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ORIGINAL ARTICLE

EFFECT OF ARSENIC ON PROTEIN AND AMINO ACIDS LEVEL IN ARIOUS TISSUES OF FRESH WATER FISH, CATLA CATLA

R. Thirumavalavan

Department of Zoology,Annamalai University,Chidambaram,Cuddalore Dist-608002,Tamilnadu

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ABSTRACT

The present study is aimed to investigate the effect of arsenic on protein and amino acids in gill, liver and kidney tissues of fresh water fish, Catla catla. The fishes were exposed to sublethal concentration of arsenic on protein and amino acids content in fresh water fish, Labeo rohita. The present stydy shows the level of protein was decreased and protein and amino acids were decreased due to arsenic

Keywords: Arsenic ,SDH, LDH, *Catla catla*, Brain,Gill, Liver Kidney

1.INTRODUCTION

Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the level of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. This is due to their persistence, as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants. Heavy metal constitutes a serious type of pollution in fresh water and being stable compounds; they are not readily removed by oxidation and affect the animal (Nammalwar, 1985). Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. Heavy metal pollution of water is a major environmental problem facing the modern world (Shrivastava and Sathyanesan, 1987). Heavy metals have a unique property of accumulation over a period of time, along a food chain and a very high level can be accumulated in an organism from very low level concentration in water and sediments (Shrivastava and Sathyanesan, 1987; Bose *et al.*, 1994).The toxicological effects of pollution are due to their high persistence and accumulation in the organisms (Goyer, 1996). Although suitable concentration of heavy metals plays a vital role in metabolic pathways when their concentration exceeds the threshold level, they act as physiological biochemical and behavioral inhibition in the organisms.
Metals are elements found naturally in aquatic ecosystems due to various processes such as weathering and erosion (Viljoen, 1999). Some of these metals are essential to living organisms in trace amounts (for example copper and zinc). Essential trace elements have a narrow optimal concentration range for growth and reproduction, and both excess and shortage can be detrimental to organisms (Pelgrom *et al.*, 1994), with unusually high concentrations becoming toxic to aquatic organisms (Wepener *et a.*, 2001). Other metals (for example cadmium and lead) have unknown biological function (Seymore, 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel chloride and chromium (Abel, 1989; Viljoen, 1999). Metal uptake by aquatic organisms is a two-phased process, firstly involving rapid adsorption or surface binding, followed by a slower transport into the cell interior. Transport of metals into the intracellular section may be aided by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezonik *et al.*, 1991; Wepener *et al.*, 2001).

Arsenic is widely distributed in the environment including water resources and animal tissues and occurs as a variety of organic and inorganic compounds (Webb,1966). The concentration of arsenic in the environments is of great concern as thus element is recognized as a cumulative poison to animals. Arsenic is mainly released in to the environment through intestinal process during the preparation of base metals and thermal power generation. The arsenic and its compounds are used as pesticides herbicides, insecticides and fungicides [Webb, 1966]. The environment as a natural

Corresponding author ***Dr.R.Thirumavalavan****, Asst. Professor, Dept. Of Zoology, Annamalai University-608002*

component of soil and in water in inorganic form [ATSDR, 1999], and its toxicity has been known since ancient times. The animals are exposed to inorganic arsenic through drinking well water, food, air and are occasionally exposed occupationally through arsenic fumes or dust (National Research Counsil, 1999). Arsenic is normally in the pentavalent inorganic arsenate form in drinking water, but upon consumption by animal, it rapidly undergoes metabolic conversion that include reduction of arsenate to arsenates [Yamanuchi and Yamamura, 1979]. In developing countries arsenic contamination of ground water remains a crucial water ground water remains a crucial water quality problem in particular, in developing countries. Acute and chronic poisoning of arsenic has occurred as a result of consumption of high level of arsenic contaminated well water, and causes numerous disease including specific causes numerous disease including specific cancers, Hypertension (Chen *et al*, 1995), respiratory system dysfunction (Mazumder *et al*., 2000).

