et al.,* 2011).

CONCLUSION

The findings of present investigation demonstrate a direct correlation between Cr(VI) concentrations and histological disorder in tissues of gills.

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