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Histopathological alterations induced by chromium in the muscle of fresh water teleost fish, *Catla catla*

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Abstract
Catla catla, a fresh water edible carp fish, the fingerlings, when exposed to different sublethal concentrations of chromium for a different periods1, 8, 16, 32 days of time brought changes in the structure and morphology of the nonosmatic organs such as muscle. The severe destruction of muscle tissue in the fish was evident by the splitting in the muscle fibers, karyolysis, nuclei exploitation atrophy, hypertrophy, hemorrhagic condition and ultimate wavy appearance of muscle fibers suggest significant concentration of metal accumulation in this effector target organ. All the changes which take place affect the contractile ability of the muscle fibers.

Keywords: alterations induced, fingerlings, karyolysis, hypertrophy

Introduction

Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in polluted environment (Duruibe JO, 2007) [4]. 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) [24, 25, 18]. These pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic organisms (Farkas *et al.* 2002) [5]. Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of waste water (Labonne *et al*., 2001; Goodwin *et al*., 2003) [11, 8]. The natural aquatic bodies have extensively been contaminated with heavy metals released from domestic, industrial and other man-made activities (Conacher *et al*., 1993; Velez & Montoro, 1998) [2, 23]. Bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies (Karadede-Akin and Unlu, 2007) [10] that could be a useful tool to study the biological role of metals present at higher concentrations in fish (Dural *et al*., 2007) [3].

Catla catla is endemic fish to the riverine systems in northern India, Indus plain and adjoining hills of Pakistan, Bangladesh, Nepal and Myanmar, and has been introduced later into almost all riverine systems, reservoirs and tanks all over India. The fish catla is a surface, column feeder with its upturned mouth and large gill rackers are adapted to feed on numerous organisms floating in water. Several workers reported on the effects of metals or chemicals or pesticides and pointed out the architectural damage to brain, gill, liver, kidney, heart, lung, muscle, testis, intestine in various animals (Jayantha Rao, 1982; Radhaiah, 1988; Vijay Joseph, 1989; Vani, 1991; Badri Sriman Narayanan *et al*., 1993; Manoranjitham *et al*., 1993; Pondy *et al*., 1997a, 1997b; Shukla *et al*., 2001, Glynn, 2003; Garg *et al*., 2004; Madhaveelatha, 2006; Sivaiah, 2006; Rajendra Prasad, 2007; Sukanya, 2007; Kishandar, 2007; Madhava Rao, 2007; Nagarjuna, 2007; Rajeswari, 2008) [9, 22, 1, 14, 19, 7, 6, 13, 20, 16, 21, 12, 17].

Exposure of animals to contaminated water also causes severe pathological changes at the tissues level. 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). Various histopathological responses at sublethal exposure to metals could bring a relationship between the level of accumulation of the metal and to the various physiological and biochemical activities of the animal (Paulose, 1989). The dysfunctioning of muscle fibers due to severe structural deformities was also reported in Cyprinus carpio under acute mercury stress (Viorica and Murcoci, 1986). 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 muscle of fresh water fish *Catla catla* exposed to both trivalent and hexavalent chromium stress.

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, 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), LC50S/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 muscle under laboratory conditions for biochemical analysis and histopathological studies. The tissue 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 muscle were taken by adopting the procedure as described by Humason (1972). Photomicrographs of the section preparations were taken using Magnus photomicrography equipment. Photographs were taken.

Plate - 10

Fig: IV Fish Control Muscle, showing compactly packed muscle fibers (PMF), intermuscular spaces, round to spindle shaped nuclei (SSN), no splitting in muscle fibers intermuscular spaces filled with viscous fluid, with lower magnification (10X); and Higher magnification (40X).

Plate - 11

Fig: IVa Fish exposed to sublethal conc. of trivalent chromium at day1, compactly packed muscle fibers (PMF), intermuscular spaces, round to spindle shaped nuclei (N), no splitting in muscle fibers with lower magnification (10X); and Higher magnification (40X).

Fig: IVb. Fish exposed to day 8, granular cytoplasm, pyknotic nuclei (PN), splitting of muscle fibers, thinning of muscle fibers karyolysis, with lower magnification (10X); and Higher magnification (40X).

Fig: IVc. Fish exposed to day 16, granular cytoplasm, pyknosis in the nuclei (PN), isolated muscle fibers, thinning of muscle fibers, and karyolysis(k) occurred, with lower magnification (10X); and Higher magnification (40X).

Fig: IVd. Fish exposed to day 32, splitting of muscle fibers, Pycnotic nuclei (PN) exfoliated, fibers appeared thin, compactly packed with less intermuscular spaces, with lower magnification (10X); and Higher magnification (40X).