Fishes are sensitive to contamination of waters and the pollutants may damage certain physiological and biochemical processes when they enter the organs of fishes [Tulasi *et al*., 1992]. Fishes are being at the higher level of the food chain accumulate large quantities of thesis semeiotics and the accumulation depends on the intake and the elimination from the body [Karadede *et al*., 2004].The gills are the first target organs in the heavy metal accumulation because they are directly in contact with water [Dubale and shah, 1979]. The gills, which serve as the primary uptake site in the fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals [Newman and Jogoe, 1997]. Liver is one of the most multi faceted and active organ in higher animals. In a vertebrate body, the liver is most important target organ as it is the chief metabolic and detoxification center [Bhattacharya and Mukherjee, 1976] The kidney is the most sensitive organ with respect to overt toxicity following exposure to heavy metal. The kidney is the main excretory organ, is mainly concerned with removar of waste materials [Lawrence and Mc Cabe, 2002].

2.MATERIALS AND METHODS

Procurement of experimental animal

The fresh water fish, *Catla catla* were collected from the fish farm located in Puthur,Nagai District, 15 Km away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanks (100X175cm) of 500liters capacity containing chlorine free aerated well water.

Acclimatization of animals

The fresh water fish, *Catla catla* were acclimatized for a minimum period of 15 days in the laboratory conditions at room temperature (28±1ºC) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack. The fresh water fish, *Catla catla* were critically screened for the signs of disease, stress,physical damage and mortality. The injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by Arrora et al., (1972). During the acclimatization, the fishes were reared in tank until there was less than 10 percent mortality in 4 days perior to the beginning of the test as suggested by Anderson(1977). The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

Metal for toxicity studies

The toxicant sample, arsenic trioxide, was used for the present experimental studies and it has the following characteristics:

Estimation of LC_{50} value

Perior to the commencement of the experiment, 96 hr medium lethal concentration as (96 hr LC_{50}) of mercuric chloride for *Oreochoromis mossambicus* was estimated (Hamilton *et al* 1977). And 24 hrs renewal bioassay system and was found to be 60 ppm after 5% trimming.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 2 groups of 10 fish each were exposed separately and arsenic trioxide (2.73ppm : 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 6 ppm having dissolved oxygen 5.8 ppm, PH7.4, water hardness 30.3mg/l (APHA et al., 1992) and water temperature 28± 2 C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaric containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours. Before the reneval of the medium through out the tenature of the experimental.

BIOCHEMICAL STUDIES

After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The brain gills, liver and kidney were isolated from the fish and used for various study.

Estimation of tissue protein

Protein content in the tissues were estimated by the method of Lowry *et al*.(1951). The tissues (brain, gill, liver, and kidney) were isolated and 2% homogenate was centrifuged at 3,000 rpm for 15 minutes. the supernatant was discarded and the reside was suspended in 1.0 ml of 0.1 N sodium hydroxide solution. 0.5 ml of this solution equivalent to 10 mg of tissue was transferred to a clean test tube and 4 ml of copper carbonate solution was added. The contents were mixed by lateral shaking and 0.4 ml of folin phenol (1:1 dilution) reagent was added. The thoroughly mixed contents were kept at room temperature for 30 minutes, the colour developed was read at 600 nm against a reagent blank in UV sisible spectrophotometer (Jasco Model-650). Bovine serum albumin (Sigma Chemical Co.) was used to construct expressed in mg/g wet weight of tissues.

Estimation of total free amino acids in tissues

Total free amino acids and content of the tissue were estimated by the method of Moore and Stein (1954). The tissues) liver, muscle, kidney and gill) were isolated in ice, quickly weighed in an cold room and immediately homogenized in cold 10 per cent TCA. The homogenate contains 10 mg of tissues).One ml of the clear supernatant was taken into a clean test tube and 2.0 ml of ninhydrin reagent was added. The mixture was cooled immediately under running rap water and the intensity of the colour was read at 570 nm in a UN-visible spectrophotometer (Jasco, model 650). Tyrosine was used to construct the standard graph and the values were expressed mg/g wet weight of tissue.

