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CHANGES IN CERTAIN ENZYMES OF THE OVARY AND LIVER IN *CHANNA PUNCTATUS*

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Abstract: Alterations in certain enzymes have been examined in the liver and ovary of a fresh water murrel, *Channa punctatus* (Bloch), after exposure to 10 mg/l (Group II), 15 mg/l (Group III) and 25 mg/l (Group IV) zinc for 8,10 and 15 days. Untreated fish (Group I) served as control. Acid phosphatase (ACP) was inhibited both in the liver and ovary. Alkaline phosphatase (ALP) decreased in the liver while it increased progressively in the ovary. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) enzymes showed a significant increase with an increase in dose and duration of the experiment. Secretion of these enzymes indicates significant pathological changes in the liver and ovary. These changes would also have an adverse influence on the functional capacity of these organs.

Key Words: Zinc, fish, Enzymological changes.
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Introduction

Fish constitute a valuable commodity from the standpoint of human consumption; aquatic pollution undoubtedly affects fish health and survival. Heavy metals are common pollutants of the aquatic environment because of their persistence and tendency to concentrate in aquatic organisms (Hoo *et al.* 2004; Ayas *et al. 2007*; Kumar and Achyuthan, 2007, Shukla *et al.* 2007, Verma and Srivastava, 2008a,b and Srivastava and Verma, 2009). Most heavy metals released into the environment find their way into the aquatic system as a result of direct input, atmospheric deposition and erosion due to rainwater. Therefore, aquatic animals are often exposed to elevated levels of heavy metals. Zinc is an essential heavy metal and it plays an important role in various biological processes including oxidative phosphorilation, gene regulation and free radical homeostasis as an essential cofactor (Feder, 1996). However, when its concentration exceeds metabolic requirements it becomes toxic (Gupta and Srivastava 2006; Srivastava *et al.* 2006 and Srivastava, 2007 and Srivastava and Verma, 2009).

Disturbances in metabolism can easily be identified by estimation of enzyme activity (Humtsoe *et al.* 2007), therefore, in this study enzymological changes have been recorded in the liver and ovary of *Channa punctatus* after exposure to sub- lethal concentrations of zinc. Enzymes of the liver were examined because liver plays a primary role in metabolism and excretion of xenobiotic compounds (Humtsoe *et al.* 2007 and Shukla *et al.* 2007). Simultaneously, enzyme activity in the ovary has been estimated because any anomaly in normal metabolism of gonads in fishes can influence its reproductive capacity and progeny.

Methods and Materials

Live specimens of fresh water fish, Channa punctatus were collected from local water bodies and acclimatized for 2 weeks under laboratory conditions. The physico-chemical characteristics of water were analyzed as per methods given in APHA *et al.* (2005) (Table- 1).

Table-1 Physico- chemical charecteristics of water

Temperature(^oC)	14 to 22
Ph	7-7.2
Dissolved oxygen (mg/l)	6.62-6.67
Hardness (mg/l)	15-18
Chloride content (mg/l)	30-35
Alkalinity (mg/l)	62-68

Fish were exposed to three sub-lethal concentrations of zinc i.e. 10 mg/l (Group II), 15 mg/l (Group III) and 25 mg/l (Group IV) for 15 days. Group I served as control. Observations were recorded on day 8, 10 and 15.

The liver and ovary were excised, weighed and processed for biochemical studies. In both tissues acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined by the method given by Kind and King (1954). Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were estimated quantitatively by Colorimetric method given by Reitmen and Frankel (1957). Levels of significance at $P< 0.05$ and $P< 0.01$ were statistically calculated by using student 't' test.

Results

Changes in enzymes of the ovary and liver are presented in Table 2. Ovarian acid phosphatase (ACP) shows a non- significant decline in group II at day 8 but it is significant at day 10 and highly significant at day 15. In group III, the decline is significant at day 8 and is highly significant at day 10 and 15.However, the decline is highly significant at all three intervals in group IV. On the contrary, hepatic acid phosphatase (ACP) shows a non-significant decline in groups II and III at day 8, it is significant at day 10 and highly significant at day 15. In group IV the decline in hepatic ACP is significant at day 8 and highly significant at day 10 and 15 (figures 1-6).

Contrarily, ovarian alkaline phosphatase (ALP) shows a highly significant increase in all treated groups at all intervals, whereas hepatic alkaline phosphatase (ALP) shows a decline. At day 8, it is non- significant in groups II and III but significant in group IV.A significant decline is noted in hepatic ALP in all groups at later intervals.

