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Chromium, Nickel and Zinc Induced Histopathological Alterations in the Liver of Indian Common Carp *Labeo rohita* (Ham.)

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ABSTRACT: Degenerative histopathalogical alterations were seen in liver due to chronic exposure of the fish, *Labeo rohita* to chlorides of chromium, nickel and zinc for 30 days. However, more severe degenerative changes were observed in case of zinc chloride exposed fish than nickel chloride and chromium chloride treated fishes, indicating more toxic nature of zinc chloride. There were enlarged nuclei, condensation of cytoplasm and disarray of hepatic cords blood congestion in sinusoids, vacuolation of hepatocytes and necrosis. Heavy metal salts get entered in the cells by readily crossing the cell membranes where interfere with the enzyme systems in the cells resulting into morphological damage and / or their vital functions could be in jeopardy. @JASEM

Keywords: Chromium, Nickel, Zinc, *Labeo rohita*, Liver.

Industrial effluents contain a large number of toxicants such as salts of heavy metals, acids, organic matter, pesticides and even cyanides which deteriorate the physico-chemical characteristics of water. These pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic organisms (Farkas *et al.* 2002). Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution (Kock *et al.* 1996).

Chromium plating is one of the major uses of chromium. Steel fabrication, paint, pigment manufacturing and leather tanning constitute other major uses of chromium. Effluents from these processes are strongly acidic and may contain the toxic hexavalent chromium or the less toxic trivalent form. Hexavalent chromium is the more toxic form. It is derived from the oxidation of ores and also from the combustion of the fossil fuels, wood and paper and is relatively stable in water. It causes irritation to sensitive epithelial lining and results into ulceration (Goyer, 1991).

Nickel finds numerous applications in many industries because of its corrosion resistance high strength and durability, pleasing appearance, good thermal and electrical conductivity and alloying ability. The production of alloys accounts for approximately 75% of total nickel consumption (Wilson *et al.,* 1986, Nriagu and Pacyna, 1988, Nicolaidou and Nott, 1989). The pathogenesis of nickel toxicity is relatively complex because of the large number of chemical and physical forms.

Zinc, an essential trace metal becomes toxic when the nutritional supply becomes excessive. The main uses of zinc are in the manufacture of galvanized iron, bronze, paint (white), rubber, glazes, enamel glass, paper, as a wood preservative ($ZnCl_2$, fungicidal action), petrochemicals, and fertilizers and in steam generation power plants etc. (Nriagu and Pacyna, 1988). Some zinc is released into the environment by natural processes, but most comes from activities of people like mining, steel production, coal burning, and burning of waste. It attaches to soil, sediments, and dust particles in the air. Zinc compounds can move into the groundwater and into lakes, streams, and rivers. Most of the zinc in soil stays bound to soil particles. Moderately increased zinc concentrations in water stemming from the release of zinc from drainage pipes due to corrosion. It builds up in fish and other organisms (Joshi 1990).

Fish is high in omega-3 and protein that the human body needs to stay healthy. However, potentially dangerous heavy metals are absorbed into the body tissues of fish that are transferred to humans when fish is eaten. Good quality of food for consumption can only be produced in an environment free from contamination. Fish, having great economic importance, are affected immensely by various chemicals including heavy metals directly or indirectly in various ways. Several reports indicate high mortality of juvenile fish and reduced breeding potentiality of adults after long term exposure to heavy metals (Olojo *et.al.*, 2005). The freshwater fish, L*abeo rohita* 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 heavy metal contaminations.

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Histopathological studies on fish are a noteworthy and promising field to understand the structural organization that occurs in the organs due to pollutants in the environment. These structural changes vary with the body parts, nature of the pollutant, medium and duration of exposure (Vijaymadhawan and Iwai, 1975). Water quality characteristics also influence histopathological manifestations of toxic effects (Galat *et al.,* 1985). The structural changes in the organs at microscopic cellular and organ level leads to alterations of the function systems (Rana *et al.,* 1981).

