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EFFECT OF HEAVY METAL INDUCED ANTIOXIDANT MECHANISMS IN FRESH WATER FISH *CLARIAS BATRACHUS*

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ABSTRACT

In this work we analaysed protein content of tissue like gills and liver. Heavy metals are enter into aquatic bodies through runoff water metals (Hg and Cd) are affected the fishes present in fresh water like *Clarias batrachus.* Metals were anaysed by atomic absorption spectrophotometry with flame & flamers atomization. The LC/EC values were determine for 24, 48, 72 & 96 hours. Protein was estimated from gills & liver which reveals that protein content was not reduced significantly ($p<0.05$) when compare to control.

KEYWORDS: Protein, Metals (Hg & Cd), Gills and Liver.

INTRODUCTION

Fish constitutes an important aspect of human food due to the high level of quality protein, calcium, phosphorus and essential amino acids for the proper growth and functioning of body muscles and tissues. *Clarias batrachus* inhabit freshwater. It is suitable species for aquaculture because it grows fast and feeds on a large variety of agricultural by-products and can tolerate adverse water quality conditions. They contain up to 18.22% of protein and Vitamin A so it is highly valuable as nourishing food for convalescing patients. Asian cat fishes of commercial importance include *Clarias macrocephalus* and *Clarias lazera* (African cat fish or Nile fish) and *Pangasius sutchi* (Lee, J.S 1973; Kloke and Potaros, 1975; Chaturvedi, 2003; and Jain, 2003).

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru 2005; Dirilgen, 2001; Voegborlo *et al*., 1999; Canli *et al.*, 1998). The natural aquatic systems may extensively be contaminated with heavy

metals released from domestic, industrial and other man-made activities (Velez and Montoro, 1998; Conacher, *et al*., 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al*., 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005). Fishes are commonly situated at the top of the food chain and therefore, they can accumulate large amount of toxicants (Yilmaz *et al*., 2007).

Among animal species, fishes are the inhabitant that cannot escape from the detrimental effects of heavy metals aqutic ecosystem (Olaifa *et al*., 2004; Clarkson, 1998; Dickman and Leung, 1998). The study has been carried out on various fishes, it shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Basa and Rani, 2003; Canli, 1995; Tort and Torres, 1988).

The heavy metals reached health threatening proportions. In general, they are not biodegraded and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Das and Kaviraj 2000; Laxi, 2005; Jayakumar and Paul 2006; Kumar *et al*., 2007; Kumar *et al.*, 2008; Kumar *et al*., 2009). Uptake of heavy metals like zinc, copper and lead through food chain in human being may cause various physiological disorders like hypertension, sporadic fever, nausea, renal damage, cramps etc. (Bhattacharya AK, Mandal SN and Das SK, 2006).

Mercury (Hg), the black listed element by environmentalists is released into the environment by several sources, such as mining and fossil fuel combustion, thermal power projects, by the use of fungicides, bactericides and pharmaceuticals (Khangarot, 2003).). Most of the mercury compounds, natural or anthropogenic in origin have various capacities in crossing the barriers separating the internal part of the organisms from the outside world such as skin, epithelium of gills and walls of the digestive system. Mercury is predominantly accumulated in gills of fish and also deposited in liver, muscles and mucus to a small extent.

Cadmium is considered as one of the most toxic metal and highly toxic for all mammals and fish. Accumulation of cadmium in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly. By contrast, the excretion of cadmium from

living organisms is a slow process. In fish, cadmium can cause a number of structural and path morphological changes in various organs. The highest cadmium levels were detected in the kidneys and liver of fish (Thophon *et al*., 2003). fish often responds to toxicants in a similar way to higher vertebrates, they can be used to screen xenobiotic that are potentially toxic. Micronuclei (MN) are formed by chromosome fragments or whole chromosomes that lag at anaphase during nuclear division due to the lack of a centromeres or kinetochores or spindle in the case of chromosome loss (Haddle *et al*., 1991).