Elevation in SGPT and SGOT is dose and duration dependent. A significant increase is noted in SGPT and SGOT in group II at day 8 whereas it is highly significant in remaining groups at all intervals.

Table.2- Changes in enzymes of the ovary and liverof *Channa punctatus*

Days	Doses (mg/l)	Groups	ACP		ALP		SGOT	SGPT
			Ovary	Liver	Ovary	Liver	Liver	Liver
Day 8	Control	Group I	5.35±0.19	8.57±0.32	8.43± 0.48	12.85±0.33	79.01±1.29	82.04±1.23
	10	Group II	4.95±0.15 n.s.	8.32±0.19n.s.	10.56± 0.32**	12.42±0.19n.s.	83.32±1.32*	88.15±1.36*
	15	Group III	4.50±0.21*	7.95±0.21n.s.	12.92± 0.36**	11.95±0.23n.s.	92.82±1.98**	97.88± 2.24**
	25	Group IV	4.01± 0.23**	7.61±0.21*	17.58± 0.79**	11.41±0.29 *	101.42±2.42 **	106.75±2.69**
Day 10	Control	Group I	5.37± 0.21	8.58±0.33	9.12± 0.43	12.86±0.30	79.95±2.30	82.95±1.42
	10	Group II	4.52±0.17 *	7.69±0.20*	12.92± 0.38**	11.69±0.23 *	89.85±1.84**	95.22±1.78**
	15	Group III	3.98±0.25 **	7.35±0.24*	13.89± 0.52**	11.35±0.32*	99.82±2.52**	106.95±2.39**
	25	Group IV	3.48± 0.26**	7.12±0.26**	18.72± 0.71**	10.98±0.34**	115.42±2.98**	116.72±2.98**
Day 15	Control	Group I	5.40±0.23	8.60±0.35	10.03± 0.45	12.88±0.32	81.65±2.69	83.59±1.84
	10	Group II	4.01± 0.21**	6.92±0.23 **	14.21±0.58 **	11.12±0.26**	99.85±2.54**	109.42±2.32**
	15	Group III	3.47±0.29 **	6.2 5±0.25 **	15.24± 0.62**	10.59±0.33**	112.56±2.89 **	125.82±2.56**
	25	Group IV	2.92±0.28 **	5.99±0.28**	25.36± 1.23**	9.89±0.36**	134.04±3.47 **	138.65±3.21**

