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BIOCHEMICAL VARIATION DURING OVARIAN VITELLOGENIC GROWTH IN A HILL STREAM TELEOST *GARRA MULLYA* (SYKES) DUE TO CADMIUM TOXICITY

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ABSTRACT

Some biochemical variations during ovarian vitellogenic growth in hill-stream teleost *Garra mullya* due to sublethal concentration of cadmium has been discussed. Total protein, cholesterol and glycogen in ovary and liver along with gonadosomatic index (GSI) and hepatosomatic index (HSI) in Cd-treated fish exhibited significant decrease while liver glycogen remained unaltered.

INTRODUCTION

Most of the fish species are seasonal breeders and the breeding period is marked by complex physiological processes in the body which result in remarkable biochemical changes in fish tissues specially liver and gonad. Ovarian vitellogenic growth is initiated by gonadotropins secreted by pituitary which stimulate the ovary to secrete estrogen. Much of the yolk material of eggs in fishes is synthesized by liver under the influence of estrogen, as a complex lipophospho-protein precursor known as vitellogenin (Emmersen and Emmersen, 1976; Tata, 1978). This is carried to the ovary by the blood to be incorporated there during vitellogenesis as a phosphoprotein-lipoprotein complex (Emmersen and Emmersen, 1976; Medda and Das Mahapatra, 1980; Quinitio *et al.*, 1989) which eventually forms much of the dry matter of the ovary. As a result the phase of vitellogenic growth of fish is accompanied by changes in cellular constituents of liver and ovary, specially protein, glycogen and cholesterol (Emmersen and Emmersen, 1976; Bano, 1977; Medda and Das Mahapatra, 1980).

During the last few decades the multidimensional human activities has resulted in enormous pollution problem of water bodies from industrial, mining and other sources. Consequently heavy metal toxicity has become an alarming problem for water bodies and cadmium is one of them. Cadmium has been found to affect the physiological and biochemical constitution of various fish species (Ball, 1967; Banerjee *et al.*, 1978; Wani and Latey, 1982, 1983). However, the information about the effect of cadmium on biochemical composition of hill-stream fishes from Chhotanagpur plateau is still inadequate though the rivers and streams of this region often get contaminated by heavy metals due to various industrial and mining effluents.

In the present communication, an attempt has been made to study the effect of Cd-toxicity on the ovarian vitellogenic growth in a hill-stream teleost *Garra Mullya* which is a common food fish of this region specially in rural areas.

MATERIAL AND METHODS

Mature *Garra mullya*, (length, 14-15 cm; average weight, 33.2 g) were used as test fish. Fishes were collected from survarnarekha river in the Getalsud region, 30 kms west of Ranchi town with the help of local fishermen and were acclimatized to laboratory condition for a week before start of the experiment. Probit analysis method (Finney, 1971; Fisher and Yates, 1974) was applied to determine the 96 h Lc_{50} value. Experiments were carried out during April, May and June separately which represents the period of maximum ovarian vitellogenic growth from the site mentioned above (Khan and Mehrotra, 1991). One dozen individuals were kept each in Cd-containing and control waters. Experimental group was lodged in water containing 4 mg/litre cadmium chloride ($CdCl_2$) on the second day of each month from April to June and were sacrificed on the 30th day of the respective months i.e. after 28 days of Cd-exposure. Control groups were maintained in identical conditions without $CdCl_2$. During experiment the fishes were fed with *Hydrilla* leaves. Size of the aquarium was 40 cm x 40 cm x 40 cm. The physico-chemical characteristics of water did not vary significantly during the three months of experiment and were : temperature 24.7 - 26.2° C; pH 7.2 - 7.5; total hardness 61 - 72 ppm; total alklinity 107 p - 116 ppm and dissolved oxygen 6.5 - 7.5 ppm

At the end of experiment (28 days) in each month the liver and ovary were quickly dissected out and gonadosomatic index (GSI i.e. ovary wt./ 100 g body wt.) and hepatosomatic index (HSI i.e.

liver wt./100 g body wt.) were determined. Tissues were transferred to chilled fish saline and were processed for the estimation of total protein, cholesterol and glycogen. Methods of Munro and Fleck (1966) modified by Abalain *et al.* (1980) were applied for the extraction and the method of Lowry *et al.* (1951) was applied for the estimation of protein. The method of Kabara (1962) was used for extraction and estimation of cholesterol. The method of Kemp *et al.* (1954) modified by Krishnaswami and Srinivasan (1961) was applied for extraction and estimation of glycogen. Statistical analysis of the data was done by student's 't' test (Bruning and Kintz, 1977).

RESULTS

Results of the experiments are summarised in Tables I and II for liver and ovary respectively. Protein and cholesterol contents of liver and ovary were found to decrease significantly in Cd-treated group in comparison to control during April, May and June along with corresponding fall in HSI as well as GSI. Decrease of GSI was more significant in April and May ($p<0.001$) than in June ($p<0.02$). Similarly HSI exhibited more significant decrease in May and June ($p<0.001$) than April ($p<0.05$). Bothe liver and ovary proteins recorded highly significant fall ($p<0.001$) in Cd-treated specimens during all the three months of experiment.

Cholesterol content also exhibited highly significant decrease during all three months in both liver and ovary of Cd-exposed specimens ($p<0.001$). Glycogen content recorded significant fall ($p<0.001$) in ovary of Cd-treated fish whereas in liver the change of glycogen was statistically insignificant.

