Biochemical perturbation in the ovary of sexually mature Channa punctatus Bloch challenged by heavy metal ions

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Abstract

The cytogenous and endocrine functions in the ovary of teleost fishes are distinctly compartmentalised as they are in other vertebrates. The 'milieu interior' of the ovary contains a vast array of substrates e.g., carbohydrates, protein, lipids and enzymes. Enzyme-substrate interactions are responsible for maintenance of 'pools' of such vital precursors as amino acids, sugars (C_5 and C_6), nucleotides and cholesterol. Growing, dividing, vitellogenic and maturing cells of the ovary have the inherent albeit variable potential to utilize these via enzymatic intervention. Specific protein, lipid, carbohydrates, steroids etc. are thus bio synthesized which facilitate the successful completion of oogenesis, folliculogenesis and vitellogenesis. Deleterious effects of heavy metal ions on ovarian metabolism have been fragmentarily studied using some species of oviparous and viviparous teleost. The present report deals with the biochemical changes in the amounts of total protein (TP) and triglycerides (TG) in the ovary of sexually mature breeding females of Channapunctatus (weighing 60.00 $\pm$ 5.00 gm) challenged by sub lethal concentration of $Pb(NO_3)_2$ (10 mg/L) and $ZnSO_4.7H_2O$ (20 mg/L) for 7, 21 and 35 days. The results showed varying degrees of time dependent changes. Pb and Zn initially (after 7 days) caused increase in TP. However, on day 21, their amounts were decreased. The increase in TP values were observed in the day 35 of treatment in response to both Pb and Zn. This may be due to bioaccumulation of these cations on the ovaries. Ovarian TG values manifested differential shifts in response to Pb and Zn. A decremental trend was observed on day 7 and 35. However, on day 21, the TG values were higher than control in both cases.

Key Words :- Carcinogenic, teratogenic, Biochemical, TP, TG, toxicity, rebound, folliculogenesis, vitellogenesis, decremental, mature oocytes, Channa punctatus, maturation, Sub-lethal, gonadotropins.

Introduction

Heavy metal ions e.g., lead, cadmium, mercury and zinc form an important class of toxicants which induce a variety of histopathologies in somatic and gonadal tissues. Further, they also cause severe aberrations in the metabolic pathways, DNA-RNA amounts and protein synthesis leading to cell death[1.2]. Salts of lead are known to have mutagenic, carcinogenic, co-carcinogenic and teratogenic effects.

Gametogenic activity is reported to be severely disturbed due to exposure to lethal and sub lethal doses of these cations for an extended period of time[3-4]. Compared to lead salts, salts of zinc have been shown to express their effects only when they are deficient or in excess.

Zn-deficiency has a marked effect on fecundity and cellular metabolism. High doses of this metal induce significant histo and cyto-pathologies. In addition, Zn has been shown to have a protective function by competing with such deleterious metals as lead by dislodging them from subcellular binding sites[5-9].

The present study reports about the biochemical perturbation in the amounts of total, protein (TP) and triglycerides (TG) in the ovary of sexually mature breeding females of Channa punctatus challenged by sub-lethal concentrations of $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ for varying duration of time.

Material and Methods

Sexually mature breeding females of C.punctatus (60.0 $\pm$ 5.0 gm)were collected from the non-polluted aquatic reservoirs in the vicinity of Udaipur during their breeding season (May-September). They were acclimated to laboratory conditions as described before[10].

Preliminary toxicity tests indicated that 10mg/l $Pb(NO_3)_2$ and 20 mg/l $ZnSO_4.7H_2O$ were the sublethal concentration to this fish for 35 days. Control and experimental groups of fishes (3-6) were set up as described before[10].

Fishes from control and experimental groups were sacrificed by cephalic stunning after 7, 21 and 35 days of exposure. The paired ovaries were dissected out surgically under semi sterile conditions. They were freed of excess fascia, blood clots and washed in chilled physiological saline (at 4^oC).

For biochemical estimations, pieces of ovaries weighing 50.0 mg were homogenized in 1.0 ml of chilled physiological saline. The homogenate was centrifuged at 3000 r.p.m. for 20 min. The supernatant was used for the biochemical estimation of TP[11] and TG[12].

