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Effects of Heavy Metal in Pisces

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Physico-Chemical Analysis of Test Water

Various physico-chemical characteristics of the test water have been enlisted in Table-I. The values obtained clearly reflected that the diluents water was quite suitable for fish and its quality conformed to the specifications outlined for static bioassay in standard methods.

Static Acute Bioassays

Toxicity of the heavy metal salt, copper sulphate, in terms of LC₅₀ value for different time intervals (24, 48, 72 and 96 hrs.) obtained under static system along with their regression equations, fiducial limit (95%) and slope function (S) are mentioned in Table –II and Figures A – E. The 24, 48, 72 and 96 hr. LC₅₀ values calculated were 30.30; 28.60; 24.70; and 19.0 mg/L respectively.

The results of acute bioassay in this study denoted a progressive increase of toxicity with increasing time intervals as is apparent from the amount of copper sulphate required for half kill decreased by nearly 38% over a period of 96hrs. as compared to that of 24hr.

Behavioural Response

On exposure to different lethal concentrations of copper sulphate exciting and agonistic behaviour was reflected by the fish. Positive symptoms.

Sublethal or Test Concentration

By applying the formula of Hart *et al.*, (1945), mentioned in the Material & Methods, over LC₅₀ values calculated during this study, the sub lethal or experimental dose of copper sulphate to the fish *H. fossilis* for chronic exposure was determined to 7.70 mg/L.

Histopathological Observations

Fish, upon exposure to 7.70 mg/L copper sulphate for 30 days, developed severe lesions in all the tissues/organs examined during the course of present study. Tissue/organ wise findings are elaborated below.

A Stomach Pathology under Chronic Stress of Copper Sulphate

Severe atrophic changes took place in the histological structure of the stomach following 30 days exposure of the fish to 7.70 mg/L of copper sulphate. It looked crumpled, shrunken, deshaped and autolysed. Vascularity and general texture of muscle layers were lost considerably. The epithelium of the mucosal fold was damaged, ruptured and disorganized. The columnar epithelial cell in general and nuclei in particular became necrosed and the erosion of mucosa was evident. Mucous goblet cells & gastric gland cells were autolysed. Submucosa also showed fibrotic and edematous changes.

C Control

The liver lobules are distinct in the fish *H. fossilis* demarcated well by central vein Each lobules is formed of polyhedralk hepatocytes arranged in groups enclosing bile passage. Each hepatocyte has granular cytoplasm and centrally placed spherical nucleus containing prominent nucleoli. The parenchymal cells form hepatic tubules which contain blood sinusoids. The H.S.I (Mean \pm SE or 5 fish) was 1.935 ± 0.027 .

Stomach

A. Normal or Contro Stomach

The stomach of the normal fish *H. fossilis* revealed usual four layers viz. mucosa, submucosa, muscularis and serosa. The stomach could be differentiated into cardiac and pyloric regions on the basis of presence of gastric glands in the former region.

Serosa forms the outermost thin layer followed by a thick layer of muscularis consisting of an outer longitudinal and inner circular muscle fibres. Muscularis is succeeded by highly vascular submucosa with abundant blood vesssels. Mucosal layer is lined by columnar epithelial cells raised into many

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primary and secondary folds or villi. The nuclei of columnar epithelial cells are almost centrally located. Mucus secreting cells, in between columnar cells, are also present at the crypts of the fold. Several simple tubular gastric glands below

Copper Stressed Fish Intestine

Server pathological changes were noticed in the intestinal tissue of copper sulphate treated fish *H. fossilis*. The villi lost their typical architecture and were ruptured leading to exudation of mucus into lumen and disintegration of columnar epithelium and necrosis Lamina propria got highly inflamed and elongated. Histolysis of columnar cells. Loss of brush border and fusion of columnar cells and appearances of small vacuolar spaces in longitudinal muscle cells were quite obvious.

Control

The kidney of *H. fossilis* is composed of large number of uriniferous tubules or nephrons surrounded by evenly distributed interstitial haematopoietic tissue. The tubular system consists of neck, proximal tubule, distal tubule and collecting duct. The neck segment connects the Bowman's capsule to that of proximal tubule. Histologically, proximal tubules are having columnar epithelial cells with central nuclei and can be distinguished on the basis of brush border towards lumen. Distal tubules are also formed of occupied by connective tissue, blood-capillaries and interstitial cells. Testis showed cells in various spermatogenic stages. The spermatogonium or sperm mother cell could be characterized by its large size and round shape, central nucleus and relatively clear cytoplasm. The primary spermatocytes are similar than spermatogonia but larger than secondary spermatocytes. The primary spermatocytes contain deeply stained nuclei whereas secondary spermatocytes have large clumpy nuclei. Both primary and secondary spermatocytes are abundant in the peripheral region of the lobule. Spermatids, present near the central region of the lobule, have basophilic oval or spherical nuclei. A few sperm cells are also present which could be located on account of their smallest size and almost central position. The gonadosomatic index (G.S.I) of the normal testis was 0.263 ± 0.0076 (Mean \pm SE of five fish). Thickness of lobular wall and diameters of lobule, sperm mother cell, primary spermatocytes, secondary spermatocytes and interstitial cells have been shown.

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