

Chromium Toxicity Induced Histopathological Alterations in The Liver of Fresh Water Carp Fish, *Catla Catla*

Dr.P.Ranganatham,
Department of Zoology,
The Adoni Arts and Science College, Adoni

Abstract :- *Catla catla*, the fresh water carp fish, fingerlings when exposed to different sublethal concentrations of chromium for a different periods 1, 8, 16, 32 days of time brought changes in the structure and morphology of the nonosmatic organs such as liver. In trivalent chromium exposed fish, in the liver a few pathological changes were seen during the initial days of exposure and then structural reorganization observed in later days of exposure, The degenerative changes observed in the liver of the fish exposed to the sublethal concentration of hexavalent chromium were the disarray of liver cords, dilation of sinusoids, vacuolization in liver tissue, etc., all these changes confirmed structural disruption by hexavalent chromium ions on prolonged exposure.

I. INTRODUCTION

Freshwaters are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of accidental discharges or criminal negligence (Vutukuru, S. S., 2003). The contamination of freshwater with a broad spectrum of pollutants has become a matter of concern all over the world (Voegborlo *et al.*, 1999; Vutukuru, 2005; Rauf *et al.*, 2009). 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).

Heavy metals toxicity received considerable attention in aquatic organisms especially in fish (Javed, M, 2002). The metals enter fish bodies through body surface, gill or the digestive tract (Vincent, S., 2002). The alteration in biochemical contents in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by number of workers Verma *et al.*, (1983); Gupta *et al.*, (1987); Khan *et al.*, (1992); James & Sampath (1995); Das *et al.*, (1999); Khare and Singh (2002); Desai *et al.*, (2002); Remia *et al.*, (2008); Hadi *et al.*, (2009) and Ganeshwade, (2011).

The higher levels of trace elements such as lead and chromium in liver relative to other tissues may be attributed to the affinity or strong coordination of metallothionein protein with these elements (Ikem *et al.*, 2003). Toxicological histopathology gives useful data concerning the changes induced by toxicants at cellular levels. 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 finer cytoarchitectural changes produced during chemical intoxication can be traced by microscopic examinations of the tissues; such studies may explain to certain extent the tissue specificity of the drug action. Their field of study is called histotechnology (Merck Source, 2002 and Stedman's medical dictionaries, 2005).

II. 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. 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 ± 2 gms 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 ± 3 oC 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, PH, 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), LC50/96 hours of trivalent and hexavalent chromium are considered as lethal concentrations. So, about 1/10 th of the 96 h LC50 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.

The effects of sublethal concentrations of trivalent and hexavalent chromium on the fish were studied at different periods of exposure in order to understand the influence of time over toxicity. Thus 1, 8, 16 and 32 days were chosen to observe the short term and long term effects of trivalent and

hexavalent chromium on the fish *Catla catla*. After the completion of stipulated exposure period, the fish were sacrificed and isolated tissues such as liver 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. The histological sections of the liver were taken by adopting the procedure as described by Humason (1972). Photographs were taken.

PLATE - 7

Fig: III Fish Control Liver showing continuous mass of large hexagonal cells laminae, granular cytoplasm distinct nuclei, hepatic labarynth of laminae hepatocytes (HC), blood sinusoids (S), with lower magnification (10X); and Higher magnification (40X).

