



Toxic effect of chromium on liver of Cyprinid Fish, *Cyprinus Carpio*

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Abstract: Studies to know the impact of chromium on the liver of *Cyprinus carpio* was made. In this context, *Cyprinus Carpio* body lengths (4-5cm) were brought live in the laboratory. After acclimatization the fish were exposed to four different concentrations 25mg/l, 30mg/l, 35mg/l and 40mg/l. The results were matched with the control. The findings of the present studies indicate that the effect of chromium is dose dependent. The hepatocytes arranged the hepatocords become narrow and compressed lobular boundaries and the kupffer cells disintegrated and the picnosis was also noted in the hepatocytes at the lower concentration of chromium (Cr). The liver of the fish exposed to higher concentration (35 and 40mg/L) showed the appearance of mass and vacuolation. These changes probably related to the hepatoma.

Keywords: Chromium, Liver of *Cyprinus carpio*

1. **INTRODUCTION**

Water is essential for life on the earth and universal solvent. Increasing number of industry waste is resulting of aquatic pollution (Seğlamtimur *et al.*, 2003). The contamination of fresh water with wide range of heavy metals is burning issue of the world. Some toxic metals are lead, nickel, cadmium, mercury, arsenic, copper, zinc and chromium is one of them, originated from electroplating, dyeing, tanner, hard-alloy steel and stainless steel industry (Wepener *et al.*, 2001; Mansour and Sidky, 2002; Costa, 2003; Yilmaz, 2003; Karadede-Akin and Ünlü, 2007).

The fauna that is living in the water is affected drastically due to change in the quality of water. Adverse effects caused by heavy metals on metabolism, growth and reproduction of the fish reported by (Canli and Furness, 1993; Parashar and Banerjee, 2001; Daksh and Capoor, 2011 and Hassan, 2011). The freshwater fish, common carp (*Cyprinus carpio* L.) is of great commercial importance because it is the most common fish widely consumed worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. The investigation of historical changes in organs of fish is an accurate way to assess effect of xenobiotic compound in experimental study.

Despite reported studies on the toxicity of chromium and effect on the vital organs like liver very few studies have been undertaken on the commercial fishes. For the proposed study, an attempt was made to know the *Cyprinus carpio*, which is very widely distributed throughout Pakistan. If the stress of the

chromium, which is highly toxic heavy metal exist continuously for longer period, that can affect the fish drastically and will create a big loss to the economy of the country and also diminish the dietary requirement. The result of proposed studies will definitely help the farmers by knowing the level of loss / stress of the chromium contained in water.

2. **MATERIALS AND METHODS**

Fish Sampling and Acclimation

100 specimens of gulfam, *Cyprinus carpio* (4-5cm TL) were obtained live from fish hatchery Chilya (District Thatta). The fish were brought to the laboratory in the plastic bags, filled with oxygen. The fish were kept in glass aquaria for acclimatization to the laboratory condition for a week before the start of experiment. The fish were fed daily with the pellet feed containing rice protein.

Test chemical

The potassium dichromate ($K_2Cr_2O_7$) (MERCK) was used as the test compound for determination of LC_{50} of chromium. The stock solution for different concentration was prepared by diluting the compound in distilled water.

Estimation of LC_{50}

For estimation of LC_{50} , group of 10 fish were kept four different glass aquaria. Each tank contained 20 lit. of water and exposed to increasing concentrations of chromium {(Potassium dichromate) ($K_2Cr_2O_7$) from 15mg/L, 25mg/L, 35mg/L, 45mg/L. Different concentrations of the chromium tested in LC_{50} . Experiment was terminated after 96 hours. Water in the aquarium was changed on daily and fresh solution of

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chemical was added. During the experiment the activities of the fishes were recorded daily.

2.4 Experimental Design:

The fish were divided in five groups each contain 10 fish one group served as control. Other groups were exposed to the different concentration of potassium dichromate (25mg/l, 30mg/l, 35mg/l and 40mg/l. The experiment continued for 30 days through June to July 2011. Maintenance and feeding was same as above in estimation of LC_{50} . At the end of the experiment fishes were dissected. Tissue sample of liver and gills were taken and fixed in Bouin's fluid for the purpose of histological studies. Since the gills and septum of the gills are cartilaginous and need to remove the calcareous material so that the tissue may be made soft enough for the further process. In this context, the gills were kept in decalcifier for 4-5 days and then washed in tape water and dehydrated. The tissue were infiltrated in Benzene and embedded by putting the tissues in melted wax for 1-2 hours in oven at a particular melting of the paraffin wax. The embedded tissues were prepared for section cutting. 6µm thick sections of the tissues were made by using rotatory microtome (Ricerd Jung) and stretched on the albumenized glass slides. The stretched sections were kept in safe place for 24 hours to ensure proper sticking. The section were deparaffinized in xylene and processed them for staining in Haematoxylin (Gurr, 1956) and Eosin (Putt, 1948) and mounted with Canada balsam and covered the sections by glass cover slip. The gills and liver of the fish, were studied under light microphotography on Leica DM 2500. (Transmitted light microscope with digital camera 4LAS software).

