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HEAVY METAL, MERCURIC CHLORIDE($Hgcl_2$) INDUCED BIOCHEMICAL CHANGES IN THE INDIAN MAJOR CARP *LABEO ROHITA* (HAMILTON)

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ABSTRACT
The sublethal and lethal toxic potential of heavy metals mercuric chloride ($Hgcl_2$) were shows its effect on some biochemical parameters (Glycogen, Proteins, Carbohydrates and Lipids,) in an Indian major carp *Labeo rohita* was investigated under laboratory conditions. The Acute toxicity value (96 h LC50) of mercuric chloride was found to be 0.25 mgl-1 calculated by Finney's probit method. In present investigation the fish *lebeo rohita* was exposed to different concentrations of 1/10th 96 h LC50 (0.025 mgl-1). Fishes were divided in five groups(G1-G5) to experiment was carried for 1day and 10days recovery period. A significant ($p < 0.05$) decline for selected biochemical parameters was observed in mercuric chloride to exposed fish during lethal and sublethal concentrations. The reduction in biochemical component of different organs of fish was expressed in terms of percent change over the control. The maximum % change in total glycogen was observed in liver (66.75) during 1 Day lethal exposure and the maximum % change in total proteins was observed in intestine (68.12) during 10 Day sublethal exposure. The maximum % change in total glycogen, total proteins and total lipids was observed in liver i.e., 64.10,56.92 and 63.63 respectively during 10 Day sublethal exposure. Whereas the minimum % change in total glycogen, total proteins, and total lipids was observed in intestine i.e., 25.55,34.50 and 35.63 respectively during 1 Day sublethal exposure. The minimum % change in total lipids was observed in gill (23.89) during 1 Day sublethal exposure. Thus, the minimum % change in biochemical constituents was observed during 1 Day sublethal exposure.

KEYWORDS: Biochemical, *Labeo rohita.* Total Glycogen, Total Proteins, Toxicity.

INTRODUCTION

Heavy metals as lead and zeolite effect biochemical parameters and also accumulate in muscle (Yacoub et al., 2012). Water pollution by industrial effluents containing organics and heavy metals pose a serious hazard to the aquatic biota and public health (Velma *et al*., 2009). An incredible discharge of untreated industrial and domestic waste waters, over the past few decades, has certainly resulted in an increased flux of metallic ions and their compounds in the rivers of the Andhra Pradesh and Tamil Nadu province (Javed *et al*., 2009). Heavy metals have devastating effects on ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al*., 2007). During the last decade, energy demand across the globe has led to the massive expansion of thermal based power plants which have been adding increasing loads of carbon dioxide and mercury as coal combustion by products to the environment, evoking major environmental and health concerns (Chen Lie *et al*., 2007; Milena Horvat *et al*., 2003; Caballeria and Fernandez, 2002; Kotnik *et al*., 2000). After entering fresh water bodies and the oceans, or settled into sediments and soils, mercury undergoes microbial transformation into the highly toxic form of methyl mercury, and it eventually bioaccumulates in the fish tissues after bioaccumulation, methyl mercury is introduced into the human population through the dietary intake of fish and seafood products (Parvinder Kaur *et al*., 2011).

Generally, heavy metals exert their toxic effects in organisms by generating reactive oxygen species, causing oxidative stress therefore, most of the heavy metals are toxic or carcinogenic in nature, posing threats to the human health and the environment (Farombi, Ajimoko *et al*., 2007). Fish are ideal sentinels for assessing the perturbations in behavior, oxygen uptake and biochemical profiles under toxic chemical exposure (Dube *et al*., 2010). The biochemical studies are good parameters and serve as bioindicator of water quality, which help to observe the adverse effect of toxicants on metabolic activities of the fish. (Kajare *et al*., 2000). Heavy metals accumulate in the tissues of the fish and cause cumulative deleterious effects at various functional levels ultimately leading to their death (Vutukuru *et al*., 2009). *Labeo rohita* is a commercial fish and widely preferred as edible fish in India and it is very important to evaluate edible

organisms. The objective of the present work was to observe the estimate and to the know percent change of biochemical parameters like glycogen, proteins, lipids, carbohydrates and free amino acids in different tissues i.e., gill, intestine, muscle, kidney, liver and brain.

