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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 3, Issue, 3, pp.230-236, March, 2011

ISSN: 0975-833X

RESEARCH ARTICLE

QUANTITATIVE VARIATION OF PROTEIN IN THE TISSUES OF A FRESH WATER FISH *Clarias batrachus* EXPOSED TO MERCURY AND CHROMIUM

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ARTICLE INFO

Article History:
Received 19th January, 2011
Received in revised form
11st February, 2011
Accepted 17th March, 2011
Published online 30th March, 2011

Key words:
Clarias batrachus,
Mercury,
Chromium,
Protein,
Bradford

ABSTRACT

The present study was carried out to determine the effects of sub-lethal concentration (96 h-LC_{50}) of mercury (0.026ppm, 0.040ppm and 0.080ppm) and chromium (3.4ppm, 5.1ppm and 10.2ppm) for 28 days in *Clarias batrachus* and the protein content in the brain, gills, liver, kidney and muscle were quantified Bradford method. The levels of protein content in the liver, kidney, brain, muscle and gill showed fluctuations of decreasing and increasing trend in fishes exposed to different concentrations of mercury and chromium in a time–dose dependent manner.The protein variation in the tissues of *C.batrachus* were significant when exposed in mercury and chromium. The variations observed in the total protein quantified in the *C.batrachus* as a general indicator of pollutant induced stress response is discussed.

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INTRODUCTION

The contamination of aquatic ecosystem with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with of the damage caused to the aquatic life. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents (Canli and Kalay, 1998).

Mercury (Hg) is a highly toxic, nonessential, persistent, immutable and non-biodegradable metal that undergoes many changes during transfer through different trophic levels of the food chain. Hg occurs naturally in the environment and exists in several forms such as metallic mercury, inorganic mercury and organic mercury. Chromium is one of the heavy metal pollutants produced from ferrochrome, tanning and pigment industries. Chromium in nature exists in toxic Cr+4 and Cr+3 forms. The Cr+6 ion is known to affect several plant processes. Cr+3 is readily oxidized to

Mn, which serves as the electron acceptor in the reaction. Heavy metals can be absorbed from the soil and atmosphere, accumulate in the organs of the plants and show their phytotoxic effects (Parmar and Chanda, 2005). *Clarias batrachus* (Linn.) is an air-breathing catfish, *C. batrachus* occurs in fresh and brackish waters throughout India. It is highly valued as a table fish throughout the Indian subcontinent and is preferred for culture even in muddy and shallow waters (Debnath and Surajit, 2009). Protein plays a vital role in the physiology of living organisms. All biological activities are regulated by enzymes and hormones, which are also proteins. Therefore the assessment of the protein content and amino-acids of the various internal organs can be considered as a diagnostic tool to determine the physiological phases of the cell (Kapila and Ragothaman, 1999). Dinodia *et al.* (2002) observed marked reduction in residual protein levels in *Labeo rohita, Cirrhinus mrigala* and *Cyprinus carpio* due to cadmium toxicity. Srivastava *et al.* (2002) recorded changes in protein biochemistry in the liver and muscle of *Channa punctatus* due to zinc toxicity. Protein content was significantly decreased in liver and muscle of *C. carpio* (Dhanapackiam *et al.,* 2000) and *C. batrachus* (Aruna *et al.,* 2000) under stress.

Studies by Neha *et al.* (2004) on teleost *Labeo rohita* exposed to chromium chloride showed that there was continuous time dependent depletion in the protein content and maximum protein was depleted after the exposure to longer duration. However, in nickel chloride and zinc chloride treated fish, there was a decrease in protein content initially but after the longer exposure period; increase in tissue protein was noted. It has now been well established that heavy metals even in traces interfere with various physiological and metabolic processes.