n.s.=non-significant
* = significant at P< 0.05
**= significant at p< 0.01

Fig.1

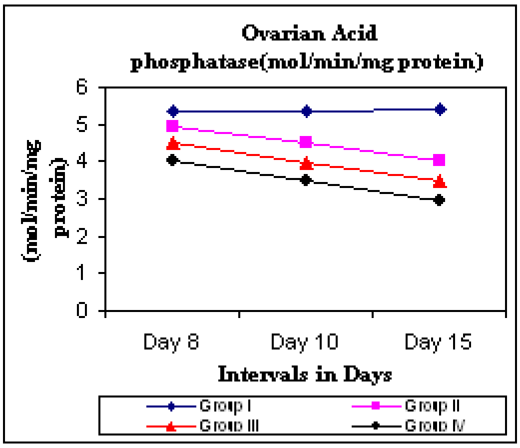

Fig.2

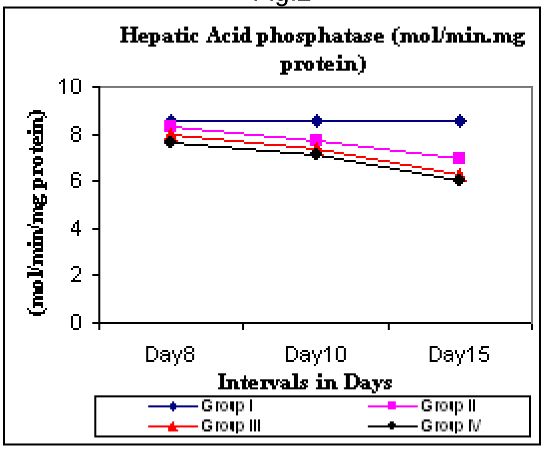

Fig.3

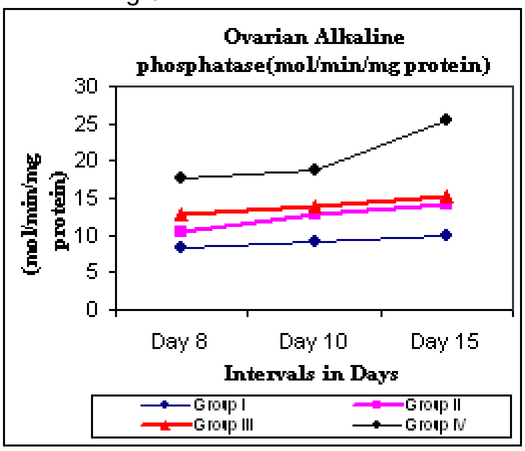

Fig.4

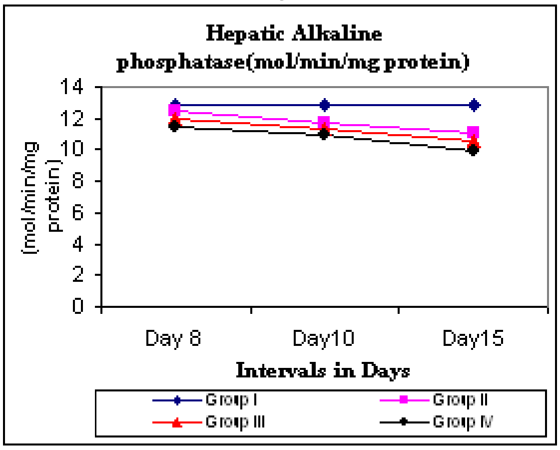

Fig.5

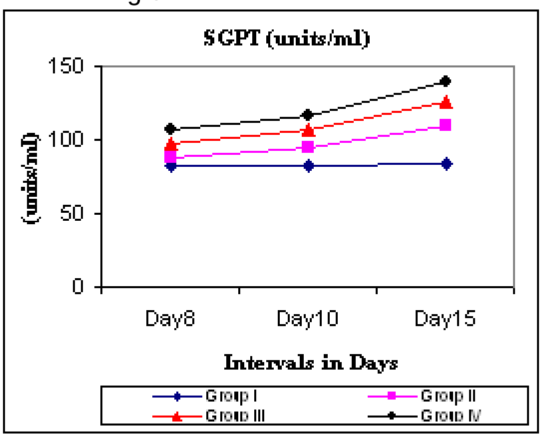

Fig.6

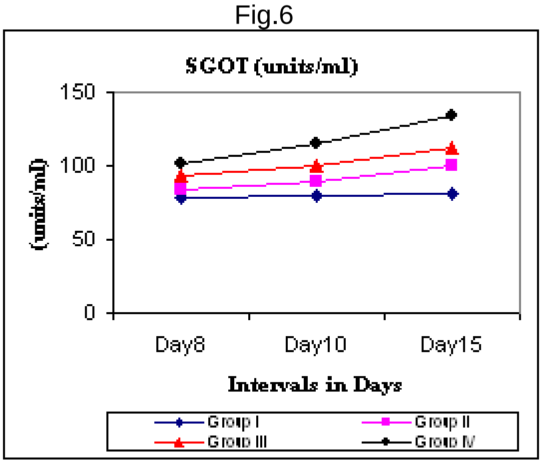

Fig 1-6 changes in enzymes of liver and ovary after sub-chronic zinc exposure.

Discussion

In the present study, a decline is reported in ovarian and hepatic ACP and hepatic ALP after exposure to Zinc for 15 days; ovarian ALP registers an increase. Similar to the present study, a significant reduction in ovarian and hepatic ACP and hepatic ALP has earlier been reported in *Heteropneustes fossilis* after Cd treatment for 15,30 and 60 days (Sastry and Subhadra, 1985). Naidu *et al.* (1984) also reported a significant decline in hepatic ACP and ALP of *Sarotherodon mossambicus* after an acute exposure (96 hrs.) to mercury. According to them impaired oxidative and transphosphorylative activities and utilization of carbohydrates during mercury toxicosis causes this depletion. Supporting the above view, Bhatnagar and Bana (1993) reported a decrease in hepatic acid phosphatase in *Channa gachua* after exposure to thiodan and rogar for 30 days; they suggested that the decline may be due to uncoupling of phosphorylation. In the present study, the decline noted in ovarian and hepatic ACP and hepatic ALP reflect the effects of Zinc toxicity, which could cause impairment of phosphoprylation or changes in permeability and disruption of lysosomes and mitochondria. This view has also been suggested by Sarasus and Andal (2005) and Humtsoe *et al.* (2007). Sarasus and Andal (2005) reported a decrease in hepatic ACP and ALP in *Hypophthalmichthys molitrix* and *Catla catla* after exposure to distillery effluent for 30 days. Decrease in enzyme activities in the present study can also be directly correlated with metal uptake. Humtsoe *et al.* (2007) reported significant reduction in hepatic ACP and ALP after exposure of *Labeo rohita* to Arsenic for 30 days. In the present study, the decline in ovarian and hepatic ACP and hepatic ALP could be a result of increased concentrations of Zinc in the ovary and liver since significant accumulation of Zinc has been reported both in the ovary and liver of fish in an earlier study (Verma and Srivastava, 2008a,b). Humtsoe *et al.* (2007) also subscribe to this view.