MATERIALS AND METHODS

Acclimatization of fish and LC50 value

The fresh water fish, *Labeo rohita* (Rohu) (8-10 cm length and 28 ± 0.6 g weights) was used five batches of fish (15fishs in each batch) for the toxicity tests. These fishes were collected from local fish ponds at Nuzividu village in Guntur district in Andhra Pradesh, India (latitude: 16.1808576.N, longitude: 80.6646.E). The fishes were acclimatized to the laboratory conditions for 15 days. The tub containing fish was aerated with rich oxygen. The hygienic conditions were maintained by renewing water after every 24hrs and, fish were daily fed with rice bran and fish pellets. The dechlorinated tap water was used throughout the course of the experiments and the physico-chemical parameters of water determined according to American Public Health Association (APHA).

The values are as follows: temperature, 28 ± 2°C; pH, 7.12; total hardness, 170 mgl-1 (as CaCO3); total suspended solid (TSS), 4 mgl-1; turbidity, 7.5 Silica units and dissolved oxygen concentration, 5-6 mgl-1. The LC50 value was estimated in the laboratory conditions as per the method of Finney's probit analysis (1971) starting with minimum range for acute toxicity trials. The acute toxicity for 96 h LC50 was found to be 0.25 mg/l and 1/10th of 96 h LC50 was 0.025mgl-1. The concentration of 1/10th of 96 h LC50 was taken as sub lethal for experimentation.

Experimental Groups

The total acclimatized fish of seventy-five *L. rohita* fingerlings were selected for the present study and were divided into five experimental groups each containing 15 fingerlings. The group-1 was exposed to lethal concentration of 0.25 mgl-1 of mercuric chloride for one day,

Group-2and 3 were exposed to lethal and sub lethal concentration of 0.025 mgl-1 of metal exposer for one day and 10 days.

Group-4th and 5th were maintained as control groups by adding the same volume of acetone using free tap water for one day and ten days respectively.

The exposed and control groups were fed with palliated feed prepared in the laboratory (rice bran and peanut waste in an equal ratio) twice a day, and the water in the large circular tubs was changed every day (for 24 hours) to maintain a constant concentration of Phenthoate during the period of exposure. Continuous aeration was provided to each tank. No mortality was observed in all the groups during the entire experimental period except in one day lethal exposure. The total fish was taken from each group and were scarified on day 1 and day 10. The gill, liver, kidney, muscle and intestine tissues were separated and frozen in ethanol until used (not more than 1 h). Then toxicant impact on biochemical parameters was estimated by following the standard methodologies.

Estimation of Total Glycogen

The glycogen was estimated by the standard method of Kemp (1954) 5% homogenate of gill, muscle and intestine and 2% homogenate of liver and kidney tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloroacetic acid (TCA), boiled for 15 minutes at 1000C, and then cooled in running water. The solution was made up to 5 ml with TCA to compensate the evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H2SO4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose by using the aforesaid method. The glucose was converted to glycogen by the multiplication factor of 0.98 and is expressed as mg of glycogen/g wet weight of the tissue.

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Estimation of Total Protein

The total Protein content of the pesticide exposed tissue samples were estimated according to modified standard method of Lowry (1951) the Quantity of 5% homogenate of muscle, gill and 2% of kidney, liver and intestine tissue were isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 1 N NaOH solution and 0.2 ml of extract taken into test tube and mixed with 5 ml of alkaline copper solution (mixture of 2% sodium carbonate and 0.5% copper sulphate in 50:1 ratio) was added. Then samples were allowed to stand for 10 min, at the end of which 0.5 ml folin phenol reagent (diluted with double distilled water in 1:1 ratio before use) was added. After 30 minutes, the optical density was measured at 540 nm in a spectrophotometer (Elico Model SL207) against a blank. The standard graph was plotted using bovine serum albumin (BSA) as standard. The values were expressed as mg/g wet weight of the tissue.

