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Toxicity assay of chromium on fish *Puntius Sophore (Hamilton)*

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Abstract
Hexacovalent Chromium is used in carpet, textile, paper and pulp, tanning, ink, dyes and paint industry and poured into environment as industrial effluent. The freshwater fish *Puntius Sophore (Hamilton)* was subjected to 0.5 ppm hexacovalent chromium stress for 75 days and transmission electron microscopic studies of gills and intestine revealed that mitochondria clustured, mitochondrial membrane fused and at some points are broken, disintegration of cristae occurs; endoplasmic reticulum shows broken and disintegration, ribosomes also reduce and disintegrate; lysosome disintegrates; nuclear membrane breaks and disintegrates, chromatin material condenses, disintegrates and nucleolus disappears; Golgi bodies also disintegrate; due to disintegration of cytoplasm, the vacuoles; and large Lipid droplets appear and in some cells segregate near nucleus. This shows that hexacovalent chromium hits the chemicals constituting these organelles resulting in the breaking or disintegration.

Keywords: Toxicity, Hexacovalent Chromium, *Puntius Sophore (Hamilton), disintegrates*

Introductions
Chromium is usually released into water bodies through various industrial practices like chrome plating, tanning and in connection with the inhibition process of cooling tower system in addition to carpet industry. Effluent disposal from widely established carpet washing and dyeing as well as tanning industry in India has considerably increased the Chromium content in their adjacent water bodies. Guru Prasad Rao and Nand Kumar (1981) [12], Nand Kumar and Rajendra Babu (1984) [17] found traces of Chromium and high salinity in water bodies which has been attributed to which seepage of tannery effluents. Handa *et al.* (1983a and 1983b) [13, 14], have reported the presence of Chromium in soil, effluents and water bodies of Bhadohi-the carpet belt of Eastern U. P. Sriwastwa reported the presence of Chromium traces in the fish pineal body of this area. Therefore, the study of the effect of Chromium stress on animal tissue becomes imperative and becomes the need of the time in the area.
Since the work of Ellis (1937) [9] toxicity bioassay studies of Chromium components on aquatic organisms, including fish, have been carried out by Benoit (1976) [6]. Chromium induced histopathological, enzymological and biochemical changes have also been worked out Boge *et al.* 1986 [7] and Nath and Kumar (1987) [18]. There are definite evidences regarding Chromium accumulation in fish as is evident in references by Freeman (1980) [11]. It was therefore considered prudent to study the ultrastructural changes under Chromium stress under electron microscope.

Materials and Methods
Live *Puntius Sophore (Hamilton)* were collected from local ponds of Gyanpur. Bhadohi and acclimated in laboratory conditions for a week. The fish was then subjected to 0.5 ppm Chromium stress for 75 days. After this, the fish was anaesthesised in MS 222 and dissected to take out gill lamella, kidney, liver and a portion of intestine. The tissues were fixed in 3% gluteraldehyde and 2% paraformaldehyde for 2 hours at 4C. The material was washed in 0.1 M buffer solution prepared by cacodylate at 4C the material was post fixed in 1% Osmic acid solution for 2 hours at 4C the material was then washed by 1.OM buffer solution for 2 hours at 4C. The material was embedded in Epoxvrasin and the mixture was kept in oven at 50C so as to thoroughly mix it.
The specimen with rasin was inserted into dry gelatin capsule (Beem). The Beem was kept for 72 hours at 500 the gelatin capsule block was cut into 500A thick sections that were placed on copper grid. They were stained with Uranyl acetate and Lead citrate and dried on

filter paper the grids were observed under transmission electron microscope CM 12 Phillips and photographs were taken.

Observation

Transmission Electron Microscopy

Transmission Electron Microscopic study was made on four tissues of *Puntius Sophore (Hamilton) viz.* liver, intestine, gill and kidney. The results obtained have been presented in 25 microphotographs of these five pertain ti liver, six to intestine, seven to gill and seven to kidney. The magnification ranges from x 7500; 10 x85800.