3. RESULTS

Exposure of the fish *Cyprinus carpio* the sublethal concentration of potassium dichromate 25mg/L, 30mg/L, 35mg/L and 40mg/L separately for thirty days result into sever alteration in the histological makeup of the liver and the result in the form of photographs. The structure of liver of the control fish *Cyprinus carpio* consist of the hepatic lobules, hepatic cords containing hypatocytes. The luman is present in the centre which recives hepatic plates containing all the secretions released by the hepatocytes in liver. The kupffer cells and blood vessel are also present

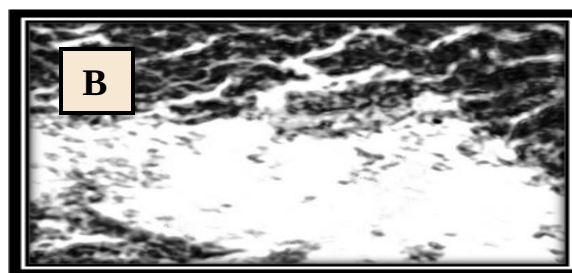


Fig.1

The results of 25mg/l concentration of chromium revealed that the hepatic cords have become narrow, compressed and elongated. The lobular boundaries disintegrated. The kupffer cells have also become changed in shape as compared to the control (Fig.1A). The picnosis was also noted in the hepatocytes present in the lobules (Fig.1B).

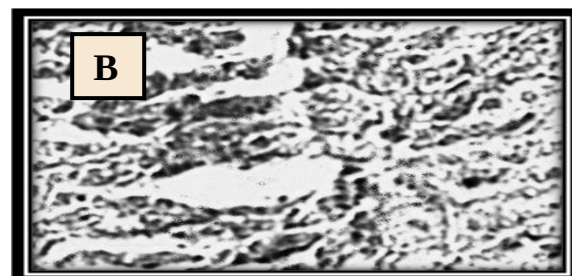
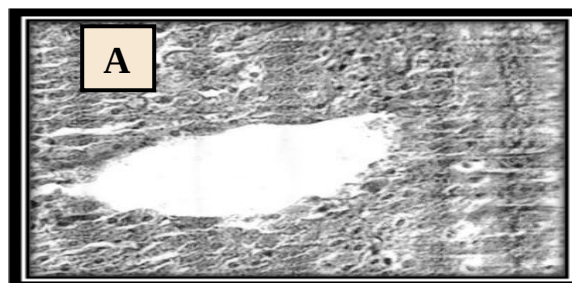
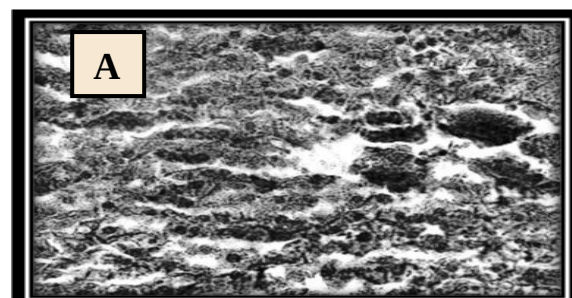
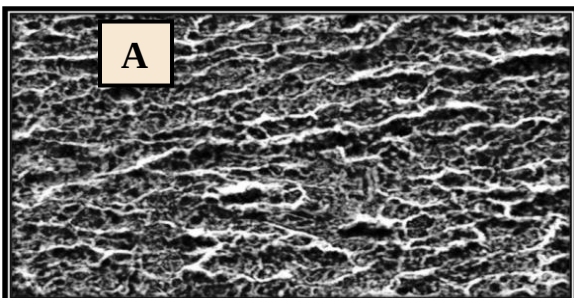


Fig.2

At 30mg/l Concentration of chromium expoure liver shows that the hepatic artery has become widened/enlarged probably due to severity of the action of Cr. The hepatic cords also have become compressed because of the regression of hepatocytes (Fig.2A) Vacuolation was noted in certain parts of the lobules that indicates the tumor formation. The vacuolation was further elongated as noted in one portion of the liver exposed to same concentration of Cr (Fig.2B).



