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Research Article

Cadmium-induced alterations in bio-chemical constituents of *Labeo rohita* and *Cyprinus carpio* fry: A comparative study

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Abstract: Cadmium is a well-known heavy metal toxicant which can interfere with metabolism of carbohydrates, proteins and lipids by inhibiting the enzymes involved in the processes. In the present investigation, the effect of cadmium was evaluated on these biochemical constituents in two fresh water fish fry *Labeo rohita* and *Cyprinus carpio*. Fish fry of *Labeo rohita* and *Cyprinus carpio* were exposed to their respective sub-lethal concentrations ($1/5^{th}$ of LC_{50}), 0.1998 ppm and 4.938 ppm of cadmium for a period of 20days. Total carbohydrates, proteins and lipids were estimated by standard biochemical procedures in control and exposed fish fry. Results showed significant fall in all the biochemical constituents and energy levels of exposed fish fry, indicating that the organism is under toxicant stress.

Key words: Cadmium; Biochemical constituents; *Labeo rohita* and *Cyprinus carpio*.

Introduction

Rapid industrialization and urbanization results in substantial increase of liquid wastes which are being released into open land or nearby natural water bodies causing a threat to plant and animal life (Beaumont *et al.*; 2000; Rani *et al.*; 2001; Desai *et al.*; 2002; Joshi *et al.*; 2002; Saxena; 2002). Heavy metals as pollutants of aquatic environment constitute a major hazard because of their toxicity; non-biodegradability and persistence in environment and affect the organisms by interacting with biochemical molecules and enzymes forming metal ion complexes. These metal complex formations disturb the physiological and biochemical mechanisms (Paterson and Usher; 1971; Fowler and Gould; 1988). Cadmium is one of such heavy metals; which is more toxic and widely distributed in the earth's crust and aquatic environment. Due to extensive use in paints; dyes; cement and fertilizers; its concentration is increasing in the aquatic system (Bryan and Langston; 1992; Jarup; 2003). The problems of environmental pollution and its deleterious effects were studied on fishes by earlier workers from various aquatic systems (Sobha *et al.*; 2007; Bais and Lokhande; 2012; Kawade and Khillare; 2012; Parthiban and Muniyan; 2011). Therefore; fishes have received extreme focus during the last few decades for trace metal pollution and risk potential of human consumption (Jagadeesan *et al.*; 2001; Rashed; 2001).

East and West Godavari districts of Andhra Pradesh; India are rich in aqua culture and are potential grounds for fish breeding and rearing ponds. These rearing ponds are richly supplied with Godavari canal water and Kolleru Lake water. Studies on these water sources revealed the presence of trace and heavy metals in both in water and sediment much above the EPA threshold levels which are of biological concern. Hence; the present experiment has relevance as the select freshwater fish fry *Labeo rohita* and *Cyprinus carpio* have been used for aqua culture in Kadiyam and Dwarapudi rearing ponds where they generally use water from Godavari canal and Kolleru Lake (Adhikari *et al.,* 2009; Rao and Rao; 2001).

Several investigations have been undertaken to study the effect of metals on biochemical constituents of fishes and reported significant changes in different biochemical constituents in cadmium-exposed fish (Larson, 1975). Carbohydrate; protein and lipid contents of fish *Oreochromis mossambicus* showed a decrease on exposure to sub lethal concentrations of cadmium (Hameed and Muthu; 2006). Various studies on *Cyprinus carpio* have concluded that sub lethal concentrations of lead; zinc and cadmium can alter the biochemical profiles (Schmitt *et al.*; 2005; Maruthappan; *et al.*; 2005).

The present investigation was carried out to determine the changes in the major bio-chemical constituents i.e. total carbohydrates; proteins and lipids in fish fry of *Labeo rohita* and *Cyprinus carpio*; in relation to sub-lethal concentration of cadmium for a period of 20 days. The carbohydrate/protein; carbohydrate/lipid ratios and energy levels were

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also calculated from bio-chemical constituents of the fish fry exposed to sub-lethal concentrations of cadmium.

