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ORIGINAL ARTICLE

EFFECT OF LEAD ACETATE ON ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (AST) IN THE SELECTED TISSUES OF FRESH WATER FISH, CATLA CATLA

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ABSTRACT

The present study is carried out to investigate the enzymological paprameters such as Aspartate aminotransferase (AST) and Alanine aminotransferase (AST) in the gill,liver and kidney tissues of fresh water fish, *Catla catla* exposed sublethal concentration of lead acetate. The present study shows the level of Aspartate aminotransferase (AST) and Alanine aminotransferase (AST was increased in gill,liver and kidney tissues of fresh water fish, *Catla catla* due to exposure of lead acetate.The present study concludes that the lead acetate affect the tissue damage in fresh water fish, *Catla catla.*

Keywords: Lead acetate, AST, ALT, *Catla catla*

1.INTRODUCTION

Heavy metal alter the physiology of the organism by affecting important aspects of the cellular metabolism [Janardana Readdy *et al*., 1998]. The sources of pollution are varied and different such as geologic weathering, industrial processing of ores and metals, the use of metals and metal components leaching of metals from garbage and solid waste dumps, animal and human excreta [Gawtham and Khan, 1998]. 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 [Nammalwar, 1985]. The sources of heavy metals are industry, municipal waste water, atmospheric pollution, urban runoff, river dumping and shore erosion. Heavy metals in surface water systems can be from natural sources [Guilizzoni, 1991]. Heavy metals have many sources from which they can flow into the water body through natural and industrial sources. Metals are found throughout the earth, in rock, soil and introduce into the water body through natural processes, weathering and erosion and industrial processes particularly these concerned with the mining and processing of metal ores, the finishing and plating of metals and the manufacture of metal objects.

Lead (Pb) is an immunotoxicant which through human exposure results in immune function changes and has the potential to adversely affect human health. It has many uses in industry including pipes, paints, enamels, glazes, motor industry and others. The major hazard in industry arises from the inhalation of dust and fume but the organic compounds may also be absorbed through the skin. It induces a broad range of physiological, biochemical and neurological dysfunctions in humans (Nordberg *et al*., 2007). Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb (Ademuyiwa *et al*., 2007; Reglero *et al*., 2009; Abdallah *et al*., 2010; Mirhashemi *et al*., 2010). The liver plays a major role in lead's metabolism (lead poisoning cause adverse effects to hepatic cells) because after Pb exposure liver is one of the major organs involved in the storage, biotransformation and detoxification (Sivaprasad *et al*., 2004). In fish Pb is accumulated mostly in gill, liver, kidney and bone. Fish eggs show increasing Pb levels with increased exposure concentration and there are indications that Pb is present on the egg surface but not accumulated in the embryo (Birge *et al*., 1979). The toxicity of Pb and other heavy metals, their

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accumulation in edible tissues, their effects on growth and metabolic processes of carb and fish were previously studied (Andreji *et al*., 2006; Ashraf *et al*., 2006; Has-Schon *et al*., 2006; Agah *et al*., 2009).

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical process when they enter the organs of the fish [Tulasi *et al*., 1992]. The heavy metal in the tissue of fishes may cause various physiological defects and mortality [Torres *et al*., 1987]. The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bio indicator of environmental pollution [Dautrempuits *et al*., 2004; Lopes *et al*., 2001]. 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 Jagoe, 1994]. Liver is one of the most multi faceted and active organ in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center [Bhattacharya and Mukherjee, 1976]. Liver is the main site for all the metabolic activities and also for all detoxification reactions. The kidney is the most sensitive organ with respect to overt toxicity following exposure to heavy metal [Bhattacharya and Mukherjee, 1976]. Kidney is an organ is mainly concerned with removal of waste materials.

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.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 3 groups of 10 fish each were exposed separately and lead acetate (5.8 ppm : 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving lead acetate 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 aquatic 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.

Estimation of LC_{50} value

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

BIOCHEMICAL STUDIES

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

Estimation of serum Aspartate amino transferase (AST) and Alanine amino transferase (ALT)

The activity of AST and ALT was determined by adopting the method of King (1965).

Principle for AST

The amount of oxaloacetate was measured by converting it into pyruvate treating with aniline citrate and then made to react with 2,4 dinitro phenyl hydrazine. The absorption of the brown colour was read at 520 nm.

L-aspartate+α-ketoglutarate AST Oxaloacetate + L-glutarate

Principle for ALT

The amount of pyruvate formed was measured by treating the pyruvate with 2,4 dinitrophenyl hydrazine. The brown colour developed was read at 520 nm

L-alanine+α-ketoglutarate ALT Pyruvate+ L-glutamate

1 ml of substrate (AST-1.33g of L.aspartic acid and 15 mg of α- Ketoglutaric acid were dissolved in 20.5 ml of phosphate buffer and 1N sodium hydroxide to adjust pH 7.5 and made upto 50 ml with phosphate buffer; ALT – 1.78g of DL-alanine and 30mg of α-ketoglutaric acid were dissolved in 20 ml of buffer. The pH was adjusted to 7.5 with 1N sodium hydroxide and made upto 100 ml with buffer. A few drops of chloroform was added) was taken in a clean test tube and it was incubated for 5 minutes at 37°C. Then 0.2 ml of serum

was added in the test tube and incubation was maintained for an hour in the case of AST and 30 minutes for ALT. The reaction was arrested by adding1.0 ml of DNPH reagent and then the tubes were kept at room temperature for 20 minutes. Then 10 ml of 0.4N sodium hydroxide solution was added and the colour developed was read at 520 nm against a reagent blank in UV spectrophotometer. Pyruvic acid was also treated in similar manner for the standard.

