Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

HEAVY METAL, CADMIUM CHLORIDE INDUCED BIOCHEMICAL CHANGES IN THE INDIAN MAJOR CARP *CIRRHINUS MRIGALA* (HAMILTON)

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Received for publication: March 06, 2013; **Accepted:** April 16, 2013

Abstract: The fresh water fish *Cirrhinus mrigala* (Hamilton) was exposed to the heavy metal Cadmium chloride for 24, 48, 72, and 96 h, and the consequential LC_{50} values were calculated using Finney's probit analysis. The LC_{50} values obtained for 24, 48, 72, and 96 h were 317.5, 316.5, 316.0 and 315.5 respectively. Later the fish were exposed to 96 h acute lethal and sub-lethal concentrations and the biochemical changes of glycogen, proteins, and nucleic acids of DNA, and RNA in the vital organs viz, Gill, Brain, Liver, Muscle and Kidney of the test fish were estimated and compared with the control fish. The present study revealed that Cadmium chloride is highly toxic to the test fish and the extent of toxicity increased with the increase in the exposure period. During the test period it was also observed that the fish showed erratic movement, restless, and surfacing phenomena engulfing air. A thin film of mucus was formed on the surface of gills and body. In the results of the biochemical changes it was observed that the total glycogen content and proteins decreased in all the tissue of the organs, whereas in the nucleic acid content heterogynous changes were observed. The increase in DNA content of gill may be due to hypertrophic nature of chloride cells. The results obtained in all were discussed at length with the available literature.

Keywords: Cadmium chloride, *Cirrhinus mrigala,* $LC_{50,}$ biochemical changes.

INTRODUCTION

Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among all types of aquatic pollutants, heavy metals are of greatest concern. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna (Nriagu and Sprague, 1987; Nriagu, 1996; Mason, 1996; Kotsanis and Georgudaki, 1999; Zyadah and Abdel-Bakey, 2000; Lliopoulou-Georguadaki and Kotsanis,n 2001; Verma *et al.*, 2005; Samanta *et al.*, 2005; Sharma and Agarwal, 2005). Being intrinsic components of the earth crust, nature has not provided effective control mechanisms for these metals. The problem increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications (Burman and Lal, 1994; Sanders, 1997; Pitter, 1999; Lodhi *et al.*, 2006).

Emissions of heavy metals to the environment occur via a wide range of processes and pathways, including to the air (e.g. during combustion, extraction and processing), to surface waters. Cadmium occurs naturally in ores together with zinc, lead and copper. Cadmium compounds are used as stabilizers in PVC products, color pigment, several alloys and, now most commonly, in re-chargeable nickel–cadmium batteries. Metallic cadmium has mostly been used as an anticorrosion agent (cadmiation). Cadmium is also present as a pollutant in phosphate fertilizers. Cadmium is discharged in large quantities from battery and inverter manufacturers, dyeing, printing and electroplating units. It tends to accumulate in tissues of biotic, flora- fauna and has deleterious effect on fish (Barman and Lal, 1994). Fish, as they come into intimate contact with large amounts of polluted water, can be used as early warning biological indicators of polluted environment (Sherry and Abidi, 2002; Pasco *et al.*, 1986; Ashram *et al.*, 2003; Shastry *et al.*, 1997; Shukla and Shastry, 1998).

MATERIALS AND METHODS

The fish *Cirrhinus mrigala* (Hamilton) of size 5-7 ± ½ cm and weight 5-6 ± ½ gm weight were brought from fish hatcheries of Nandivelugu, Tenali Mandal, Guntur district, Andhra Pradesh, India which is 20 km away from the University, and acclimatized to the laboratory conditions for about a week in plastic pools of 200 liters capacity. Washed thoroughly prior to conduction of fish to prevent the fungal disease. Tap water was used for maintaining the fish in the fish tanks had a pH 7.2±0.1, dissolved oxygen 8.0±0.3 mg/L and bicarbonates 95.0±5.0mg/L at 28 ± 2°C. During acclimatization, fishes were fed with groundnut oil cake and rice bran, water was renewed on every day to maintain water quality. Pilot experiments were

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conducted to choose the mortality concentration at which the fish respond. For each test, five concentrations were chosen with 10 fish in each concentration. Static renewal tests were conducted to determine the LC_{50} values. The LC50 was calculated by Finney (1971) probit analysis method. The respective probit values for respective per cent mortalities were taken from Fisher and Yates (1938). In the present investigation, 96 h LC_{50} was taken as lethal and $1/10^{th}$ of this as sub-lethal concentration to study the effect of Cadmium chloride on biochemical constituents such as total protein, glycogen and nucleic acid in fish.