Statistical analysis

Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) Duncan (1957).

3.RESULTS

Level of total protein in brain tissue

In the brain tissue of normal fish, the level of protein was 56.18 ± 1.07 mg/g wet wt. of tissues. During the sublethal concentration of arsenic, the level of protein was decreased upto 20.65 ± 1.86 mg/g wet wt. of tissues when compared to control. The percent change over control was – 63.24(Table 1).

evel of total protein in gill tissue

The level of total protein was 54.17 ± 1.25 mg/g well wt of tissue I in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of protein (22.39±1.77mg/g wet wt. of tissue). The percent change over the control was – 58.66(Table 1).

Level of total protein in liver tissue.

In the normal liver tissue, the level of protein content was 75.21 ±1.21.mg/g wet wt. of tissue when the fish exposed to arsenic, the level of protein content was decreased upto 49.29±1.17mg/g wet wt. of tissues. The percent change over control was-34.46(Table 1).

Level of total protein in kidney tissue

The level of protein present in the kidney tissue of normal fish was 58.27±1.80 mg/g wet wt of tissue. The level of protein was decreased upto 20.65±1.37 mg/g wet wt. of tissue when the fish exposed with sub lethal concentration of arsenic. The percent change over control was – 64.56(Table 1).

Level of protein in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of arsenic

Tissues	Control	21 days	% COC
Brain	56.18±1.07	20.65±1.86*	-63.24
Gill	54.17±1.25	22.39±1.77*	-58.66
Liver	75.21±1.21	49.29±1.17*	-34.66
Kidney	58.27±1.80	20.65±1.37*	-64.56

Mean ± S.D. of six individual observations;* Significance ($p<0.05$) Group I compared with group II; Values are expressed as (mg/g wet wt. of tissue)

Table 2Level of amino acid in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of arsenic

Tissues	Control	21 days	% COC
Brain	6.79±1.10	9.85±1.88*	45.06
Gill	4.21±1.66	6.62±1.61*	57.24
Liver	12.26±1.69	21.69±1.72*	76.91
Kidney	7.14±1.12	12.86±1.62*	80.11

Mean ± S.D. of six individual observations;* Significance ($p<0.05$) Group I compared with group II; Values are expressed as (mg/g wet wt. of tissue)

Level of amino acid in brain tissue

In the brain tissue of normal fish, the level of amino acid was 6.79±1.10 μmg/g wet wt. of tissues. During the sublethal concentration of arsenic, the level of amino acid was increased upto 9.85±1.88 μg/g wet wt. of tissues when compared to control. The percent change over control was 45.06(Table 2).

Level of amino acid in gill tissue

The level of amino acid was 4.21±1.66 μg/g wet wt. of tissue in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the increased trend of amino acid (9.85±1.88μg/g wet wt. of tissue). The percent change over the control was 57.24(Table 2).

Level of amino acid in liver tissue.

In the normal liver tissue, the level of amino acid content was 12.26 ±1.69μg/g wet wt. of tissue. When the fish exposed to arsenic, the level of amino acid content was increased upto 21.69±1.72μg/g wet wt. of tissues. The percent change over control was 76.91(Table 2).

Level of amino acid in kidney tissue

The level of amino acid present in the kidney tissue of normal fish was 7.14±1.12 μg/g wet wt of tissue. The level of amino acid was increased upto 12.86±1.62μg/g wet wt. of tissue when the fish exposed with sub lethal concentration of arsenic. The percent change over control was 80.11(Table 2).

4.DISCUSSION

Protein is the most important and abundant biochemical constituent present in the animal body. It plays a major role in the synthesis of microtonal detoxifying enzymes and helps to detoxify the toxicants which enter the into the animal body (Ramasamy, 1987). Proteins are important organic constituents of the animal cells. It plays a vital role in the process of interactions between intra and extra cellular media being a part of cell membrane and enzymes (Ramalingam *et al*., 2002). The amino acid and the building blots of protein. There are number of amino acids present in the animal body and these very in accordance with the number and sequence of amino acids (Linder, 1985).