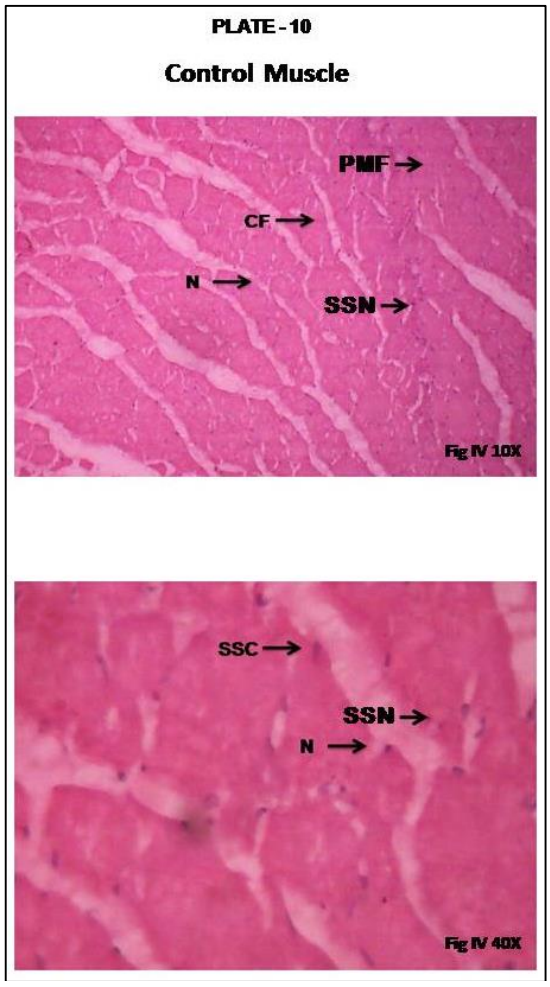
Plate - 12

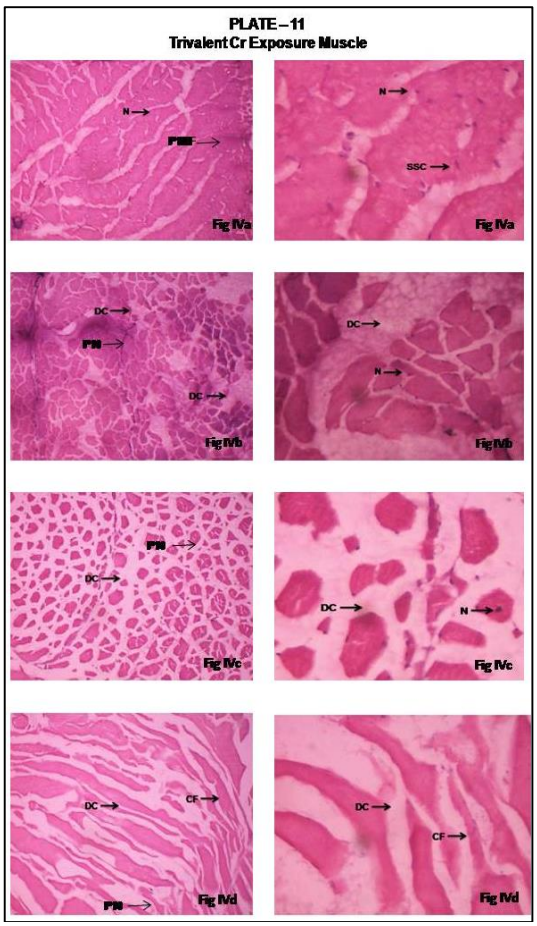
Fig: IVe. Fish exposed to sublethal conc. of hexavalent chromium for day 1, packed muscle fibers (PMF), intermuscular spaces, round to spindle shaped nuclei (N), no splitting in muscle fibers, with lower magnification (10X); and Higher magnification (40X).