The biochemical constituent's viz., Glycogen, Total proteins, Nuecleic acids (DNA & RNA) changes in protein profiles were estimated by standard procedures in tissue of five organs viz., Muscle, Gill, Liver, Brain and Kidney of the healthy fish (Control) and those of from the fish exposed to sub-lethal and lethal concentrations of Cadmium chloride (Merck). One-tenth of the 96 h lethal concentration was taken as sub-lethal dose and the fish were exposed to sub-lethal dose for a period of 96h. The fish were sacrificed for the biochemical analysis. The glycogen content, proteins, and nucleic acids were estimated by methods of Kemp *et al*.(1954), Lowry et al. (1951), and Searchy and MacLnnis (1970).

RESULTS

The LC50 values along with regression values obtained in the present study were presented in table-I. The percent mortality obtained for *Cirrhinus mrigala* (Hamilton) for different exposure periods at different concentrations of cadmium chloride were also presented in tableII. The LC_{50} values were determined by Finney (1971) probit analysis for 24, 48, 72, and 96 h were 317.5, 316.5, 316.0 and 315.5 respectively. The regression values obtained were x=23.23 and y = 0.085x - 26.38 respectively and regression values were 0.9897, 0.9826, 0.9868 and 0.9897 respectively for 24, 48, 72 and 96h. In the present study it was observed that Cadmium chloride is highly toxic to the test fish *Cirrhinus mrigala.* It was also observed that there was an inverse relationship between exposure concentration and the LC50 value.

Table. I: Calculated LC50 values and regression values for the toxicity data of exposure concentration and percent mortalities.

Hours of Exposure	LC50 values in ppm	Regression Values (R^2)
24	317.5	0.9897
48	316.5	0.9826
72	316	0.9868
96	315.5	0.9897

Table. II: Exposure of *Cirrhinus mrigala* to Cadmium chloride to different time periods and the Per cent mortalities of the fish.

24 h		48 h		72 h		96 h	
Dose In ppm	Percent Mortality	Dose In ppm	Percent Mortality	Dose In ppm	Percent Mortality	Dose In ppm	Percent Mortality
315	20	314	30	314	30	313	20
317	40	316	40	316	50	315	40
319	60	318	60	318	60	317	60
321	70	320	70	320	80	319	70
323	90	322	80	322	90	321	90

The LC50 values and their confidence limits of Cadmium chloride for *Heteropneustes fossilis* were reported by Deepak Kasherwani *et al*., (2009) for 24, 48, 72 and 96h as 434.74, 409.88, 401.31 and 392.92 mg/l, respectively and reported an inverse relationship of exposure duration and concentration. Smet and Blust (2001) observed 100% mortality in *Cyprinus carpio* after 21-29 days of exposure to 20 mm of Cd. The lethal effects of heavy metals (Hg, Cu, Cd, Zn and Pb have been described to coagulation of mucus (i.e., precipitation of insoluble metal proteins compounds) on gill surface, damage done to gill tissues and consequently result to the respiratory failure Tilak, *et, al.* (2005).

Glycogen:

The results of the changes in total glycogen content under exposure to Cadmium chloride sub-lethal and lethal concentrations over the control for 96 h along with percent change and standard deviation in the selected tissue viz, Liver, Brain, Muscle, Gill and kidney of the test organism Cirrhinus mrigala were presented figure-5. The glycogen content in control fish was highest in liver followed by muscle, gill, brain and kidney. Under exposure to lethal and sub-lethal concentrations of Cadmium chloride, the percent change over the control was highest in gill in both lethal and sub-lethal concentrations, followed by kidney, muscle, liver and brain. The decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused by the test toxicant. Increase in serum glucose levels in fish under stress was reported by Chowdhury et al. (2004). Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehninger, 2004).

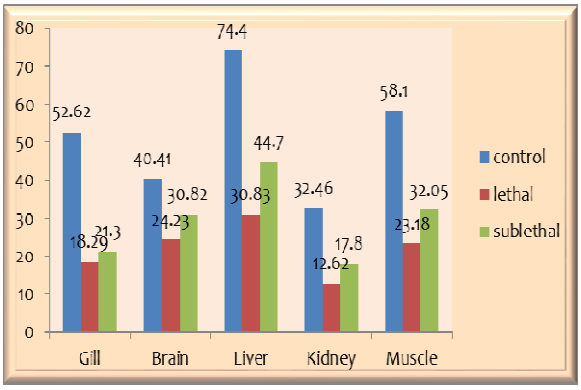

Fig.5: Changes in the glycogen content (mg/gram wet weight of the tissue) and % change over the control in different tissues of *Cirrhinus mrigala* exposed to lethal and sub-lethal concentration of Cadmium chloride for 96h.

A fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands in toxicant treated animals through glycolysis or hexose monophosphate pathway (Cappon and Nicholas, 1975). In the present study, it was observed that exposure to lethal and sub-lethal concentrations of Cadmium chloride in the fish *Cirrhinus mrigala* caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism.