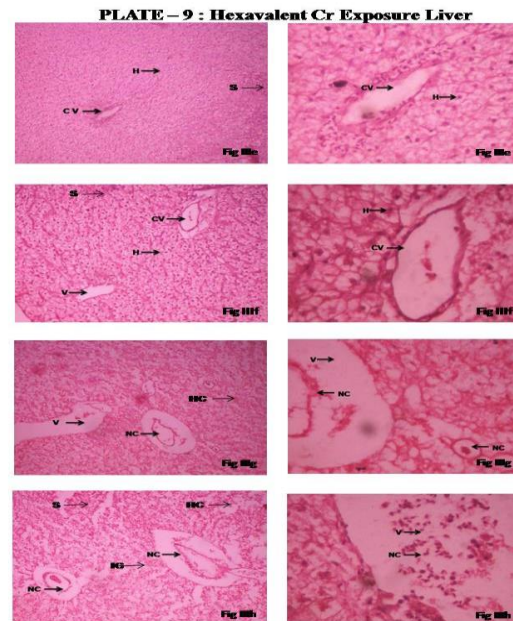
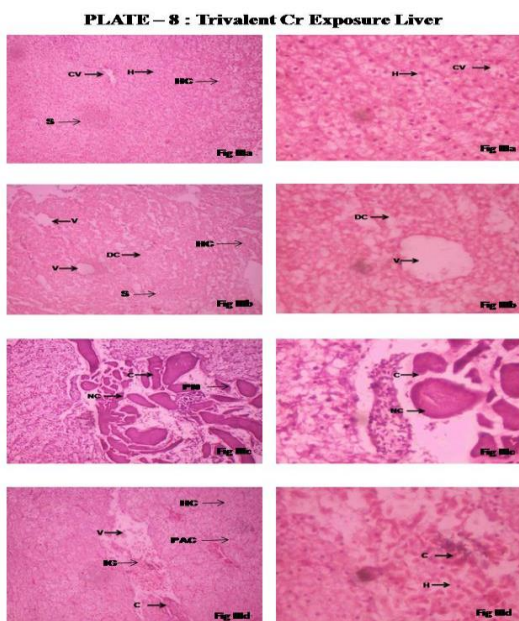
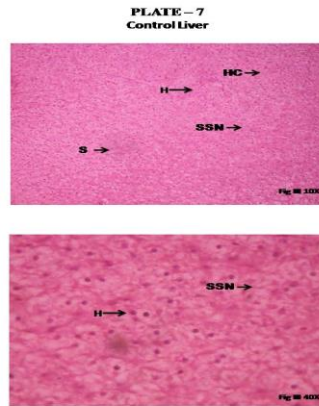


PLATE - 8

Fig: IIIa Fish exposed to sublethal conc. of trivalent chromium at day1, large hexagonal cells, granular cytoplasm distinct nuclei, hepatic labarynth hepatocytes (HC) blood sinusoids (S), with lower magnification (10X); and Higher magnification (40X).

Fig: IIIb. Fish exposed to day 8, vacuolization in hepatic cells (HC), dilation of blood sinusoids (S), Liver cords disarranged, atrophic changes in hepatocytes (HC), with lower magnification (10X); and Higher magnification (40X).

Fig: IIIc. Fish exposed to day 16, hepatocytes (HC) with vacuoles, globular bodies, polygonal hepatocytes hypertrophied, cell membranes thickened necrosis in pancreatic acini. with lower magnification (10X); and Higher magnification (40X).

Fig: III d. Fish exposed to day 32, sinusoids degenerated, intercellular gaps (IG), pancreatic acinar cells (PAC) and degeneration of hepatic cells (HC), with lower magnification (10X); and Higher magnification (40X).

PLATE - 9

Fig: IIIe. Fish exposed to sublethal conc. of hexavalent chromium for day 1, near to normal liver hexagonal cells, granular cytoplasm distinct nuclei some blood sinusoids (S), with lower magnification (10X); and Higher magnification (40X).

Fig: III f. Fish exposed to day 8, dilation of blood sinusoids (S), hypertrophic nuclei, atrophic changes, vacuolization in liver tissue, granular degeneration and lamellar structure (LS) dissolution, with lower magnification (10X); and Higher magnification (40X).

Fig: III g. Fish exposed to day 16, vacuolization in hepatocytes (HC), loss of polygonal shape hypertrophy, cell membranes thickened, pancreatic acini around blood capillaries necrosed, with lower magnification (10X); and Higher magnification (40X).

