Alterations in biochemical profile of liver and ovary in zinc-exposed fresh water murrell, *Channa punctatus* (Bloch)

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**Abstract:** Sub-chronic exposure (15 days) of *Channa punctatus* to sub-lethal concentrations of zinc (10, 15 and 25 Zn mg l⁻¹) resulted in a significant decline of hepatic and ovarian glycogen, cholesterol, total proteins and total lipids. DNA and RNA contents were also estimated in the ovary. These parameters simultaneously decline with an increase in dose and duration of the experiment. A decline in all parameters studied thus reflects an adverse influence of Zn exposure on metabolism as well as reproductive activity of the fish.

**Key words:** Fish, Zinc, Biochemical changes

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**Introduction**

Fish are the richest source of an essentially healthy diet. They are however, endangered by water borne pollutants transferred along the food chain (Hoo et al., 2004; Ayas et al., 2007; Kumar and Achyuthan, 2007; Shukla et al., 2007; Srivastava and Srivastava, 2008).

Amongst various water pollutants, heavy metals poses a great threat to fishes. Heavy metal, zinc is used in various industrial operations forms and excessive zinc finds its way into lakes and rivers. Although, small quantities of zinc are required for normal development and metabolism of organisms, if levels exceed the physiological requirements, zinc can act as a toxicant. Exposure to excess zinc has been reported to bring about biochemical as well as histological changes in various organs of fishes (Agrawal and Srivastava, 2003; Gupta and Srivastava, 2006).

However, a study of the changes brought about in reproductive organs and reproductive activity are very few. Hence biochemical changes in the ovary of *Channa punctatus* have been recorded along with RNA and DNA after exposure to zinc for a short period. Simultaneous biochemical alterations have also been recorded in the liver with a view to correlate ovarian alterations with general metabolic changes in the fish.

**Materials and Methods**

Adult specimens of *Channa punctatus* (average length 18-20 cm. and average weight, 65 to 70 g) were procured from nearby water bodies (Jalmahal, Ramgarh and Nerva lakes). They were acclimatized for 15 days in 500 liter glass aquaria containing water from a tube well (Temperature- 14 to 22°C, Dissolved oxygen- 6.62 to 6.76 mg l⁻¹, Alkalinity- 62 to 68 mg l⁻¹, CO₂ NII) (APHA, 2005). Water was analyzed for physicochemical properties prior to setting up the experiment and on each autopsy interval. Fish were fed minced goat liver twice a week during acclimatization and the water of aquaria was changed on the following day. Fish were not fed during experimental regime. Fish were examined on alternate days and found to be healthy and uninfected. They were however, treated with mild KMnO₄ solution when brought to the laboratory. Thirty fish were taken both in control and treated groups. Control fish (Group-I) were put in normal tap water or tubewell water (480 liter). Zinc sulphate (ZnSO₄ 7H₂O), Mol. wt. 287.55, E. Merck, India (Ltd.) was used for the experiment containing 65.38 Zn. A stock solution was prepared in 500 ml distilled water containing 50 g Zn by diluting 219.937 g ZnSO₄. Further working dilutions were made accordingly. Zinc dilutions added to 480 liter aquaria water so as to give the following zinc concentrations - 10 mg l⁻¹ (Group II), 15 mg l⁻¹ (Group III) and 25 mg l⁻¹ (Group IV). It works out to be approximately 20, 25 and 50% of LC₅₀ 56.52 mg l⁻¹.

Five fish from each group were sacrificed on day 8, 10 and 15. The liver and ovary were excised, weighed and ten samples were processed for biochemical studies. Glycogen, cholesterol and total proteins were estimated by methods given by Oser (1965); total lipids were determined by the colorimetric method given by Fring and Dunn (1970). Ovarian DNA and RNA content were determined by the method given by Ceriotti (1952, 1955). Level of significance at p<0.05 and p<0.01 was, statistically calculated by using student t-test (Sendecor and Cochran, 1967).

**Results and Discussion**

Results reveal dose and duration dependent decrease in biochemical parameters of the liver and ovary (Fig. 1a-j).