Proteins:

The results of the changes in total protein content under exposure to Cadmium chloride sub-lethal and lethal concentrations over the control for 96 h along with percent change and standard deviation in the selected tissue viz, Liver, Brain, Muscle, Gill and kidney of the test organism *Cirrhinus mrigala* were presented in figure-6. The protein content in control fish was highest in liver followed by muscle, gill, brain and kidney. Under exposure to lethal and sub-lethal concentrations of Cadmium chloride, the percent change over the control was highest in gill in both lethal and sub-lethal concentrations, followed by kidney, muscle, liver and brain.

Gill tissues of *Cirrhinus mrigala* evidenced a decrease in the protein content under sub-lethal concentrations of Cadmium Chloride followed by kidney. Biochemical changes in the muscle and liver of the fish suggests that they are relatively less affected than other tissues under Cadmium Chloride toxicity. 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 keto-acids through gluconeogenesis pathway for the synthesis of glucose; or due to the directing of free amino acids for the synthesis of necessary proteins, or, for the maintenance of osmotic and ionic regulation (Robberecht *et al.*, 1982).

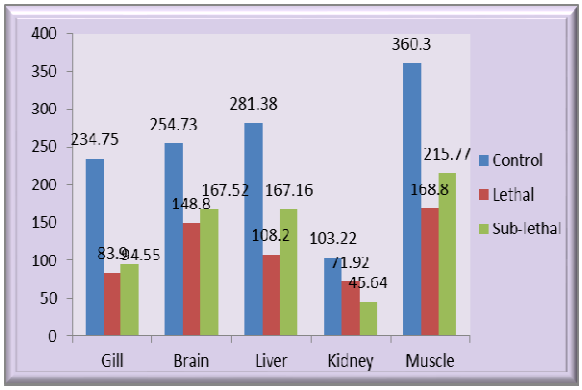

Fig.6: Changes in the Protein content (mg/gram wet weight of the tissue) and % change over the control in different tissue of *Cirrhinus mrigala* on exposure to lethal and sub-lethal concentration of Cadmium chloride for 96 h.

Nucleic Acids (DNA & RNA):

The calculated values of nucleic acids along with standard deviation and the per cent change over the control were given in Fig.7 and 8.

The DNA content in control fish *Cirrhinus mrigala* in different tissues was in the order of:

Gill > Brain > Kidney > Liver > Muscle

The RNA content in control fish *Cirrhinus mrigala* in different tissues was in the order of:

Liver > Muscle > Kidney > Gill> Brain

Under exposure to lethal and sub-lethal concentrations of Cadmium chloride, the amount of DNA was found to decrease in most of the tissue of *Cirrhinus mrigala* except in liver and gill. The leotropic gradation series in terms of decrement are:

Lethal: Brain > Kidney > Muscle
Sub-lethal: Muscle > Brain > Kidney

Under exposure to lethal and sub-lethal concentrations of Cadmium chloride the amount of RNA decreased in most of the tissue of *Cirrhinus mrigala*. The leotropic series in terms of decrement in RNA content is

Lethal: Brain > Gill > Muscle > Liver> Kidney
Sub-lethal: Muscle > Liver > Brain > Kidney > Gill

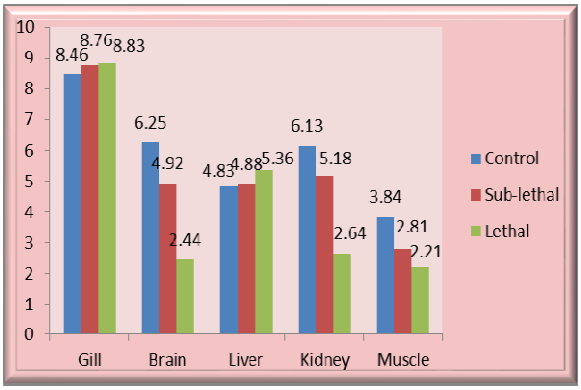

Fig.7: Changes in the amount of Deoxy ribonucleic acid (DNA) (mg/gram body wet weight of the tissue) and % change over the control on exposure to sub-lethal and lethal concentration of Cadmium chloride for 96 h.

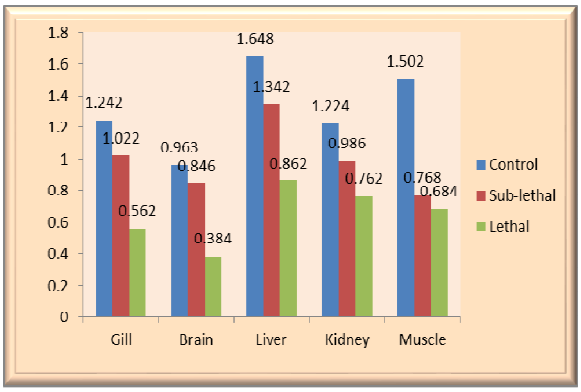

Fig.8: Changes in the amount of Ribonucleic acid (RNA) (mg/gram body wet weight of the tissue) and % change over the control on exposure to sub-lethal and lethal concentration of Cadmium chloride for 96 h.