Results and Discussion

The ovary of sexually mature C.punctatus exposed to $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ for varying durations exhibited characteristic variations in the biochemical milieu of TP and TG (Table 1). These results are discussed in the light of available literature which is scant and fragmented.

Total ovarian protein showed a marked increase on day 7 and 35 of treatment. However, on day 21 of challenge their amounts were much lower vis-a-vis control. The early increase (i.e., after 7 days) in the amounts of TP signifies considerable degenerative changes in the ovarian cell types. Although by day 21 some 'rebound' recovery appeared. The increase in TP became significantly evident on day 35 in response to both Pb and Zn. This may be due to bioaccumulation of cations in the ovaries which resulted in their subsequent deleterious effects. Banerji[13] observed that $Pb(NO_3)_2$caused significant decremental changes in the total ovarian protein of C.punctatus after 15 days. In the present study such changes seen after 7 days. This may be due to those differences. No further biochemical information is available on the ovarian TP values shifts in response to heavy metal ions. Results of other studies do suggest that heavy metal ions may act either by inactivating intracelluar proteins[14] or by inhibiting protein synthesis at the transcriptional or translational levels[15-17]. Loss of hydrophilic property and susceptibility for easy precipitation of protein may occur due to binding of heavy metal ions. This may be true both in case of Pb and Zn. Insufficiencies of protein may irretrievably and detrimentally affect folliculogenesis and vitellogenesis[18]. Results of the present studies appear to substantiate this. Ovarian TG of C.punctatus manifested differential shifts in responses to Pb and Zn. A decremental albeit differential trend was observed on day 7 and 35 of treatment. However, on day 21 the TG values were higher than control in both cases.This build up may be due to 'rebound' recovery or inability of the cells to utilise these substrates. Lack of lipid or its hydrolysates may mean loss of alternate source of energy as well as non-availability of precursors needed for steroidogenesis. The latter may adversely affect folliculogenesis and vitellogenesis which needed a constant supplication of these steroids. A comparison of these findings with other shows some differences as well as compatibilities. Thus, Banerji[13] found Pb to cause decremental changes in ovarian TG of C.punctatus after 30 days of treatment which were further aggravated by day 60. These data are at variance with the present studies. Katti and Satyanesan[19] also observed a significant reduction of TG values due to $Pb(NO_3)_2$ in Clarias batrachus. No tangible information is available on the Zn-induced changes in the ovarian TG of teleosts.

There is considerable debate and conflict in the literature on the mechanism(s) involved in the depletion of lipids in ovarian cell types due to heavy metal ions. The vitellogenic and mature oocytes have been reported to contain maximal amount of TG[20]. Gonado tropins and sex steroids have been implicated in facilitating the transfer of phospholipids and lipids from the liver to oocytes that enter vitellogenic stages. The decline of lipids as observed in the present studies on day 7 and 35 may be due to aberrant oscillations in the titers of gondotropins/ or sex steroids;these may not only affect vitellogenesis but also the intricate processes of maturation.

Table -1

Effect of $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ on the ovarian biochemical milieu of sexually mature breeding females of Channapunctatus.

S.No.	Duration	TP mg/dl%)	TG mg/dl%
1.	$Pb(NO_3)_2$		
(a)	7 day Control Experimental	 196.10±1.82 **290.00±0.07 (+47.88)	 66.32±1.24 ***52.51±1.24 (-20.82)
(b)	21 day Control Experimental	 193.21±1.30 **109.10±2.11 (-43.53)	 62.04±1.11 ***78.56±1.22 (+26.62)
(c)	35 day Control Experimental	 195.05±1.85 ***320.40±1.80 (+64.26)	 60.58±0.09 ***29.96±0.08 (-50.54)
2.	$ZnSO_4.7H_2O$		
(a)	7 day Control Experimental	 192.12±1.03 ***340.01±1.80 (+76.98)	 66.98±1.52 60.01±0.09 (-10.40)
(b)	21 day Control Experimental	 196.28±1.41 **119.42±2.52 (-39.16)	 64.53±0.05 ***88.93±1.12 (+37.81)
(c)	35 day Control Experimental	 191.19±1.78 ***290.33±2.62 (+51.85)	 66.21±1.81 61.51±2.28 (-7.10)

*****P<0.01, ***P<0.001, ± S.E., ( ) values in parenthesis are decrease or increase vis-a-vis controls.***

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