Glycogen decline is non-significant at day 8, in both tissues of Group II, whereas it is significant at day 10 and highly significant at day 15. However, in Groups III and IV the decline is highly significant from day 8 onwards. Similarly a decline in glycogen has been observed in the liver of *Laboe rohita* by Bengeri and Patil (1986) and in *Channa punctatus* by Srivastava et al. (2002) after exposure to zinc for 96 hr and 15 days respectively. Glycogen also declines in the liver of *Cirrhinus mirgala* (Ham.) after exposure to lead for 30
Fig. 1 a - j: Shows biochemical profile of liver and ovary in zinc-exposed fish, Channa punctatus

Levels of significance: * = Significant at p<0.05, ** = Significant at p<0.01
Biochemical profile of liver and ovary in C. punctatus

and 60 days (Kumar et al., 2005) and in the ovary of Clarias batrachus after exposure to methyl mercruic chloride for 15 days (Verma et al., 2002). These results, therefore, indicate a similar effect of Hg, Pb and Zn on glycogen metabolism. Lowering of hepatic glycogen reflects reduced glycogenesis or increased glycogenolysis. Depletion of glycogen in liver may further be correlated with a high demand of glycogen for excess energy requirements, as corroborated by excessive movements and surfacing frequency in the experimental fish.

Reduced ovarian glycogen may be attributed to its utilization for maturation and development of oocytes as indicated by ongoing histological study; depletion in glycogen may also be a result of higher production of energy molecules required for metabolic activities of the ovary.

The decline in cholesterol of both tissues is non-significant at day 8, in Group II, significant at day 10 and highly significant at day 15. In Groups III and IV, however, similar to glycogen, this decline is highly significant from day 8 onwards. A significant reduction in liver cholesterol content has been reported after Cd treatment of Garra mullya for 4 months (Sinha et al., 2001). Sindhe et al. (2002) also reported a decline in cholesterol of both the liver and ovary of N. notopterus after exposure to Cd and Hg for 2 months. Observations in the present study also indicate that sub-chronic zinc exposure influences the cholesterol level in a similar manner to that noted for exposures to Hg and Cd.

Lowering of cholesterol may be attributed to an increase in lipid utilization for meeting additional energy requirements under stress conditions, as suggested by Srivastava et al. (2002). The reduction in hepatic and ovarian cholesterol content may also result in altered vitellogenesis and steroidogenesis, as suggested by Sindhe et al. (2002) in N. notopterus.

The decline in total proteins and total lipids is similar, both in liver and ovary. It is non significant at day 8, significant at day 10 and highly significant at day 15. In Groups III and IV, it is highly significant at all intervals. Sindhe et al. (2002) observed a decline in liver and ovarian proteins of Notopterus notopterus after exposure to various concentrations of cadmium chloride and mercuric chloride, individually and in combination, for 2 months. Exposure of Channa punctatus to Ni for 30 days (Desai et al., 2002) and Zn for 15 days (Srivastava et al., 2002) also results in a similar decline in the liver. Exposure of Cyprinus carpio and Cirrhina mrigala to zinc for 60 days (Dhawan and Kaur, 1997) also results in a decline of total proteins in the ovary. The decline noted may be due to blocking of protein synthesis, as suggested by Vijayram et al. (1989). Hepatic proteins may be depleted due to proteolysis by increased activity of lysosomal enzymes (Dhanapakiam and Ramasamy, 2001; Desai et al., 2002) or they may be mobilized for yolk formation in the developing oocytes.

A decline in the lipid content of liver was observed in female H. fossilis exposed to nickel chrome-electroplating effluent for 120 days (Gupta, 1991). Similarly a decline in the lipid content of the ovary of Cyprinus carpio and Cirrhina mrigala have been reported after exposure to zinc for 60 days (Dhawan and Kaur, 1997). The reduction in total lipid content may be a result of disturbed vitellogenesis, steroidogenesis and/or reduced enzyme activity (Sindhe et al., 2002). Mercury is reported to reduce gonadotrophin release which causes impairment in yolk formation by the oocytes of Channa punctatus (Ram and Sathyanesan, 1984). Zinc, being a heavy metal, may also have a similar influence on the hypothalamic centers in Channa punctatus.

Contrary to the above parameters, ovarian DNA and RNA show a significant decline from day 8 onwards, in all groups. A significant reduction in ovarian RNA/DNA ratio has been reported after mercury treatment of Labeo rohita for 30 days by Aditya et al. (2002). Similarly, present results also show a decline in RNA/DNA ratio as well as DNA and RNA content. This decline may be a result of chromosomal aberration and fragmentation as reported by Gupta et al. (2006), in a fresh water teleost. Heteropneustes fossilis (Bloch) treated with zinc. Lowering of RNA/DNA ratio and RNA and DNA content also indicates an increased catabolism of proteins which corroborates well with the decline in proteins noted in the present study.

Present study clearly indicates that a short exposure to sublethal doses of zinc significantly reduces the functional capacity of the liver and ovary which will inturn hamper metabolic pathway and reproductive capacity of the fish. Genotoxic nature of zinc is also indicated by the present study.

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References