MATERIALS AND METHODS

Animal Collection

Healthy living specimens of ***C. batrachus*** were procured from commercial farm and transported in a plastic container to the Laboratory. The test organisms were of the size 25 ± 5 cm and weight was 190 ± 20 gm. The test organisms were kept in a large pre-washed glass aquarium and there after acclimatized for a period of 2 weeks. During this period of acclimatization, 24 hours renewal bioassay was employed and fish were fed daily with chopped chicken.

Rearing of animals in laboratory

Metal Analyses

Metals (Hg and Cd) in water, from the site of collection were analysed by atomic absorption spectrophotometer with flame (IL model S11 with deuterium background corrector) and flameless atomization (Varian Spectra 300 Zeeman).

TOXICANT AND TOXICITY TEST

Mercury Chloride

The heavy metal toxicants selected for exposure were Mercury Chloride and Cadmium Chloride (HiMedia). After the acclimatization period, *C. batrachus* was placed in eight different plastic containers containing well aerated bore-hole water. Renewal bioassay test was employed in the experimental set up. Fishes were divided into eight groups. The first group served as a control (0.00) and other seven as experimental groups were exposed to different concentration such as 0.1 mg/L, 0.5 mg/L, 0.6 mg/L, 0.7 mg/L, 0.8 mg/L, 0.9 mg/L, 1 mg/L and mortality was recorded. By observing mortality 0.540 mg/L HgCl2 (LC_5/96 hours.) is sublethal concentration for 96 hours. of exposure.

EPA PROBIT ANALYSIS PROGRAM USED FOR CALCULATING LC/EC VALUES

Table 1: Estimated LC/EC Values and Confidence Limit.

$HgCl_2$			
Estimated LC/EC Values and Confidence Limits			
Exposure 95% Confidence Limits			
Point	**Conc.**	**Lower**	**Upper**
LC/EC 1.00	0.488	0.227	0.579
LC/EC 5.00	0.540	0.305	00.621
LC/EC 10.00	0.571	0.357	0.646
LC/EC 15.00	0.592	0.396	0.664
LC/EC 50.00	0.691	0.585	0.785
LC/EC 85.00	0.807	0.725	1.107
LC/EC 90.00	0.837	0.748	1.224
LC/EC 95.00	0.884	0.780	1.427
LC/EC 99.00	0.979	0.839	1.917

Cadmium Chloride

For the treatment of $CdCl_2$, renewal bioassay test was employed in the experimental set up of second set of $CdCl_2$. Fish were divided into Eight groups, with the first group serving as a control (0.00) and the other Seven as experimental groups exposed to different seven concentration as 5 mg/L, 10 mg/L, 12 mg/L, 14 mg/L, 16 mg/L, 18 mg/L, 20 mg/L and mortality was recorded. By observing mortality 10.248 mg/L $CdCl_2$ (LC5/96 hours) sublethal concentration is estimated for 96 hours of exposure. The fishes were then exposed to concentrations of 0.540 mg/L $HgCl_2$ and 10.248 mg/L ($CdCl_2$) with parallel control without feeding was maintained.

EPA PROBIT ANALYSIS PROGRAM

USED FOR CALCULATING LC/EC VALUES

Table 2: Estimated LC/EC Values and Confidence Limits.

$CdCl_2$			
Estimated LC/EC Values and Confidence Limits			
Exposure 95% Confidence Limits			
Point	**Conc.**	**Lower**	**Upper**
LC/EC 1.00	8.933	3.357	11.132
LC/EC 5.00	10.248	4.978	12.196
LC/EC 10.00	11.026	6.124	12.843
LC/EC 15.00	11.585	7.028	13.327
LC/EC 50.00	14.276	11.816	16.584
LC/EC 85.00	17.592	15.443	26.548
LC/EC 90.00	18.484	16.074	30.374
LC/EC 95.00	19.888	16.970	37.268
LC/EC 99.00	22.815	18.636	55.141

Protein Estimation

Animal were dissected and required **(**liver and Gills**)** tissues were removed and used for protein estimation by Lowry method.