Fig: III h. Fish exposed to day 32, atrophy of liver cords, shrinkage of hepatocytes (HC), granular degeneration, cytoplasmic disintegration hepatocytes (HC) and the sinusoids (S) highly dilated, with lower magnification (10X); and Higher magnification (40X).

III. RESULTS AND DISCUSSION

The structure of the normal liver of the fish consists of a continuous mass of large hexagonal cells forming laminae. These cells contained granular cytoplasm with distinct nuclei either eccentric or slightly centrally placed. Suspended in the hepatic labarynth were seen some blood sinusoids. Each lamina of the liver was separated by the thick wall of the peripheral cells (Fig III).

No significant changes were observed in the structure of the liver of the fish exposed for a period of 1 day to the trivalent chromium (Fig IIIa). However, in the fish after 8 days of exposure to trivalent chromium, the liver exhibits some degenerative changes like hypertrophy in the nuclei, vacuolization in the hepatic cells and dilation of blood sinusoids. The parenchymatous tissue was greatly disrupted. A mild degree of granular degeneration was evident in most of the hepatocytes. A very small degree of atrophic changes were seen in the liver cords with the shrinkage of hepatocytes (Fig IIIb). On exposure for a period of 16 days, hepatocytes with widespread vacuoles and appearance of some typical globular bodies, which may be suspected as, filtered fats were observed. Few hepatocytes lost their polygonal shape as they were hypertrophied. The cell membranes of the hepatic cells were found to be thickened. Necrosis was observed in the pancreatic acini around blood capillaries (Fig IIIc). After 32 days of exposure, sinusoids were found to be degenerated resulting into bleeding in the intercellular gaps. Degeneration of hepatic cells was seen prominently (Fig III d).

No significant changes were observed in the structure of the liver at day 1 of the fish exposed to hexavalent chromium, as compared to control (Fig IIIe). On exposure of the fish for a period of 8 days, the liver was greatly disrupted with dilation of blood sinusoids. Nuclei became hypertrophic. Severe degree of atrophic changes was noticed in the liver cords; vacuolization was heavy in the liver tissue with exploitation of hepatic nuclei. Granular degeneration and gradual dissolution of lamellar structure were noticed (Fig III f). On exposure of the fish for a period of 16 days, to sublethal concentration of hexavalent chromium, widespread vacuolization was observed within hepatocytes, some hepatocytes lost their polygonal shape because of hypertrophy. The cell membranes of hepatic cells were found to be thickened. The pancreatic acini around blood capillaries were necrosed (Fig III g). On further exposure for a period of 32 days, there appeared severe degree atrophy of the liver cords, shrinkage of hepatocytes, granular degeneration and cytoplasmic disintegration. The liver was mostly disrupted due to the rupture of the cell membranes of the hepatocytes. The sinusoids were highly dilated (Fig III h).

IV. CONCLUSIONS

The degenerative changes observed in the liver of the fish exposed to the sublethal concentration of hexavalent chromium were supported by the metabolic disorders observed in it. The disarrayed structure of liver cords, hepatic cells vacuolization sinusoids dilation, severe degree of nuclear atrophy, coagulation of blood cells followed by the hepatocyte shrinkage and dissolution of laminar structure suggest that the depletion in glycogen reserves. All these biochemical disorders observed in the liver of the fish exposed to the sublethal concentration of hexavalent chromium could be due to its gradual structural disorganization. Bengeri and Patil (1986) stated that the concentration of a metal is more

important in bringing the histological changes in the liver of the fish; hence these changes could be used as a tool for assessing the toxic effects of heavy metals in aquatic environment.