Liver

In a control fish the liver cell shows clear cell boundaries with nucleus, mitochondria, endoplasmic reticulum and glycogen. Mitochondria and Golgi bodies are clearly organized. The nucleus shows lamellar nuclear membrane like lamellar cell boundaries. The chromatin material is darkly stained and present in small clusters. In the case of experimental fish, the liver cell boundaries show disorganization and the lamellar structure is broken at several places.

A wavy feature is seen in the lamellar texture of cell membrane as well as fusion and break down in the cell membrane. This is also seen in case of nuclear membranes. At some places shrinkage and bursting of nuclei are quite apparent. In some areas near the periphery of cells and mitochondria and other membranous structures appear to be clustered and disintegrated. The disintegration of mitochondria and endoplasmic reticulum is also visible. Cell organelles are in the process of disintegration. However, the chromatin material is darkly stained and more granular in the case of control fish. Moreover, the nucleolus is clearly visible in the liver cells of control fish and this is not seen in the case of an experimental fish. In the liver of an experimental fish, colored dense particles (probably droplets) are seen to be deposited around the nucleus after exposure to Chromium stress.

Intestine

In control fish, the microvilli of the intestinal epithelial cell surface, facing the lumen, are long, straight, dense, thick and parallel almost touching each other Cap between the two adjacent microvilli is less and more color sensitive materials are present in the form of large or small thin dots. In case of experimental fish, however, the microvilli of the intestinal epithelial cell surface facing the lumen are short and less stretched and the gap between the two microvilli is larger and the villi are thin and sparse. The number of color sensitive large or small dots is comparatively smaller below the surface of the microvilli the cells are small and the cell membranes are clear with a definite nucleus inside. The nuclei are small with defined margins and nucleolus. The glycogen particles less towards the margins and more in the central part.

The nuclear material is compactly organized in case of a control fish, it is smoothly dispersed and less darkly stained in a treated fish in the later case, the nucleus is spherical or oval with definite margins but nucleolus is absent. Lysosomal material is more confined towards the margin of cell just below the microvilli. There is some segregation of glycogen particle in the lower portion of the cell. The cellular boundaries disintegrating in experimental epithelium but in case of control epithelium, such disorganization is not clear. Darkly stained granular structures are present over the membrane of endoplasmic reticulum in control state but after treatment these darkly stained structures disappear. Disorganization and degeneration become apparent in the endoplasmic reticulum of the intestinal cell. In the control fish, the intestinal glands are more compact, round and well organized suggesting a fully functional state of the gland in an experimental fish, there are more stretched and longitudinal perhaps in the process of being non-functional.

Gill

The gill of a control fish shows epithelial cells having spherical or irregular nuclear margins. The pillar cells also show a similar structure of the nucleus. Nuclear membranes in a control fish are smooth and chromatin material is darkly stained in small particles. After treatment, however, the nuclear membranes appear and disintegrated at a few points and chromatin material is less darkly stained and more smoothly dispersed throughout the nucleus. Darkly stained bodies (probably lipid droplets) appear surrounding the nucleus also observed in the case of experimental epithelial cells; the bursting of nucleus membrane is also seen. The mitochondria are more elongated and clustered. The mitochondrial membrane is fused at some points and broken and disintegrated at others. Disintegration of cristae is also visible. In case of the control tissue, however, such changes are not visible. In the case of the experimental fish, other cell organelles are not visible. Only some vacuolar and membranous structures are noticed.