Materials and Methods

Expermental design:

Labeo rohita and *Cyprinus carpio* fry (length: 0.8 to 1cm) were collected from fish breeding ponds of Kadiyam and Dwarapudi; East Godavari District; Andhra Pradesh; India. In the breeding ponds; they used the water which was treated and processed devoid of metal contaminants. These fry were acclimated to laboratory conditions (pH=7; temp. 29±1°C) for 48 hrs. After acclimatization for two days; healthy fry of *Labeo rohita* and *Cyprinus carpio* were isolated and divided into two groups; with the first group serving as control (without metal toxicant) and the other group as experimental or exposed group. They were fed regularly with the rice bran and groundnut oil cake powder mixture in 1:1 ratio. The water was changed every day in both control and exposed groups without causing disturbance to the fry. Analytical graded cadmium chloride was used as the metal toxicant in these experiments and it was dissolved in distilled water. Appropriate amount of this solution was added to the exposed to reach the desired sub lethal concentration of cadmium namely 0.1998ppm for the fry of *Labeo rohita* and 4.938ppm for the fry of *Cyprinus carpio*. These sub lethal concentrations were calculated from the earlier tolerance experiments conducted on these fry (Surya Kumari; 2008). These fries were exposed to their respective sub lethal concentrations for a period of 20days. Similarly, the control was maintained without metal toxicant. Samples were collected from both the control and exposed groups at intervals of 24hrs; 48hrs; 96hrs; 10days and 20days. At each interval; the fry was sacrificed and dried in an oven at 60°C for 48hrs. The dried material was homogenized into a fine powder with mortar and pestle. The powder was stored in glass vials and preserved in desiccators prior to analysis.

Estimation of Biochemical Constituents:

Total Carbohydrates:

Total carbohydrates were estimated by adopting the anthrone method (Carroll *et al.*; 1956). The dry tissue powder of the sample was homogenized in 10% tri-chloro acetic acid (TCA) and centrifuged at 3000 rpm for 15minutes. 5ml of anthrone reagent was added to the known amount of TCA supernatant and vortexed. Then they were kept in boiling water bath for 15minutes. The tubes were cooled to room temperature and the absorbance was measured at 620nm in spectrophotometer (Chemito-2000) using a blank. The amount of total carbohydrates was calculated against glucose as standard and they are presented as µg/mg dry weight of the tissue.

Total Proteins:

The total proteins were determined by following the method of Lowry *et al.,* (1951) by using Folin-phenol reagent. The pellet obtained after homogenization and centrifugation as described above; was homogenized with IN Na OH. A known amount of this solution was taken and Lowry regent was added followed by Folin Phenol reagent (1:1 Folin Phenol; distilled water). The solution was vortexed thoroughly and incubated for 30 minutes at room temperature. The colour obtained was measured at 600nm in a spectrophotometer (Chemito-2000). The values were calculated against Bovine Serum Albumin (BSA) as standard and they were presented as µg/mg only weight.

Total Lipids:

Total lipids were estimated by using sulpho phospho vanillin method (Barnes and Blackstock; 1973). The dry tissue powder of the fish fry sample was homogenized with chloroform: methanol (2:1) and centrifuged at 2500rpm for 15minutes. A known amount of the supernatant was taken in a test tube and the solvent was removed under vacuum. To the residue; 0.5ml of concentrated sulphuric acid was added and was kept in boiling water bath for 15minutes. After cooling to the room temperature; 2.5ml of the phosphoric acid-vanillin reagent was added; thoroughly vortexed and the tubes were closed with cotton wool. Then they were incubated for 30minutes at room temperature. The optical density was measured at 520nm in a Spectrophotometer (Chemito-2000). The values were calculated against cholesterol standard and they were presented as µg/mg dry wt. of tissue powder.

Statistics:

The experiments were repeated for five times and each assay was done in triplicate. In each set of experiments; the exposure values are compared with their respective controls at each interval by using students "t" test (Snedecor and Cochran; 1967).

Results

Total Carbohydrates:

The total carbohydrate levels from control and exposed fish fry of *Labeo rohita* and *Cyprinus carpio* were presented in Fig. 1. A significant reduction in total carbohydrates was observed in exposed fish fry of both the species compared with that of control. The maximum reduction (47.4%) in total carbohydrates was recorded in *Labeo rohita* fry at 20days. While in *Cyprinus carpio*; similar trend was observed in total carbohydrate levels; however, the percent reduction was 33.36% at 96hrs which was less when compared with that of *Labio rohita* (42%) (Fig.1). 20days exposure showed 25.03% reduction in total carbohydrates in fry of *Cyprinus carpio*. This

indicates that compensatory mechanisms are in progress to mitigate the metal induced stress in the fry of *Cyprinus carpio*.