Metals are known to change the physiology of the organism by affecting important aspects of the cellular metabolism such transport across the membrane, mitochondria function and lysosomal stability (Reddy *et al*., 1998). Heavy metal causes the biochemical changes to tissues and genes through diverse mechanisms such as interrupting intracellular homeostatic,

disrupting membrane potential, altering the protein synthesis, and interrupting excitatory amino acid pathway in many tissues (Yee and Choi, 1996).

In the present study, the level a protein decreased and the level of amino acid increased in the gill, liver and kidney tissue when the fish exposed with arsenic trioxide for 21 days. This result suggest that the decreased level of protein might be due to their catabolism to liberate energy during the stress of arsenic toxicity. Similarly, Jana and Bandyopathyay (1981) have reported the reduction in protein content in *Channa punctatus* exposed to arsenic and lead. Reddy *et al*., (1988) have reported that the fall in protein level during heavy metal exposure may be due to increased catabolism and decreased anabolism of protein. Jha and Jha, (1995) have reported that the level of protein content decreased in liver tissue of anabu testudineus exposed nickel chloride. Sen *et al*., (1992) have observed a reduction in protein level in *Channa punctatus* exposed to phenyl mercuric acetate.

Vincent *et al*., (1995) have reported that the protein content was decreased in liver tissue of *Catla catla* exposed to chromium. Baskaran *et al*., (1991) have reported that the impact of commercial detergent on protein metabolism in the fresh water fish *Oreochromis mossambicus.* Ramalingam *et al*., (2000) reported that protein content was decreased in *Cirrhinas mrigala* exposed to lead acetate. Palanichamy and Baskaran (1995) have reported a reduction in the level of protein in the muscle and liver tissue of *Channa striatus* exposed to mercury, cadmium and lead. Neff (1985) has reported that decline in protein content may be related to increased energy cost of homeostasis, tissue repair and detoxification during toxic stress. Avash Maruthi and Ramakrishna Rao, (2000) reported that the level protein decreased in liver and muscle tissue in *Channa punctatus* exposed to sugar mill effluent. Radhaiah *et al* (1985) reported that the protein content decreased in the kidney tissue. This result may be due to protein breakdown leading to increase amino acid. Ambrose *et al*., (1994) have reported that the level of protein decreased in kidney tissue of *Cyprinius carpio* exposed to tannery effluent.

James *et al*., (1991) observed a reduction in protein content in liver, gill and muscle tissue to *Oreochaomis mossambicus* exposed to zinc and cadmium. Almeida *et al*., (2001) have reported that a decrease in protein content in liver and muscle of *Oreochromis niloticus* exposed to cadmium. Bradburgy *et al*., (1987) reported that the decreased protein content might be attributed to the destruction or necrosis of cells and consequent impairment in the mechanism of protein synthesis.

In the present study, the level of amino acid content is increased in brain, gill liver and kidney tissue of *Labeo rohita* exposed to arsenic trioxide for 21 days. This is mainly a consequence of higher catabolic activity of protein to meet the high energy demand by breaking down the protein into free amino acids. Jagadeesan (1994) has observed that the mercuric chloride exposed *Labeo rohita* shows an increasing level of amino acid content in kdney, brain and liver tissues. Ramesh Kumar, (1989) has observed the amino acid content increased in cliver tissue of *Mystus vitatus* exposecd to zinc sulphate. Bass, (1962) has reported that an increase in amino acid in liver, muscle and brain tissue of *Cyprinus carpio* exposed to merculy. This might be due to enhanced proteolysis. Karuppasamy (2001) has observed the level of amino acids was in creased in liver, muscle, kidney, brain and gill tissue of *Channa punctatus* exposed to phenyl mercuric acetate.

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