Estimation of Total Lipids

Lipids were estimated according to the method of Barnes and Blackstock.(1973) 50 mg of tissue was homogenized with 10 ml water in a warring blender in chloroform: methanol mixture (2:1). The homogenates were filtered through Whatman no. 1 filter paper and the residue was re-homogenized as before and then filtered. The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCl (added as one fourth of the volume). 1 ml of filtrate was taken in a test tube and evaporated under nitrogen and 1 ml of concentrated H2SO4 was added and boiled for 10 min. For estimation of total lipid, 0.2 ml of solution was taken and 2 ml of vanillin reagent was added. The developed color was read in spectrophotometer at 520 nm against reagent blank. The standard graph was plotted by the above method with cholesterol powder. The values were expressed as mg/g wet weight of the tissue.

Estimation of Total Carbohydrates

Carbohydrates were estimated by Trevelyan and Harrison method (1951) the freshly prepared anthrone reagent (5 ml) was pippeted into thick walled pyrex tubes (150 x 25 mm) and chilled in ice water. The solution under test (1 ml) was layered on the acid, cooled for a further 5 min, and then thoroughly mixed while still immersed in ice water. The tubes were loosely fitted with corks, heated as required in vigorously boiling, constant level water bath and then cooled in water for 5min. Then it was made up to 10 ml with water and optical density was determined in a spectrophotometer. The standard graph was plotted with D-glucose by using the above said method. The values were expressed as mg/g wet weight of the tissue.

Statistical Analysis

The results are expressed as mean (X) ± standard deviation. The n values were same for control and test groups. The data was analyzed using 'Graph pad instat (Data set 1, SD) software student t-test was conducted for pairwise comparisons to determine the significant difference at 95% level of confidence.

For all the tests, values of results with $p<0.05$ were considered to be of statistical significance. The graphs were drawn using MS Excel 2007. The percent change in the biochemical constituent of the exposed fish over the control was calculated as follows:

$$\%\text{Change} = \frac{\text{Exposure value-Control value}}{\text{Control value}} \text{X100}$$

RESULTS

The estimated mean value of biochemical parameter of freshwater fish, *L. rohita* exposed to lethal (96 h of LC50, 0.25 mgl-1) and sublethal concentration (1/10th 96 h of LC50, 0.025mgl-1) of metal of mercuric chloride, Phenthoate for a period of one day and ten days; along with standard deviation (SD) and percent change over the control are represented in figure 1and 2. All the biochemical constituents in metal exposed fish decreased significantly at $p < 0.05$ when compared with the control fish without metal exposure. But the total carbohydrate content in intestine of 1 Day lethal exposed fish and 10 Day sublethal exposed fish did not show significant reduction ($p > 0.05$). The maximum % change

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in total glycogen was observed in liver (66.75) during 1 Day lethal exposure and the maximum % change in total proteins was observed in intestine (68.12) during 10 Day sublethal exposure. The maximum % change in total glycogen, total proteins and total lipids was observed in liver i.e., 64.10,56.92 and 63.63 respectively during 10 Day sublethal exposure.