MATERIALS AND METHODS

Disease-free fish, *Labeo rohita* were bathed in 1% $KMnO_4$ solution and acclimated in big glass aquarium of 400 to 450 liter capacity for a period of 15 days. Chlorine free aged tap water was used in the aquaria. The water had pH 8.2 ± 0.2; hardness 280 mg/l; D.O. 6.2 mg/l; total alkalinity 310 mg/l and temperature 25 ± 2^0C. The fish were fed with rice bran daily at 10.30 am. The water in the aquaria was changed daily after the consumption of food supplied. The healthy fish of both the sexes and uniform size and weight (125 ± 2g) were selected from the lot for experimental purpose. Initially 96h LC_{50} doses were determined for chromium, nickel and zinc heavy metal compounds by the method as described in standard methods by the APHA (1998).

Forty healthy fish from the stock were selected and were divided into four groups.
Group-I: Consisted of 10 fish in aged tap water which served as control. Group-II: Consisted of 10 fish kept in toxicant water containing 6mg/l chromium chloride for 30 days. Group-III: Consisted of 10 fish kept in toxicant water containing 4mg/l nickel chloride for 30 days. Group-IV: Consisted of 10 fish kept in toxicant water containing 2mg/l zinc chloride for 30 days.

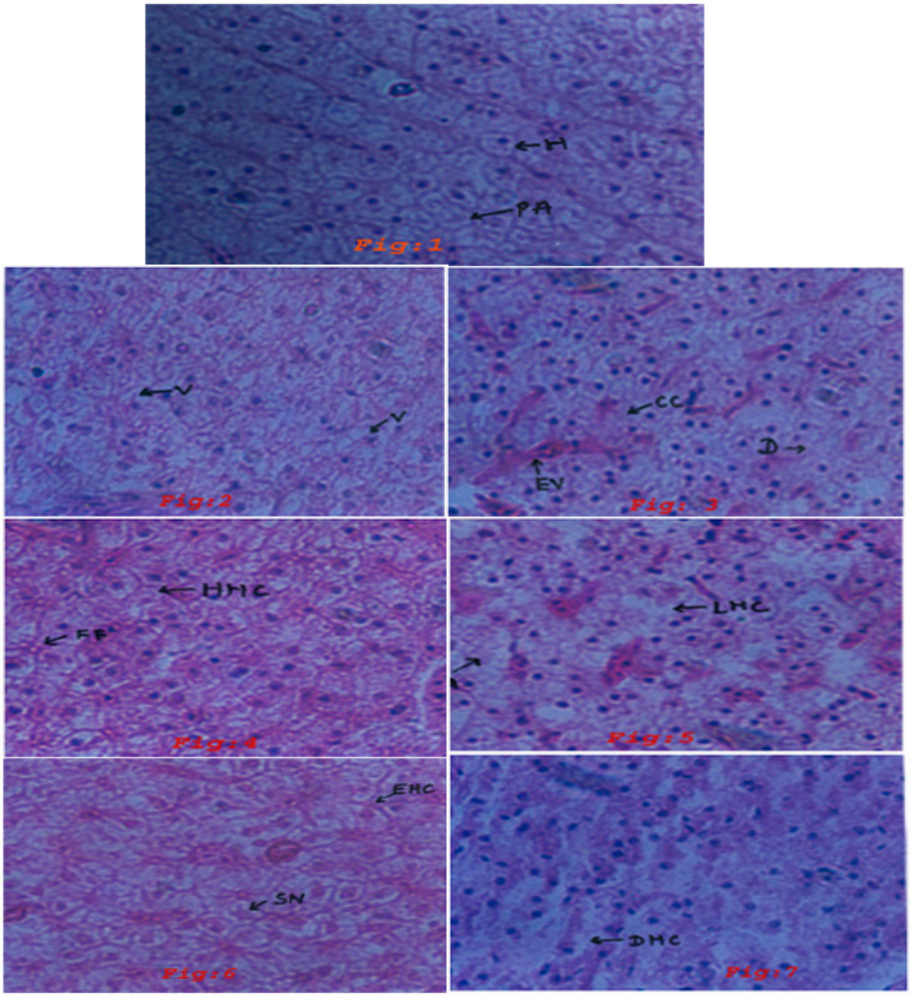
The fish were exposed to 6mg/l of chromium chloride, 4mg/l of nickel chloride and 2mg/l of zinc chloride, separately, which are $1\backslash10^{th}$ of their 96h LC_{50} concentrations. To avoid the effects of starvation, the fish were fed on the rice bran at the average feeding rate of 25mg food \ gm fish \ day. The toxicant solutions and the aged tap water (control) were renewed every day in the morning after removing the unused food, to maintain uniform test concentrations throughout the experimental period. . The controls as well as the experimental fish were sacrificed on the day 10 and 30. The liver was dissected out and rinsed in fresh water fish saline to remove the bloodstains. Then the liver tissue was cut into small pieces of desirable size and fixed immediately into aqueous Bouin's fluid. All possible precautions were taken to ensure proper fixation of tissues. The tissues were further processed by standard methods as described by Weissman (1972). The sections were cut and stained with haematoxylin-eosin, processed further, cleaned and then observed under microscope.