Table 1: Comparison of ratio of total carbohydrates/total proteins, total carbohydrate/total lipids and total energy levels in fry of *Labeo rohita* and *Cyprinus carpio* on exposure to sub-lethal concentrations of cadmium (0.1988 and 4.938ppm respectively). Each value represents the mean ± standard deviation. The values in the parenthesis represent percent decrease over their respective controls. *Significantly different from their respective controls over at P < 0.05.

Period of Exposure	Total carbohydrates/Total proteins		Total carbohydrates/Total lipids		Total energy levels (cal/mg)	
	Control	Exposed	Control	Exposed	Control	Exposed
			Labeo rohita			
24 hrs	0.0351	0.0309	0.2401	0.2706	4467.55 ± 248.88	3889.9 ± 236.41* (-12.93)
48 hrs	0.0368	0.0305	0.2185	0.2282	4663.67 ± 295.4	3721.275 ± 142.57* (-20.2)
96hrs	0.0378	0.0235	0.1877	0.1633	500.42 ± 239.39	3754.7 ± 391.98* (-24.9)
10 days	0.0418	0.0236	0.188	0.1488	4969.68± 385.59	4296.475 ± 135.75* (-14.09)
20 days	0.0418	0.0253	0.1925	0.1375	5298.1 ± 224.84	4375.68 ± 161.74* (-17.41)
			Cyprinus carpio			
24 hrs	0.06733	0.05913	0.4026	0.3505	3524.72 ±159.94	2858.22 ±165.89* (-18.91)
48 hrs	0.05829	0.06398	0.34698	0.4218	4268.53±85.73	3133.82±79.40* (-26.58)
96hrs	0.06628	0.05208	0.2921	0.2524	4713.0±190.04	3282.12±75.23* (-30.36)
10 days	0.1214	0.1217	0.4232	0.4393	5316.57±101.93	4005.18±175.94* (-24.67)
20 days	0.1297	0.1418	0.3451	0.329	6550.5±224.88	4919.55±254.11* (-24.89)

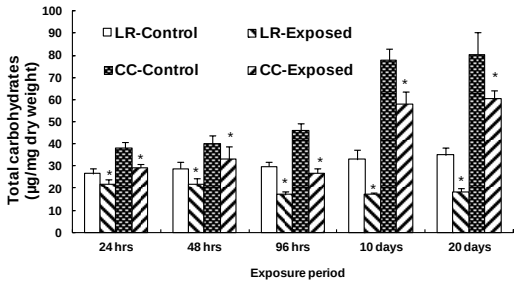

Figure 1: Comparison of total carbohydrates *Labeo rohita* and *Cyprinus carpio* fry exposed to sub-lethal cadmium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P<0.05.

Total Proteins:

Cadmium exposure significantly decreased the level of proteins in fish fry of *Labeo rohita* and *Cyprinus carpio*. The maximum reduction in protein levels was observed at 48hrs (7.84%) exposure in fry of *Labeo rohita* where as in fry of *Cyprinus carpio;* the highest decrease in protein was recorded at 20hrs (30.41%) (Fig.2).

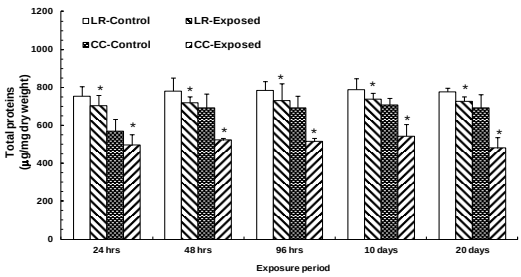

Figure 2: Comparison of total proteins *Labeo rohita* and *Cyprinus carpio* fry exposed to sub-lethal cadmium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P<0.05.

Total Lipids:

Cadmium-induced stress also reduced the level of total lipids in *Labeo rohita* and *Cyprinus carpio* fry. A maximum reduction of 33.37% was observed at 96hrs in fry of *Labeo rohita*. However; the maximum percent reduction in lipids in *Cyprinus carpio* was 32.47% at 96hrs (Fig.3).