In contrast to ovarian and hepatic ACP and hepatic ALP, ovarian ALP increases after sub – chronic exposure to Zinc. Gill *et al.* (1990) reported increased activity of ovarian ALP in Rosy barb (*Puntius conchonius*) after a short exposure of 48 hrs to $HgCl_2$. Above report indicates possibility of a similar mode of action of both metals i.e. Zn and Hg on ovarian ALP. An increase in ALP of Zn exposed ovary during this study also supports earlier reports on intestine, liver and gills of the catfish *Heteropneustes fossilis* (Bloch) by Kothari and Soni, (2004a,b) and Jat and Kothari, (2006). An increase in ALP suggests increased glucose and phosphate transfer (Jat and Kothari, 2006); it is also known to be an indicator of histological damage (Yang and Chen, 2003 and Atli and Canli, 2007). Histological damage in the ovary of this fish has earlier been reported after exposure to same Zn concentrations (Verma and Srivastava, 2008a).

An increase in SGOT and SGPT has been reported in *Cyprinus carpio* (Mathan, 2006), *Sparus auratain* (Antonella and Landriscina, 1999), *Carassius auratus gibelio* (Zikic *et al.* 2001), *Cyprinus carpio* (De la Torre *et al.* 2000) and *Clarias gariepienus* (Velmurugan *et al.* 2006) after exposures to various metals. Mathan (2006) opined that an increase these enzymes may have resulted from tissue damage and increased synthesis of the enzymes to defend against stress. Antonella and Landriscina (1999) also suggested that cadmium alters hepatocyte cell membrane structure and concomitantly induces changes in mitochondrial membranes resulting in elevation of these enzymes. Similarly, De la Torre *et al.* (2000) and Zikic *et al.* (2001) attributed increased SGOT and SGPT activity to liver cell damage with concomitant liberation of transaminases into the circulation. De la Torre *et al.* 2000 and Velmurugan *et al.* 2007 recorded an increase in transaminases and attributed it to alterations in amino acid catabolism after Cd treatment of *Cyprinus carpio* and *Clarias gariepienus* respectively. Similarly, Adham (2002) recorded an increase in SGOT and SGPT of *Clarias batrachus* in Lake Maryut (containing Zn); he suggested that it may be a sign of damaged liver which inturn leads to leakage of these cellular enzymes into the blood. Present study supports the above observations. Increased transaminase (SGOT and SGPT) activity in *Channa punctatus* exposed to zinc, may either be due to leakage of enzymes across damaged plasma membranes and / or an increased synthesis of enzymes by the liver as a defence against stress. To corroborate this view ruptured and totally damaged hepatocytes have been observed in an earlier study conducted under similar conditions (Verma and Srivastava, 2008b).

Accumulation of metals by the liver, has been reported to be an important factor influencing enzyme activity (Dubale and Shah, 1981 and Humtsoe *et al.* 2007); high accumulation of zinc in the liver has been recorded earlier during a similar investigation (Verma and Srivastava, 2008b); this could be instrumental in causing cell damage leading to an increase in transminases. In the present study an elevation of SGOT activity could also provide oxaloacetates required for gluconeogenic pathway to meet additional supply of glucose required for production of energy during oxidative metabolism (Tilak *et al.* 2005). Thus elevation in the levels of SGOT and SGPT in liver of *Channa punctatus* can be considered a response to stress, induced by zinc, for contributing to gluconeogenesis and/or energy production necessary to meet excess energy demands. This is corroborated by excessive movements noted in experimental fish.

This study conforms with earlier reports that these enzymes are a very sensitive index for hepatic and ovarian changes occurring due to adverse condition. It can also be concluded that both ovarian and hepatic functions are adversely influenced by exposure to zinc, thereby hampering metabolic pathways and reproductive capacity of the fish.

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