MATERIALS AND METHODS

Healthy living specimens of *Clarias batrachus* were procured, from Bharath Fish Seed India, Poondi, Thiruvallur district, Tamilnadu. *C. batrachus* measuring 9.5± 0.5cm in length and weighing 5 ± 0.5 gm were used in the experiment. conditions for 20 days prior to experiment in a glass aquarium (40 x 40 x 100 cm) filled with dechlorinated water. Water quality characteristics were determined. The mean values for the test water qualities were as follows, temperature 27.5±1.5◦C, pH 7.5±0.03, dissolved oxygen 6.4±0.2mg/l, alkalinity 250±2.8mg/l as $CaCo_3$, total hardness 456±3.5mg/l. Stock solutions of mercuric chloride and potassium dichromate were prepared by dissolving analytical grade mercuric chloride ($HgCl_2$ from Merck) and potassium dichromate ($K_2Cr_2O_7 \cdot 7H_2O$ from Merck) respectively in double distilled water. Acute toxicity test was conducted in accordance with standard methods (APHA, 1995). Sublethal or safe level concentrations were derived from 96h LC_{50} as per the procedure described by Finney (1971). The 96 h-LC_{50} (~10%) of were selected as sublethal concentrations of mercury (0.026ppm, 0.040ppm and 0.080ppm) and chromium (3.4ppm, 5.1ppm and 10.2ppm) and ten fishes of same size were exposed to each concentrations with three replicates for a period of 28 days. A control batch corresponding to each test group was simultaneously maintained. The experiments were repeated five times for each test concentrations. To maintain a constant toxic concentration in the media, the water medium with appropriate concentration of heavy metal was changed every day. Fish were fed with commercial pelleted diets *ad libitum.*

After 7, 14, 21 and 28 days the gills, liver, kidney, brain and muscle tissues of control and treated *C. batrachus* were dissected and homogenized thoroughly with 5 ml of phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm for 15 minutes. After centrifugation the supernatant was collected and the proteins were precipitated by adding equal volume of 10% ice cold TCA. The pellet was re-dissolved in 2 ml of 1N NaOH. To 0.1 ml of the above sample 5 ml of the protein reagent was added, mixed thoroughly and the absorbance was measured at 595 nm. Bovine serum albumin was used as standard (Bradford, 1976).The results were analyzed using SPSS 10.1 version ANOVA followed by post-hoc Tukey-HDS's test, the significant levels of difference

between control and treated fish were calculated at $p < 0\ .05$ level.

Table 1. The levels of protein content (µl/ml) in tissues of *Clarias batrachus* exposed to sublethal concentrations (% 96h LC_{50}) of mercury (ppm) toxicity (7 to 28 days).

Days	7 days				14 Days				21 Days				28 Days			
Tissues	Cont	0.026	0.040	0.080	Cont	0.026	0.040	0.080	Cont	0.026	0.040	0.080	Cont	0.026	0.040	0.080
Gil	2.64 ±0.29	2.25 ±0.07	2.17 ±0.06	1.94 ±0.02	2.80 ±0.12	2.03 ±0.06	1.65 ±0.48	1.77 ±0.02	2.88 ±0.07	2.21 ±0.01	2.08 ±0.01	1.92 ±0.04	2.77 ±0.11	2.23 ±0.03	2.07 ±0.06	1.93 ±0.02
Kidney	4.18 ±0.01	3.17 ±0.03	2.86 ±0.03	2.47 ±0.02	4.18 ±0.01	2.82 ±0.01	2.59 ±0.01	1.97 ±0.12	4.13 ±0.01	3.06 ±0.01	2.61 ±0.01	2.27 ±0.02	4.19 ±0.01	3.10 ±0.01	2.86 ±0.07	2.31 ±0.02
Brain	3.88 ±0.02	2.47 ±0.19	2.2 4±0.05	2.06 ±0.02	3.82 ±0.05	2.07 ±0.02	2.09 ±0.01	1.90 ±0.01	3.78 ±0.09	2.1 ±0.02	2.11 ±0.01	2.10 ±0.01	3.87 ±0.06	2.23 ±0.03	2.14 ±0.11	2.18 ±0.07
Muscle	3.25 ±0.02	2.73 ±0.46	2.40 ±0.01	2.43 ±0.47	3.26 ±0.06	2.06 ±0.02	2.01 ±0.01	1.89 ±0.01	3.19 ±0.04	2.19 ±0.01	2.10 ±0.01	2.01 ±0.01	3.16 ±0.27	2.31 ±0.01	2.28 ±0.01	2.10 ±0.01
Liver	4.43 ±0.05	3.26 ±0.03	3.09 ±0.01	2.56 ±0.03	4.37 ±0.02	2.9 3±0.04	2.82 ±0.04	2.04 ±0.05	4.51 ±0.01	3.06 ±0.05	2.90 ±0.01	2.39 ±0.01	4.45 ±0.03	3.10 ±0.01	3.04 ±0.04	2.45 ±0.03

Cont- Control

Table 2. The levels of protein content (µl/ml) in tissues of *Clarias batrachus* exposed to sublethal concentration (% 96h LC_{50}) of chromium (ppm) toxicity (7 to 28 days).