DISCUSSION

The key product in vitellogenesis is a multicomponent lipo-phospho-protein called vitellogenin which is synthesized in the liver and transported by blood to the ovary (Emmersen and Emmersen, 1976; Quinitio *et al.* 1989). Before incorporation in the ovary as yolk granules, the vitellogenin is cleaved into yolk protein-phosvitin and lipovitellin (Tata, 1978). The process of vitellogenesis is initiated in liver under the influence of estrogen (Emmersen and Emmersen, 1976; Bano, 1977; Medda and Das Mahapatra, 1980). Deposition of yolk protein in the ovary is reflected in the increased gonadosomatic index and hepatosomatic index (Emmersen and Emmersen, 1976; Quinitio *et al.*, 1989). A similar result of GSI and HSI increase was observed during the present study among control group of fishes

TABLE I : *Variations in the protein content, cholesterol and glycogen in the liver and of hepatosomatic index in Garra mullya due to cadmium toxicity.*

Month	Total Protein (Percent)		Cholesterol (mg/g tissue)		Glycogen (mg/g tissue)		Hepatosomatic Index (HSI)	
	Control	Treated	control	Treated	control	Treated	control	Treated
April	28.50 ±0.37	18.10 ±0.29	10.80 ±0.26	5.90 ±0.22	5.70 ±0.29	5.40 ±0.21	2.60 ±0.34	1.00 ±0.20
	$p<0.001$		$p<0.001$		NS		$p<0.05$	
May	26.40 ±0.31	20.00 ±0.45	11.50 ±0.22	6.50 ±0.42	5.30 ±0.26	5.20 ±0.26	2.80 ± 0.26	1.40 ±0.15
	$p<0.001$		$p<0.001$		NS		$p<0.001$	
June	22.60 ±0.26	17.30 ±0.42	11.60 ±0.28	6.20 ±0.29	5.40 ±0.21	5.70 ± 0.21	2.10 ±0.17	1.40 ±0.15
	$p<0.001$		$p<0.001$		NS		$p<0.001$	

NS - Non Significant.

TABLE II : *Variations in the protein content, cholesterol and glycogen in the ovary and of gonadosomatic index in Garra mullya due to cadmium toxicity.*

Month	Total Protein (Percent)		Cholesterol (mg/g tissue)		Glycogen (mg/g tissue)		Hepatosomatic Index (HSI)	
	Control	Treated	control	Treated	control	Treated	control	Treated
April	23.60 ± 0.64	15.60 ± 0.24	9.70 ± 0.23	4.90 ± 0.26	6.20 ± 0.20	3.60 ± 0.14	6.70 ± 0.66	2.50 ± 0.80
	p<0.001		p<0.001		p<0.001		p<0.001	
May	26.80 ± 0.42	19.70 ± 0.50	13.30 ± 0.49	5.60 ± 0.18	7.50 ± 0.50	4.80 ± 0.21	10.70 ± 0.06	5.80 ± 0.24
	p<0.001		p<0.001		p<0.001		p<0.001	
June	28.30 ± 0.45	22.90 ± 0.53	14.30 ± 0.29	9.70 ± 0.23	7.80 ± 0.09	5.70 ± 0.27	11.50 ± 0.99	8.20 ± 0.72
	p<0.001		p<0.001		p<0.001		p<0.02	

(Table I and II) while a sharp decrease was noticed in the Cd-treated specimens probably due to histological damage done by toxic impact of cadmium. A significant fall in the gonadosomatic index along with disappearance of mature oocytes and liver damage has also been noticed in Cd-treated *Garra mullya* (Wani and Latey, 1982, 1983) which confirms the present finding. This change of GSI and HSI can be attributed to Cd-toxicity as cadmium shows high affinity with sulphydryl and hydroxyl groups and ligands containing nitrogen which is considered to be the mechanism of cadmium toxicity (Nilsson, 1970). Consequently the binding with such groups in biochemical systems of living tissues makes the effect of Cd-toxicity lethal even at low concetrations.

The cholesterol, protein and glycogen contents of liver and ovary have been found to increase during breeding season in various species of fish (Emmersen and Emmersen, 1976; Bano, 1977; Das Mahapatra and Medda, 1980, 1982; Sherni, 1981). Cholesterol, a major source of steroids during gonad maturation in fishes reflects the physiological state of ovary by its profiles in it as steroidogenesis via cholesterol precusor involves complex enzyme systems (Armstrong, 1968). Hence, the level of cholesterol becomes an index of functional state of ovary in fishes. During the present study the cholesterol, glycogen and protein levels along with HSI and GSI were found to be high both in the ovary and liver of control groups as the spawning months came nearer June (Table-1 and 2). However, in Cd-treated specimens all the parameters exhibited significant decrease in both ovary and liver except liver glycogen which remained unaltered, suggesting the possibility that cadmium toxicity either blocks hormone action or the complex enzyme systems responsible for vitellogenesis and steroidogenesis. An elevated level of serum glucose and depletion of liver glycogen among Cd-treated *Clarias batrachus* and *Tilapia mossambica* has been reported (Banerjee *et al.*, 1978). The retention of glycogen level even in the Cd-treated specimens as has been observed during the present study may, however, be attributed to the stress condition under which liver has a tendency to retain glycogen (Love, 1970).

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