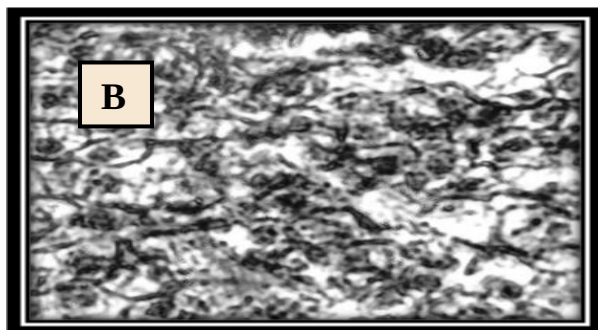


Fig. 3

The condition of liver of the fish exposed to 35mg/l concentration indicates that beside the regression of hepatic cords and hepatocytes, the kupffer cells have lost their integrity (**Fig.3A**). The hepatocytes in the liver of the fish has hypertrophied and lost their boundaries. The ratio of the vacuulations has been increased as compared to the liver exposed to 30mg/L concentration of chromium (**Fig.3B**).

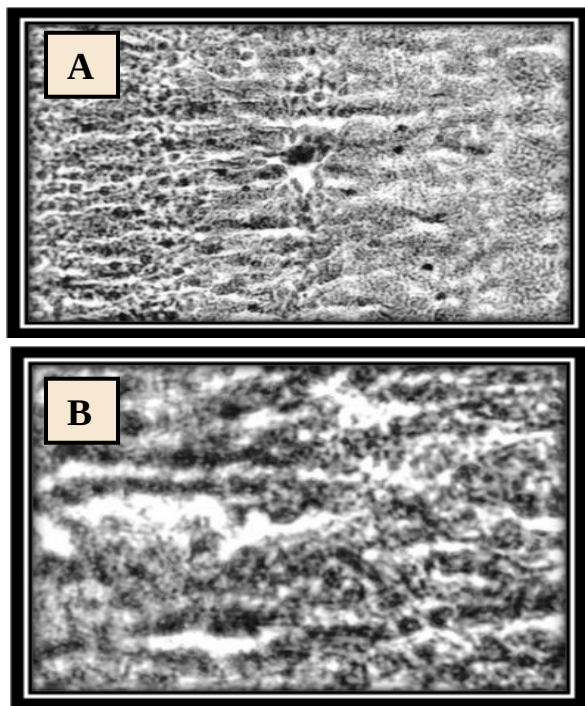


Fig.4

The liver exposed to 40mg/l concentration has become totally disintegrated. There are no lobular demarcation left. The kupffer cells showed an abnormal look (**Fig.4A**). Lumen in the lobules has disappeared because of hypertrophy and the nuclei proliferation was noted (**Fig.4B**).

4.

DISCUSSIONS

Many heavy metals are cause of water pollution and studies has also been reported by many workers (Karaded and Unlu, 2007). Chromium is one of

the heavy metal and originated from electroplating, dyeing, tannery, hard-alloy, textile, steel and stainless steel industries waste cause aquatic pollution. (Ayse *et al.*, 2010; Parvathi *et al.*, 2011; Senthil Elango *et al.*, 2011). Effect of chromium on fish has been studies in a few species such as *Labeo rohita* (Jafri *et al.*, 2003), *Channa punctatus* (Mishre and Mohanty, 2008), *Oreochromis aureus* (Ayse *et al.*, 2010), *Oreochromis mosambicus* (Senthil Elango *et al.*, 2011).

Liver of the fish is another vital organ of the body responsible for metabolic activities. The liver is composed of hepatocords containing hepatocytes, hepatic plates, and the lumen arranged in the lobules. The kupffer cells are also present in the vicinity of the lobules.

The liver of the fish exposed to lowest concentration of chromium (Cr) revealed that the hepatocords become narrow, compressed and somewhat elongated. The lobular boundaries disintegrated. The kupffer cells had also lost their integrity. The liver at the higher doses showed hypertrophy at one part and the picnosis was noted in the rest of the portion. Both these conditions indicate that the liver is under attack of heavy metal and leading towards the total collapsed. This condition was noted in the fish exposed to highest concentration. The liver of this group of fishes has totally collapsed and lost its shape. The disintegration leads to the mass like structure that corresponds to the tumor formation. Such type change has also been reported in the earlier studies made by (Saleh, 1982 and Dutta, 1993). The present studies on the gradual effects of Cr on the liver are in agreement with the past finding.

(Jafri *et al.*, 2003) had reported hepatocellular alteration of liver and grade IV hyperplasia in the fish, *Labeo rohita* exposed to chromium. Enlarged nuclei, condensation of cytoplasm and disarray of hepatic cords were noted in the liver of *Labeo rohita* exposed to chromium for 30 days (Bhatkar, 2011). Moreover, it was reported in several studies that chronic heavy metals accumulation in the liver of fish cause hepatocytic break down, cirrhosis and death (Pourahamad and Brien, 2000; Varanka *et al.*, 2001; Saxena and Sexena, 2008).

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