Tissues	Cont	3.4	5.1	10.2	Cont	3.4	5.1	10.2	Cont	3.4	5.1	10.2	Cont	3.4	5.1	10.2
Gill	3.04	2.77	2.57	2.44	2.87	2.4	2.20	1.91	2.95	2.60	2.42	2.03	2.96	2.70	2.50	1.97
	±0.20	±0.02	±0.05	±0.05	±0.18	7±0.01	±0.001	±0.01	±0.30	±0.01	±0.01	±0.01	±0.28	±0.01	±0.01	±0.02
Kidney	3.86	3.3	3.09	2.77	4.27	2.85	2.64	2.57	4.27	3.05	2.92	2.67	4.57	3.11	3.01	2.70
	±1.14	6±0.03	±0.01	±0.01	±0.08	±0.04	±0.03	±0.02	±0.09	±0.03	±0.02	±0.01	±0.05	±0.01	±0.01	±0.01
Brain	3.61	2.96	2.82	2.60	3.81	2.33	2.18	1.86	3.74	2.83	2.63	2.43	4.25	3.04	2.98	2.63
	±0.15	±0.01	±0.06	±0.02	±0.10	±0.01	±0.01	±0.04	±0.12	±0.04	±0.01	±0.04	±1.83	±0.02	±0.01	±0.01
Muscle	3.47	2.96	2.82	2.60	3.50	2.48	2.17	1.93	3.43	2.64	2.46	2.23	3.48	2.83	2.70	2.12
	±0.22	±0.01	±0.06	±0.01	±0.26	±0.01	±0.03	±0.02	±0.23	±0.03	±0.03	±0.05	±0.18	±0.01	±0.01	±0.03
Liver	4.61	3.47	3.23	2.98	4.67	2.94	2.74	2.67	4.51	2.83	3.03	2.76	4.76	3.27	3.12	2.91
	±0.07	±0.01	±0.03	±0.02	±0.08	±0.04	±0.04	±0.03	±0.02	±0.59	±0.02	±0.02	±0.08	±0.02	±0.01	±0.01

Cont- Control

RESULTS

The median lethal concentration (LC_{50}) of mercury to *Clarias batrachus* for 24, 48, 72 and 96 h of exposure were 1.4 ppm, 1.2 ppm, 1.0 ppm and 0.8 ppm respectively. The LC_{50} value of chromium to *C. batrachus* for 24, 48, 72 and 96 h of exposure are 120 ppm, 115 ppm, 110 ppm and 102 ppm respectively. Increase in exposure period results in decreased LC_{50} values. The sublethal concentrations (96 h-LC_{50}) of mercury (0.026ppm, 0.040ppm and 0.080 ppm) and 96 h-LC_{50}) chromium (3.4ppm, 5.1ppm and 10.2ppm) were treated to *C. batrachus* for 28 days. The highest amount of protein was observed in Liver, kidney, brain, muscle, and gills in the control group and compared with the mercury and chromium treated fishes and tabulated (Tables 1&2). Gills, kidney, brain, muscle and liver showed pronounced changes in the protein content as sublethal mercury and chromium concentration increased. As the duration of the exposure period increased, there was a sudden decrease from 7th day to the 14th day followed by slight increase from14th to 21st and to 28th day at 0.026 ppm, 0.04ppm and 0.08ppm sublethal concentrations of mercury. All the obtained values were subjected to statistical analysis and the values were significant at $p < 0.05$ level. Results indicate that sublethal mercury and chromium exposure leads to a general decline and gradual increase in the level of proteins in liver, kidney, brain, muscle and gill tissues of *C. batrachus.*

DISCUSSION

Proteins are highly sensitive to heavy metals and are one of the earliest indicators of heavy metal poisoning **(**Kakkar and Jaffery, 2005). Heavy metals may alter the protein concentration through impairing the synthesis and metabolism of protein, DNA and RNA as well as alter the activity of lysosomal enzymes (Jana and Bandopadhyaya, 1987). It is possible that pollutant stress influences the conversion of tissue proteins into soluble fractions reaching the blood and hence, decrease in protein content was observed in the tissues of liver and muscle of *Labeo rohita* exposed chronically to heavy metal salts, chromium chloride, nickel chloride and zinc chloride (Neha *et al.,* 2004). In the present study mercury and chromium sublethal toxicity stress also led to depletion of the original protein content in gill, kidney, brain, muscle and liver of *C. batrachus* was observed in the control and treated tissues (Table 1 &2). Depletion of protein content has been observed in all the organs of *C. batrachus* till 14 days of exposure followed by slight increase from 14th to 21st and to 28th day after treatment. However, this increase could not reach the level of the control values. The maximum level of protein was found in the liver and the minimum in the gills and muscles of *C. batrachus.* Increase in exposure period as well as sublethal mercury concentration led to decrease in total protein content though their levels show replenishment after 21 days of exposure to the toxicant. Similar observation was noticed in the chromium treated tissues of the experimental fish. Kapila and Ragothaman (1999) have also reported decreased tissue proteins followed by increased level of proteins in the fish, *Boleopthalmus dussumieri* exposed to mercury, copper and cadmium for prolonged periods. The initial drop in the protein content during mercury toxicity may be on account of reduced protein synthesis and an enhanced proteolysis in the various organs of fish (Jagadeesan and Mathivanan, 1999).