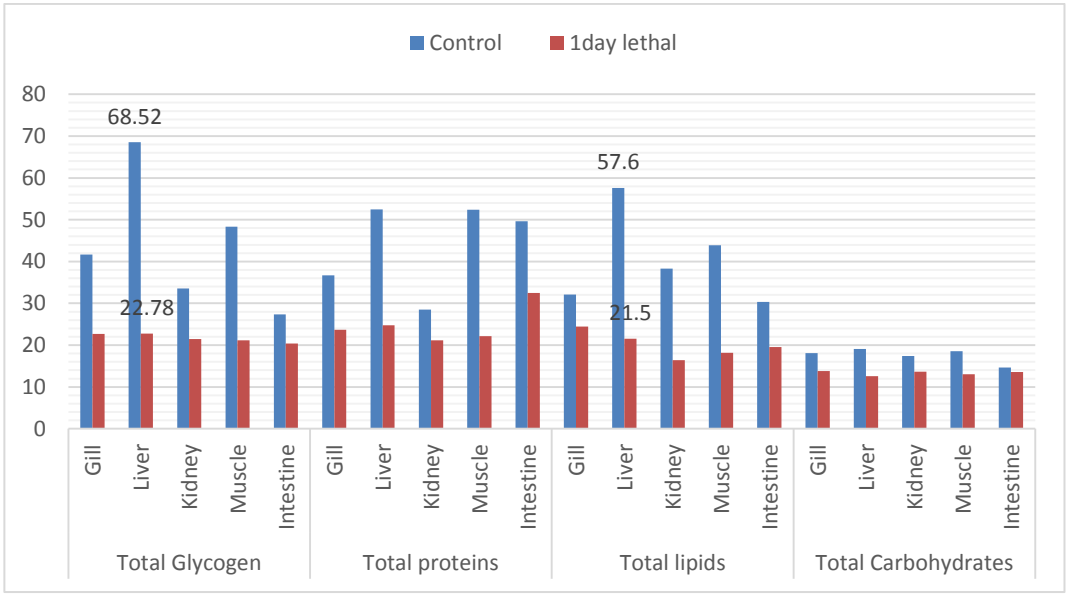

Figure 1: Changes in Biochemical constituents in the different organs of fish exposed to the toxicant for 1Day lethal concentration of Mercuric chloride($Hgcl_2$).

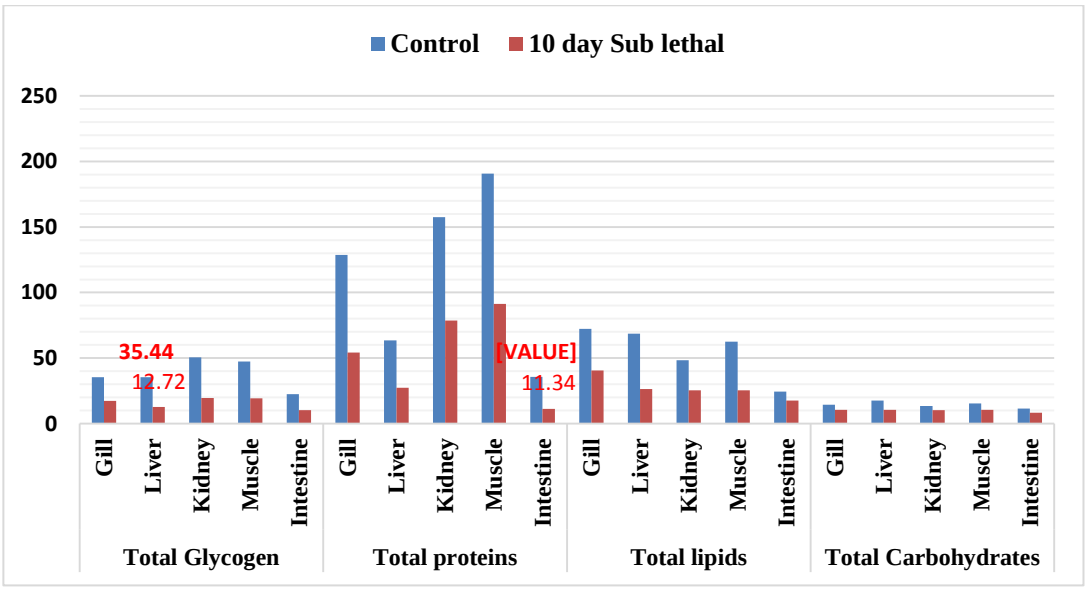

Figure 2: Changes in Biochemical constituents in the different organs of fish exposed to the toxicant for 10 Day sublethal concentration of Mercuric chloride($Hgcl_2$).