Statistical Analysis

Probit analysis was carried out as suggested by Finney (1971). Regression lines of probit logarithmic transformations of concentrations were made. Confidential limits (Upper and Lower) of the regression line with Chi-Square test were calculated by following the procedures of UNEP/FAO/LAE (1987). The data obtained were statistically analyzed by statistical software SPSS (version 7.5). The data were subjected to one way ANOVA.

RESULTS AND DISCUSSION

Behavioral Responses

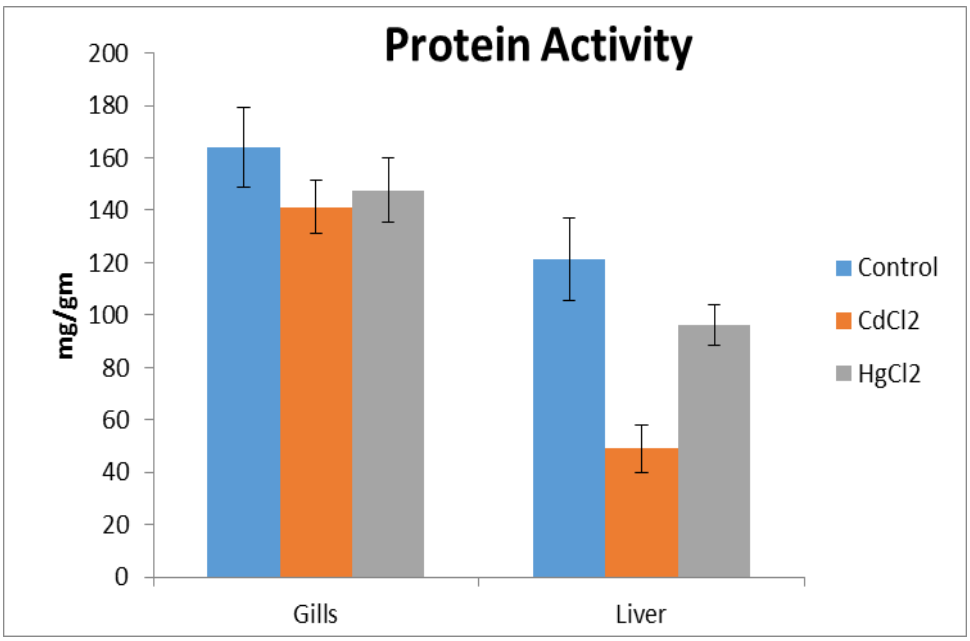
The behavior of fish remarkably changed due to the treatment of cadmium and mercury compared to the control. The various responses exhibited by fish due to sub lethal concentrations of cadmium and mercury during initial stage of exposure included, restlessness, erratic and fast swimming, abrupt change in position and direction, jumping and overall hyperactivity. The fish showed surfacing tendency throughout the experimental period. Physiological responses like rapid opercula movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional.

Protein Estimation

Protein was estimated from gill and liver(table 3) which reveals that protein content was not reduced significantly ($p<0.05$) when compared to control. ($p<0.05$ significant differences between the control and treated groups.)

Table 3: Alteration in the Protein content of *Clarias batrachus.*

Alteration in the Protein content of *Clarias batrachus* when exposed to LC_5 Concentration of Heavy metals.		
Tissues exposed	**Gills**	**Liver**
mg/g ± S.D.		
Control	163.9 ± 15.22	121.3 ± 15.60
$CdCl_2$	141.3 ± 10.23	49.01 ± 9.01
$HgCl_2$	147.6 ± 12.34	96.1 ± 7.89
Each value is mean ±SD of observations.		



Alteration in the Protein content of ***Clarias batrachus*** when exposed to LC_5 Concentration of Heavy metals.

Fall in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. Fall in protein level during exposure may be attributed to increased catabolism and decreased anabolism of protein due to toxic stress of heavy metals.

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