The depletion of protein level induces diversification of energy to meet the impending energy demands during the toxic stress. The reduction in tissue proteins reflects a prior increased energy cost of homeostasis, tissue repair and detoxification under toxic stress. It is also possible that when an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands. Hence depletion in protein level is observed (Neff, 1985). Evidently the whole energy is required to mitigate any stress condition and this energy may be derived from proteins (Shakoori *et al.,* 1992). The obvious reasons for the varying proteins levels in fish under heavy metal toxicity could be due to the rapid metabolism under heavy metal stress (Shakoori *et al.,* 1992). Reduction in protein content is a cause of decrease in RNA in the tissues of fish or amino-acid incorporation and disaggregation of polysomes (Holbrook, 1980). The induced proteolysis by the heavy metal in the respective

tissues contributes to the increase in the amino-acids (Radhaiah *et al.,* 1987; Ravichandran *et al.,* 1994). During protein metabolism the removal of the amino group from different amino-acids was observed suggesting the elevated levels of amino-acids (Begum, 2008). Both transamination and deamination seems to be enhanced at this stage. Baskaran (1991) reported depletion of protein content in the muscle and liver of *Oreochromis mossambicus, Mystus vittatus and C. striatus* exposed to fenvalerate. Jeba kumar *et al.* (1990) observed decrease in protein content of *Lepidocephalichthys thermalis* exposed to sublethal concentrations of fenvalerate. A significant decrease was reported in the protein content of almost all tissues in *Ctenopharyngodon idellus* when exposed to sublethal and lethal concentrations of fenvalerate (Tilak *et al.,* 2001). The decrease of the protein content in the liver may be due to necrosis of the hepatocytes. Kondal *et al.* (1988) attributed the decrease in protein content of the liver of *Saccobranchus fossilis* and *Heteropneustes fossilis* exposed to factory effluent and ammonia respectively, to the availability of energy required for the synthesis of proteins as a consequence of the decreased level of the key enzyme (Malate dehydrogenase) and due to enhanced catabolism of amino-acids, as a consequence of increased activity of glutamate dehydrogenase. Protein content significantly decreased in liver and muscle of *Cyprinus carpio communis* under stress (Dhanapackiam *et al.,* 2000) and *C. batrachus* (Aruna *et al.,* 2000). Initial increase of protein content after 7th day of treatment reflects an increase in total enzyme activity of the tissue in order to match the physiological demand of the system. The fish needs to balance the adverse stress by synthesizing certain molecules which appear to be proteinaceous in nature to reduce the toxicity. The increase of protein content in the kidney reflects stimulated protein synthesis of detoxification enzymes at the expense of glycogen to meet additional energy requirements in the synthetic activity of the tissues (Aruna, *et al.,* 2000). The kidney which is an important organ of excretion and osmoregulation is indirectly affected by pollution through blood circulation (Newman and MacLean, 1974). Decreased protein content in the kidney could be possible due to protein break down leading to increased amino-acid pool of tissue (Radhaiah *et al*., 1987). The decrease in the protein content of the kidney of *C. batrachus* after exposure to malathion is attributed to the impairment of protein synthesis and/or increase in the rate of their degradation to amino acids which may be fed to the TCA cycle through aminotransferase probably to cope with the stress condition (Aruna *et al.,* 2000). Kasturi and Chandran (1997) observed decreased level of protein content in the tissues of *Mystus gulio* exposed to sublethal concentration of lead. Sen *et al.* (1992) also observed decrease in protein content of brain and liver of *C. punctatus* exposed to toxic effects of zinc. These observations are in accordance with the results of the present study.

Conclusion

In this study, mercury and chromium sub lethal concentrations in different tissues of *C. batrachus* showed different elevation of the protein content which shows the toxicological effects of heavy metals on fish that can be used for monitoring the heavy metal toxicity in aquatic ecosystem.

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