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Whereas the minimum % change in total glycogen, total proteins, and total lipids was observed in intestine i.e., 25.55,34.50 and 35.63 respectively during 1 Day sublethal exposure. The minimum % change in total lipids was observed in gill (23.89) during 1 Day sublethal exposure. Thus, the minimum % change in biochemical constituents was observed during 1 Day sublethal exposure.

In the present study, the fingerlings of *L. rohita* exposed to sub lethal and lethal concentrations of mercuric chloride toxicity which generated various biochemical alterations in total glycogen, total proteins, total lipids, and carbohydrates of gill, liver, intestine, kidney and muscle tissues. A major fall in glycogen content was observed in liver and muscle; moderate decline was noticed in intestine and gill whereas slight change found in kidney and intestine during 1 Day lethal and sublethal exposures. After 10 Day chronic sub lethal exposure of mercuric chloride, significant decline in the glycogen content in all the vital tissues at a metal concentration of 0.025 mgl-1 was noticed. The aquatic organisms need large amount of energy for active functioning of vital organs, which can be supplied from reserve food material of glycogen through glycogenolysis process. These reductions might also be due to the prevalence of hypoxic or inhibition of the enzyme glycogen synthetase.

DISCUSSION

Biochemical composition of fish tissues is important to evaluate their specific physiological demands during various stages of life. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Harper *et al*., 1985). A decrease in the glycogen content of the fishes exposed to metallic stress has been reported by several authors (Hadi *et al*., 2009; Martin, Arivoli, 2008; Sobha *et al*., 2007; Emad *et al*., 2005). Recent report (Vutukuru *et al*., 2011) indicates that glycogen content decreases drastically in fish under hypoxic or anoxic conditions. The decrease in glycogen content may also be due to inhibition of the enzyme glycogen synthetase or hormones which mediate glycogen synthesis (Stamp and Lesker,1967; Edwards, 1973).

The present study was maximum % change in total glycogen was observed in liver (66.75) during 1 Day lethal exposure. The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation (Schmidt, 1975). In the present investigation, decrease was more apparent in sublethal concentrations than in lethal concentrations. The maximum % change in total proteins was observed in intestine (68.12) during 10 Day sublethal exposure. Fishes constitute very low amount of carbohydrates variable to environmental factors like seasons and physiological factors like feed intake (Nabi *et al*., 2013). The heavy metals induced changes in carbohydrates in different fishes were reported by various authors explaining that the decline in carbohydrate contents may be due to metabolic stress (Hadi *et al*., 2009). Cholesterol is important for maturation of gonads as it is a precursor for steroid hormone synthesis. It is associated with reproduction in fish (Diwan *et al*.,1989). In the present study, heavy metal $HgCl_2$ induces total cholesterol content in ovary of *Labeo rohita*. Cholesterol is significantly elevated in experimental fish as compared to controlled one (Fig1and 2). The maximum % change in total glycogen, total proteins and total lipids was observed in liver i.e., 64.10, 56.92 and 63.63 respectively during 10 Day sublethal exposure. Earlier studies also showed that appreciable decline in the protein content of vital organs like gills, liver and muscle of fishes exposed to mercury and other heavy metals (Martin *et al*., 2008; Sobha *et al*., 2007; Sneha latha Das *et al*., 2001). The decrease in the protein content observed in the present study may be attributed to its utilization in cell repair and tissue organization.

CONCLUSION

The present study indicates that heavy metal mercuric chloride toxicity caused alterations in all the biochemical parameters of fish *L. rohita*, treated at different sub lethal and lethal exposure periods in fish tissues; which shown low

levels of glycogen, protein and lipid contents when compared to untreated fish tissues, might be caused by intoxication of pesticidal stress in the intermediary metabolism of the fish. The declined biochemical values indicate the change in the rate of synthesis and degradation under the impact of accumulation of metal pollutant. Moreover, in the present investigation, mercuric chloride is showing more toxicity on biochemical parameters of fish *L. rohita*.

Hence, it is concluded that the utilization of these mercury increases then cause harmful effects to the aquatic organisms. Hence should be minimized and create the awareness to the industrialization like thermal based power plants and coal combustion by-products.

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