REFERENCES

- [1]. Akhilender Naidu, K., Abhinendar Naidu, K. and Ramamurthi, R. 1984. Acute effects of mercury toxicity on some enzymes in liver of teleost *Sarotherodon mossambicus*. *Ecotoxicol. Environ. Saf.*, 8(3):128-133.
- [2]. Farkas, A., Salanki J. and Specziar A., 2002. Relation between growth and heavy metal concentration in organs of bream, *Abramis brama* L. populating lake Balaton. *Arch. Environ. Contam. Toxicol.*, 43: 236-243.
- [3]. Gardner, G.R. and Yevich, P.P. 1970. Histological and haematological responses of an estuarine teleost to cadmium. *J. Fish. Res. Bd. Can.*, 27: 2185-2196.
- [4]. Gardner, G.R. and Yevich, P.P. 1970. Histological and haematological responses of an estuarine teleost to cadmium. *J. Fish. Res. Bd. Can.*, 27: 2185-2196.
- [5]. Garg, U.K., A.K. Pal, G.J. Jha and S.B. Jadhao 2004. Pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides on bone health of broiler chicks. *Toxicologic Pathology*, 32:364-369.
- [6]. Hayat, S., M. Javed and S. Razzaq, 2007. Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. *Pakistan Vet. J.*, 27(1): 8-12.
- [7]. Javed, M., 2005. Heavy metal contamination of freshwater fish and bed sediments in the river Ravi stretch and related tributaries. *Pakistan J. Biol. Sci.*, 8(10): 1337-1341.
- [8]. Javed, M. 2002. *J. Ani. Vet. Adv.* 1: 16-19.
- [9]. Javed, M. 2004. *Ind. J. Biol. Sci.* 1: 59-62.
- [10]. Jayantha Rao, K., Azhar Baig Md., Ramamurthy, R., Madhu, Ch. And Murthy, V.S.R. 1984. Chronic sublethal effects of phosphomidon on a fresh water teleost *Tilapia mossambica*. *Bull. Environ. Sci.*, 1:3-5.
- [11]. Jha, B.S. and Satheyendra Pandey, 1990. Toxicity of Lead nitrate to the fish, *Channa punctatus* (Bloch) *Bio. J.*, 2 (1): 151-154.
- [12]. Kasherwani, D., Lodhi, H. S., Tiwari, K.J., Shukla, S. and Sharma, U.D 2009. *Asian J. Exp. Sci.*, 23: 149-156.
- [13]. Lliopoulou-Georguadaki, J. and Kotsanis, N.: *Bull. Environ. Contam. Toxicol.*, (2001). 66: 77-85.
- [14]. Rajeswari, I.S.R. 2008. Toxicity of organophosphate Compound acephate on haematological, selected biochemical parameters and histological studies in wistar strain rats. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India.
- [15]. Rauf, A., M. Javed, M. Ubaidullah and S. Abdullah, 2009. Assessment of heavy metals in sediments of the Ravi River, Pakistan. *Int. J. Agric. Biol.*, 11: 197-200.
- [16]. Sharma, R.K. and Agrawal, M. (2005) : *J. Environ. Biol.*, 26: 301-313.
- [17]. Verma, R.S., Khan, M.A., Tripathi, R., Shukla, S. and Sharma, U.D (2005) : *Aquacult.*, 6: 57-62.
- [18]. Vincent, S., Ambrose, T. and Selvanayagam, M 2002 : *J. Environ. Biol.*, 23: 209-212. 19. Virchow, R. 1858. Die cellular pathologic in ihrur Bergundung auf physiologische and pathologische Geweibre. Berlin: A. Hirschwold.
- [19]. Vutukuru, S. S, 2003: Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton). *Bull. Environ. Contam. Toxicol.*, 70, 118-123.
- [20]. Vutukuru SS (2005) Acute effect of Hexavalent chromium on survival, oxygen consumption, Hematological parameters and some biochemical profiles of the Indian major carps, *labeo rohita*. *Int. J. Environ. Res. Public Health.* 2(3): 456-462.
- [21]. Zyadah, M.A. and Abdel-Bakey, T.C (2000) : *Bull. Environ. Contam. Toxicol.*, 64: 740-747.