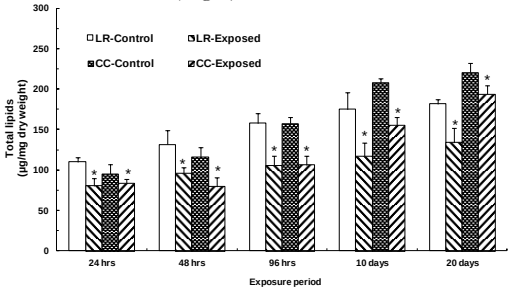

Figure 3: Comparison of total lipids *Labeo rohita* and *Cyprinus carpio* fry exposed to sub-lethal cadmium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P<0.05

Carbohydrates/Proteins; Carbohydrate/Lipid ratios and Energy levels:

The ratio of carbohydrates/proteins showed a gradual increase in control fry from 24 hrs to 20 days and an opposite trend was observed in exposed fry (Table 1). The ratio of carbohydrates / lipids decreased with increasing exposure time and was found to be more for exposed fry than their respective controls. Initially the trend was similar in both the fish fry but changed after 96 hrs exposure.

The energy levels of *L. rohita* and *C. carpio* fry showed an increase with increasing time on

exposure to sub-lethal concentration of cadmium but this increase was less in exposed fry compared to control. However; there was a significant decrease ($p<0.05$) in exposed fish fry over their respective controls at all intervals. A minimum decrease of 12.93% was observed at 24 hours and a maximum decrease of 24.9% at 96 hrs exposed in *L. rohita* (Table 1). In *Cyprinus carpio* fry; the minimum and maximum decrease levels were 18.91% and 30.36% respectively at 24hrs and 96hrs in comparison to their respective controls (Table 1).

Discussion

Heavy metals are widely distributed in free water sources and are harmful to aquatic fauna. Biochemical parameters are the best indicators of stress caused by heavy metals. In the present study; the results indicate that there was a depletion of all the three biochemical constituents on exposure to sub-lethal concentration of cadmium for 20 days in fry of *Labeo rohita* and *Cyprinus carpio*. Larson (1975) reported a similar change in different biochemical constituents of a fish exposed to cadmium.

In both the fish fry there was a decrease in carbohydrates level. Metal ions can block the active absorption of glucose by the intestinal epithelial cells. Many scientists proved in toxicity tests that the cadmium is more toxic to non-air breathing fishes. Cadmium has affinity for ligands like phosphate; cystenyle and histydyl side chains of proteins and can bind with carrier protein molecules resulting in inhibition of sugar and amino acid transport (Alva rado; 1966).

Cyprinus carpio and *Labeo rohita* fry showed a decrease in total lipids value. This can be attributed to liver function. Cadmium damages the liver; which in turn results in decrease of esterified cholesterol and hyper cholesterolenia (Kurde; 1990). Reduced lipoprotein lipase activity plays a role in the increment of plasma lipid (Asha Agarwal and Poonam Sharma 1999).

Proteins play a vital role in physiology of living organisms. All biological activities are regulated by enzymes and hormones; which are also proteins. Assessment of protein content can be considered as a diagnostic tool to determine the physiological phases of the cell (Kapila Manoj and G. Raghothaman; 1999). Cadmium competes with zinc for the same sulpha hydral group and binds more firmly. Thus; proteins are too sensitive and early indicators of heavy metal poisoning.

In the present investigation; maximum decrease was observed in the total carbohydrates followed by total lipids and total proteins in *Labeo rohita* and *Cyprinus carpio* fry exposed to sub-lethal concentration of cadmium for 20 days. The results are in corroboration with finding on other fishes where toxicants induced the stress causing alterations in bio-chemical constituents. It is clear from the energy levels that the decrease (30.36%) was marginally high in the fry of *Cyprinus carpio* than *Labeo rohita* indicating the relative effect of cadmium. The decrease in biochemical constituents observed in the investigation might be due to interaction of cadmium with bio-molecules causing inhibition or activation of different enzyme systems in metabolic pathways (Valle and Ulmer; 1972). The decrease in the energy levels might also be due to the utilization of energy by the fish fry to overcome the stress caused in the body.

The differential responses observed at 96 hrs indicate the possible operation of compensatory mechanism to mitigate the stress caused by cadmium toxicity. At 10 and 20 days of exposure; the fish fry might have developed a mechanism either to mitigate the effect of the toxicant or due to compensatory mechanisms in operation and hence; a less effect was observed.

Conclusion

From the above results, it can be justified that the fry of *Labeo rohita* is more sensitive to heavy metal cadmium than the fry of *Cyprinus carpio*. From the above observation; it has been concluded that heavy metals like cadmium cause deleterious effects on fishes and can reduce the biochemical constituents. It may not be fatal for an individual organism at sub lethal exposure but it does affect the total energy levels and growth rate resulting in disturbance of the metabolism.

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