RESULTS AND DISCUSSION

Exposure of the fish, *Labeo rohita* to the sublethal concentrations of chromium chloride (6 mg/l), nickel chloride (4mg/l) and zinc chloride (2 mg/l) separately for thirty days resulted into mild to severe alterations in the histological makeup of the liver and the results are presented in the form of actual photographs (Figures, 1 to 7). The results obtained are toxicant and duration dependent.

The T. S. of control liver shows continuous mass of hepatic parenchymal cells arranged in cords around blood vessels. The hepatic cells are polygonal in shape and with centrally placed rounded nucleus and homogeneous cytoplasm. Hepatic cells are not arranged to form distinct lobules. Pancreatic acini of exocrine function lie embedded in between the hepatic cells surrounding the blood capillaries (Fig. 1). In the ten days chromium chloride treatment group, the cytoplasmic damage was not so severe but the orientation of the cells was disrupted and vacuolation in the liver cells were found (Fig. 2). Thirty days of chromium chloride exposure also resulted in enlarged nuclei, condensation of cytoplasm and disarray of hepatic cords. Some liver cells appeared almost devoid of cytoplasmic contents (Fig, 3). The lesions are further characterized by elongations of blood vessels, necrosis and degeneration. With ten days of nickel chloride treatment, histological study of liver showed marked alterations. Widespread vacuolation within the hepatocytes and appearance of some typical globular bodies, which may be suspected as, infiltered fats (Fig. 4). Few hepatocytes lost their polygonal shape as they were hypertrophied. The cell membranes of hepatic cells were found to be thickened. The pancreatic acini around blood capillaries were necrosed. After thirty days treatments with nickel chloride, sinusoids were found be degenerated resulting into bleeding in the intercellular gaps. Pancreatic acinar cells lost their identity (Fig. 5). Degeneration of hepatic cells was seen prominently. The liver of *Labeo rohita*, after exposure of the fish to zinc chloride for ten days revealed swelling of hepatic nuclei, disorganization of hepatic cells with edematous hepatocytes and many cells were devoid

of cytoplasmic contents (Fig. 6). After thirty days entire liver tissue became a necrotic spongy mass with degeneration of sinusoids. Most of the hepatocytes lost their cell boundaries and some of them showed indistinct cell boundaries. Hepatocytes showed disintegration and at several places the nuclei could not be seen distinctly. The overall picture was one of consistent degeneration (Fig.7).



Figs. 1-7. T.S. of liver of the fish, Labeo rohita illustrating 1) the normal structure showing polygonal hepatocytes (H) and normal pancreatic acini (PA). 2) changes after exposure to chromium chloride for 10 days as evidenced by disorganized hepatic cells and vacuolation (V). 3) changes after exposure to chromium chloride for 30 days as evidenced by elongation of blood vessels (EV), degeneration (D) and condensation of cytoplasm (CC). 4) changes after exposure to nickel chloride for 10 days as evidenced by filtered fats (FF) and hypertrophy of hepatic cells (HHC). 5) changes after exposure to nickel chloride for 30 days as evidenced by degeneration of sinusoids (DS), loss of identity of pancreatic acini (PA) and lysis of hepatic cells (LHC). 6) changes after exposure to zinc chloride for 10 days as evidenced by swollen and hypochromatic nuclei (SN), disarray of hepatic cords and edematous hepatocytes (EHC). 7) changes after exposure to zinc chloride for 30 days as evidenced by degeneration of hepatocytes of advanced stage (DHC). All preparations in Iron-haematoxylin – Eosin preparation. X400