The results in Fig-7 and 8 indicated heterogeneous levels of DNA and RNA in the tissue of brain, liver, muscle gill and kidney. The level of DNA in different tissue indicates cell number (Cui *et al.*, 1997) and is constant for a species. In other tissues, no significant change was observed in DNA levels. The level of RNA in liver is higher than brain, gill, muscle and kidney. The work of Durairaj and Selvarajan also supports the present study. Most nucleic acid binding compounds are able to bind to RNA as well as DNA, some with affinities greater for RNA (Wilson *et al.*, 1993).

DISCUSSION AND CONCLUSION

The contamination of heavy metals is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain. Since, Aquatic environment is the ultimate sink for all the pollutants, the living organisms in the aquatic environment, (non target organisms) are prone to be affected by these pollutants (Murty, 1986). In recent years, Aquaculture practices are increasing on an industrial scale.

The effects of sub-lethal doses of Cadmium chloride on the fresh water fish *Cirrhinus mrigala*, (Hamilton) were studied. The effects of one to five doses were examined. Acute static tests were employed to determine the per cent mortality and the Lc50 values were calculated using Finney's probit analysis. The sub-lethal dose chosen for Cadmium chloride was 1/10 LC_{50} value.

On exposure to sub-lethal and lethal concentrations of Cadmium chloride some behavioral changes such as the increase in opercular activity, surfacing phenomenon, loss of equilibrium, hyper excitation and mucus production were observed in *Cirrhinus mrigala*. Also, a thin film of mucus was observed all over the body including the gills.

The glycogen in response to exposure to sub-lethal concentration of Cadmium chloride was gradually decreased. The depletion was attributed to the utilization of reserve carbohydrates to meet the excessive demands of energy induced by the heavy metal stress. Glycogen drain has also been suggested to be partly due to suspension of gluconeogenesis known to be induced by heavy metal.

The depletion of Proteins was more in lethal than sub-lethal when compared to control. The depletion of proteins may be due to a proteolysis' effect of the heavy metals possibly to meet the excessive energy demands under the toxic stress. The total ninhydrin positive substances (free amino acids) showing an elevation following the administration of Cadmium chloride was considered to bear a testimony to proteolysis. The depletion TNPS in all the tissue concomitant with the depletion of proteins following the administration of Cadmium chloride suggest the utilization of free amino acids for the release of energy through TCA cycle. The reduction noticed in the protein content of the tissues of treated and together with the reduction in the attributed, at least partly, to the excessive proteolysis and utilization for energy purposes.

Nucleic acids and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating different activities of cells. Their ratios also provide significant information about the way in which, or the mechanism by which, these contents regulate the multifaceted activities of cells. In the present study DNA contents were found to be increased in gill and liver tissue in response to Cadmium chloride treatment. However, the degree of elevation was tissue specific. Enhancement in the DNA level might be due to activation of some dormant regulating factors or increase in activity of the essential factors controlling DNA synthesis. The slight increase of DNA in gills

following Cadmium chloride treatment might be attributed to the hypertrophic nature of chloride cells in response to the toxicant administration.

Under exposure to lethal and sub-lethal concentrations of Cadmium chloride the amount of RNA decreased in most of the tissues of the fish, *Cirrhinus mrigala*. The decreased RNA level reflects the intensity of protein synthesis and metabolic activity of the tissue of the fish under stress conditions.

The present work revealed that the variations in bio-chemical parameters serve as indices in monitoring the pathological status of the heavy metal treated fish. These variations were found to be tissue specific and hence, can be used as meaningful indicators of heavy metal pollution. The above said parameters can be examined more critically to develop more meaningful indicators or markers to assess or to characterize a particular pollutant and its potential toxicity.

Since majority of heavy metals are released cumulatively and regularly, through the industrial and human activities their residues are known to bio-accumulate in the tissues of fish and other animals, and transfer via food chain to the human bodies, the grave risk to the health of the people who consume these fish seems to be considerable. The need to protect the people from undue exposure to the heavy metals through the food chain cannot be over emphasized. The heavy metals will also show impact on reproductive impairment of the commercially important fish and the carnivores, especially the birds.

ACKNOWLEDGEMENTS

The authors thank the Head of the Department of Zoology, Acharya Nagarjuna University for providing necessary facilities. They also thank UGC for providing equipment to carry out this work under Special Assistance Programme.

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Source of support: Nil
Conflict of interest: None Declared