Kidney

In a control fish the kidney cells show normal features with spherical or irregular nucleus, some cells are surrounded by deeply stained particles (probably lipid droplets) which are evenly distributed. In case of experimental kidney, however, the cell nucleus is dilated and chromatin material becomes condensed. In some cases the nuclear and cellular membranes are broken or disintegrated in treated kidneys. The deeply stained lipid droplets are seen heavily segregated in clear zones surrounding the nucleus, which may be due to shrinkage. In some cells vacuolation appears and nucleus as well as chromatin material shows condensation and fading. Outer nuclear membrane is also coming out Polar dilation of nuclear membrane is visible. Mitochondria disintegrate after treatment. Endoplasmic reticulum is visible in control cells but disappear after treatment Intercellular spaces are more in a control tissue but become less after treatment Degenerating organelles are visible after treatment.

Discussion

The study of chromium toxicity on fish is available from Pickering. Stevens and Chapman (1984) and Abbasi and Soni (1985) [1].

Some workers like observed the histopathological changes in fish tissue under Chromium stress Freeman (1980) [11], observed the accumulation of Chromium in the body tissue of different fish. Dyes used in dyeing industries are carcinogenic as reported by Miller (1953) [16], Decloitre *et al.* (1973) [8] and Bannirkov *et al.* (1973) [3]. Dyes slowly creep into the aquatic media and directly affect the aquatic organisms particularly fish.

The variations in toxic response may be due to differences in breathing, habits, age, sex, 1989), Sastry and Sunita The variations in toxic ambient water quality and extent of availability of the different test organisms to Chromium stress.

Abel (1975) [2] postulated that death of the fish during acute toxicity experiment was probably the result more than one mode of action. He further suggested that protein denaturation, alteration in membrane permeability and active transport due to toxic effects of chemicals were the most probable causes of death.

But no work has been done so far on the ultra structural changes in the cells of fish tissue under Chromium stress. Therefore, it was considered prudent to observe the changes under Transmission Electron Microscope.

Gill

The epithelial and pillar cells of the fish show deviation in the form of bursting of nuclear membrane, mitochondria became more elongated and clustered, mitochondrial membrane becomes fused at some points and broken at other also gets disintegrated. Disintegration of cristae is visible. The smooth nuclear membrane with darkly stained chromium in patches in control fish after treatment disintegrates at some points and the chromatin material looks less darkly stained and more smoothly dispensed throughout the nucleus. Cell organelles show disintegration after exposure to Chromium stress. Vacuolationn appears in cytoplasm and several stained large droplets (probably lipid) aggregate near the nucleus in the fish gill subjected to stress.

Intestine

The microvilli of intestine become short, less stretched, thin and sparse. Gaps between the microvilli are more and they have less color sensitive, large or small dots in experimental fish.

Intestinal glands become more stretched longitudinal process of perhaps non functional state after exposure to chromium Stress whereas in control, it is more compact round shaped and well organized.

Below the microvilli the nucleus is of spherical nature without mucieolus. The nuclear material is smoothly dispensed so that they are less darkly stained and lysosomal material accumulates more towards the margin of cell and the cellular membrane shows disintegration. There are glycogen particles in the lower portion of the cell. No clear pattern of cytoplasmic reticulum is visible in experimental fish.

Liver

In the experimental liver exposed to long term Chromium stress, the disorganization of cell boundaries, broken tamellar structure, cell membrane in the process of fusion and disintegration, bursting of nuclear membrane and shrinkage of nuclei, loss of color, dense particles probably lipid droplets) around the nucleus are seen.

Kidney

Chromium stress mostly hits the cell membrane, nuclear membrane, chromatin material, mitochondria, cytoplasmic reticulum and lysosome-the ultra structures of the cell and due to disintegration of cellular material vacuolation appears which shows that the cellular function deteriorates due to Chromium effect. The poisoning hampers all physiological functions, which may ultimately result in the death of the fish under prolonged stress condition of sub lethal doses of Chromium.

Conclusion

It seems that Chromium stress hits the chemical constituent of cell membrane, nuclear membrane, endoplasmic reticulum, lipid, glycogen, chromatin material, mitochondrial wall and cristae, breaking chemical bonds of constituent chemicals and converting them into another chemical forms resulting in degeneration of cell organelles.

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