The histological alterations noticed in the present study are in accordance to the chronic exposure to the different pollutants. The lesions developed in the liver might be due to the cumulative action of toxicant on blood and ultimately to other cellular structures. There seems to be a definite correlation between tissue damage and certain physiological alterations (Olurin *et al.* 2006). Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Ojolo *et al* 2005**;** Saxena *et al.* 2008). The hepatic lesions

observed in the present investigation are in accordance to the recordings made by different workers during acute exposure to different pollutants. Sastry and Gupta (1978); Patil (1995) have reported higher degree of liver damage in acute treatment than the chronic exposure with mercury on *Channa punctatus*. Kumari and Kumar (1997) have also reported similar changes in *Channa punctatus* collected directly from highly polluted lake. The present study also revealed that the alterations noticed in the acute exposure were in line to the observations recorded in ninety days chronic exposure to eslan, mercury and ammonia on fish liver (Banerjee and Bhattacharya, 1997). Infiltration of blood filled spaces in the liver along with disarray of cords supports the view of previous workers that heavy metals cause haemorrhage in the internal organs (Singh and Bhati, 1994; Patil, 1995). But it seems that the reported lesions are not heavy metal specific, as other workers have reported similar pathological lesions in the liver of the fish after exposure to insecticides and herbicides (Jain and Mishra, 1994; Singh and Bhati, 1994; Olurin *et al.* 2006). The present results are well in agreement with those of Bhoraskar and Kothari (1997) reported severe damage in the liver of *Clarias batrachus* exposed to 10 mg/l zinc sulphate. Histological damage due to zinc in fish liver has also been reported by several workers (Wong *et al*., 1977; Kumar and Pant, 1981). Osman *et al*., (2009) recorded congestion and hemorrhage in the hepatic sinusoids with dilation of hepatic vessels, vacuolization and degeneration of hepatic cells with fatty changes with atrophy of pancreatic acini; in liver of the *oreochromis neloticus* exposed to the polluted water containing heavy metal salts. António *et al*., (2007) studied histopathological changes in liver of Nile tilapia, *Oreochromis niloticus* exposed to waterborne copper and observed vacuolization and necrosis of the liver parenchyma. Moreover, it was also reported by several studies that chronic heavy metal accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and ultimately death (Pourahamad & O'Brien 2000, Varanka *et al.* 2001; Saxena *et al.* 2008).

The liver plays a key role in accumulation and detoxification of heavy metals (Gbem *et al*., 2001; Ojolo *et al.* 2005). Although, according to Roch and McCarter (1984), fishes are known to possess sequestering agent (metallothionein), the bioaccumulation of these trace element in the liver tissue reaches a proportion in which the function of the liver is impeded, thus resulting in gradual degeneration of the liver cells syncytial arrangement. Thus cirrhosis, the outcome of prolonged hepato-cellular injury is manifested by fibrosis of hepatic cords. Oxygen required to support the intense metabolic activity of the liver is supplied in arterial blood via the hepatic artery. In effect, necrosis of parenchyma cells had taken place (Olurin *et al.* 2006).

*Conclusions***:** Thus the heavy metal salts found in domestic waste or industrial effluent or agricultural runoff may have an adverse effect not only on the organs which come in direct contact with the toxic external medium such as scales and gills, but also on the vital organs like testis, ovary, liver and kidney as these heavy metal salts seem to get entered in the cells by readily crossing the cell membranes where they might interfere with the enzyme systems in the cells resulting into morphological damage and / or their vital functions could be in jeopardy (Bhatkar 2003; 2004; 2010; Ojolo *et al.* 2005; António *et al*., 2007; Osman *et al.*2009).

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