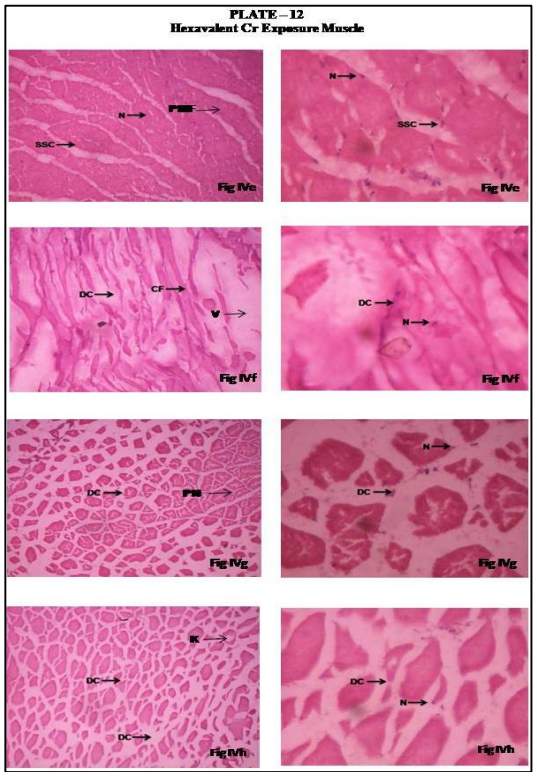
Fig: IVf. Fish exposed to day 8, muscle bundles wavy with vacuolization (V), with lower magnification (10X); and Higher magnification (40X).

Fig: IVg. Fish exposed to day 16, pyknotic nuclei (PN) and karyolysis (K) seen, with lower magnification (10X); and Higher magnification (40X).

Fig: IVh. Fish exposed to day 32, bending of muscle fibers, fibrilization loss of total muscular integrity, wavy appearance of muscle fibers and pyknotic nuclei (PN), with lower magnification (10X); and Higher magnification (40X).







Results and discussion

The muscle is an organ for maintenance of structure, movement of the organism and also a seat for many metabolic activities. Histopathological alterations in fish under the influence of heavy metals can be used as a perceptive model to keep an eye on the aquatic pollution. This study describes the effects of trivalent and hexavalent chromium on muscle of *Catla catla* exposed for 1, 8, 16 and 32 days.

The structure of the muscle of the normal control fish was seen with compactly packed muscle fibers with definite intermuscular spaces. Round to spindle shaped nuclei were found distributing all over the bundle length with occasional hyper chromasia. There was no splitting in muscle fibers. The intermuscular spaces appeared to be filed with viscous fluid (Fig IV). In the muscle of the fish exposed to sublethal concentration of trivalent chromium for a period of 1 day there was no significant variation in the structure and morphology of the muscle (Fig IVa). On exposure for a period of 8 days, granular cytoplasm, pyknotic nuclei, mild splitting of muscle fibers followed by thinning of muscle fibers was seen. (Fig IVb). For a period of 16 days, distinct granular cytoplasm with pyknosis in the nuclei, muscle fibers were isolated slightly followed by thinning of muscle fibers was seen. The muscle fibers exhibited longitudinally splitted muscle fibers. (Fig IVc). for a period of 32 days, splitting of muscle fibers with mild degree of sprain, Pycnotic nuclei which were exfoliated at certain places fibers appeared thin compactly packed with less intermuscular spaces. The fibers did not appear with normal thickness (Fig IVd).

In the muscle of the fish exposed for a period of 1 day to hexavalent chromium there was no significant variation in the structure and morphology of the muscle (Fig IVe). For a period of 8 day, muscle fibers were thinned with a tendency of coalization was seen. The muscle appeared as pallid and toneless with severe degree of atrophy. The muscle bundles became wavy with a heavy vacuolization due to muscle atrophy (Fig IVf). For a period of 16 days, splitting of muscle fibers followed by thinning of muscle fibers was seen. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei loss of proper organization, Karyolysis occurred and appeared as granular debris (Fig IVg). For a period of 32 days, further degeneration of muscle was seemed with bending of muscle fibers due to muscular sprain. There was heavy fibrilization with the loss of total muscular integrity, reticular formation with high wavy appearance of muscle fibers. The nuclei became highly pyknotic and scattered without any proper organization (Fig IVh).

Conclusions

The degenerative changes which were progressed in the muscle of the fish exposed to sublethal concentration of hexavalent chromium support the metabolic disorders observed in it. This metal enters the muscle carried over by the liver and other active tissues on failure of detoxification mechanisms. The severe destruction of muscle tissue in the fish was evident by the splitting in the muscle fibers, karyolysis, nuclei exploitation atrophy, hypertrophy, hemorrhagic condition and ultimate wavy appearance of muscle fibers suggest significant concentration of metal accumulation in this effector target organ. All the changes which take place affect the contractile ability of the muscle fibers. This indicates that the fresh water aquatic fishes which are exposed to heavy metals exhibits physiological, structural and morphological changes based on their toxicity there is bioaccumulation through biomagnifications leads to affect the health of humanbeings, so prevent the pollution of the aquatic environments from heavy metal pollutants and from other industrial effluent toxicants.

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