The activities of serum AST and ALT are expressed as moles of pyruvate formed/mg of protein/hr.

Statistical analysis

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

3.RESULTS

Level of aspartate aminotransferase (AST) in the gill tissue

Sub lethal concentration of cadmium chloride treated *Catla catla* showed changes in the level of aspartate aminotransferase in the gill. It exhibited fluctuations from the mean control level. In the treated fish, the level aspartate aminotransferase got increased for 15 days (Table 1).

Level of aspartate aminotransferase (AST) in the liver tissue

The level of aspartate aminotransferase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of lead. The aspartate aminotransferase in liver tissue was found to be increased for 15 days (Table 1).

Level of aspartate aminotransferase (AST) in the kidney tissue

The aspartate aminotransferase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of lead. The aspartate aminotransferase in the kidney tissue were found to be increased for 15 days (Table 1).

Level of alanine aminotransferase (ALT)in the gill tissue

Sub lethal concentration of cadmium chloride treated *Catla catla* showed changes in the level of alanine aminotransferase in the gill. It exhibited fluctuations from the mean control level. In the treated fish, the level alanine aminotransferase got increased for 15 days (Table 2

Level of alanine aminotransferase (ALT)in the liver tissue

The level of alanine aminotransferase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of lead. The alanine aminotransferase in liver tissue was found to be increased for 15 days. (Table 2).

Level of alanine aminotransferase (ALT)in the kidney tissue

The alanine aminotransferase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of lead. The alanine aminotransferase in the kidney tissue were found to be increased 15 days(Table 2).

Table 1The level of aspartate aminotransferase (AST) in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of lead acetate

Tissues	Control	15 days
Gill	11.92±1.02	21.66±1.19
Liver	26.02±1.08	49.17±1.82
Kidney	18.92±1.66	31.12±1.82

Mean ± S.D. of six individual observation
* Significance ($p<0.05$) Group I compared with group II and III.
Values are expressed as (µmoles of pyruvate formed / mg of protein/hr)

Table 2 The level of alanine aminotransferase (ALT) in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of lead acetate

Tissues	Control	15 days
Gill	28.66±1.86	42.89±1.26
Liver	48.50±1.86	89.16±1.27
Kidney	34.26±1.62	59.16±1.87

Mean ± S.D. of six individual observation
* Significance ($p<0.05$) Group I compared with group II and III.
Values are expressed as (µmoles of pyruvate formed / mg of protein /hr)

4.DISCUSSION

Heavy metal constitutes an important class of toxic substance which are encountered in numerous occupational and environmental circumstances. The heavy metal released the aquatic and terrestrial media by a variety of anthropogeric activities, and industrial uses [Mary Chandravathy and Reddy, 1995]. With some of these interactions there is high reactivity involving a high degree of inhibition of the specific enzyme that accounts for the effects on the whole animal.

Aspartate aminotransferase (AST) is responsible for transterring amino group form aspartate to 2-β glutasic acid forming glutamate and oxalo acetate. The rise in AST level is virtually responsible for all types of tissue damage [Tiwari and Sivastava, 2001]. Alanine aminotransferase (AST) is responsible for transferring amino group from alanine to 2-ketoglutaric acid forming glutamate and pyruvate. It is well known for tissue damage and its level rises higher in most types of hepato cellular damage [Tiwari and Srivastava, 2001]. Alterations in AST and ALT level are the biomarker for assessing the toxicity of heavy metals [Martin *et al*., 1981]

In the present study, the level of AST and ALT activity increased in the gill, liver and kidney tissues of *Catla catla* exposed to lead acetate for 15 days. This result may be due to necrosis, which causes increase in the permeability of cell membrane resulting in the damage of tissues. Similar results made reported by Hwang *et al*, [2000]. They reported that the

level of AST and ALT activities are increase due to heavy metals in chronic liver damage.

The activity of AST and ALT can be used to indicate the tissue damage of liver and kidney [Nemscoc and Boross, 1982]. Khan *et al*., (1993) reported disturbances in the liver function after heavy metal exposure reported the increase in the AST and ALT activities in the serum of animal exposed to lead. Hori *et al*., (2006) have observed the level of AST in the liver tissue of *Brycon cephalus* exposed with phenol. Alteration in the activity of AST and ALT will be reflected nitrogen metabolism on the energy yielding TCA cycle(Beyer *et al*., 1996).

Several investigators also reported that heavy metal intoxication showed a significant increase in AST and ALT activities in the liver tissue of animals (Rana *et al*.,1996; Khandelwel *et al*.,2002). The hepatocellular necrosis is generally associated with alterations in the liver tissue and serum (Zimmerman,1978). The elevated level of AST and ALT indicate stopped up transmutation where feeding of amino acids into the TCA cycle occurs in order to cope up the energy crisis during cypermethrin toxicity [Philip *et al*., 1995]. The significant increase of these enzyme in the tissues seems to indicate possible dysfunction, taking place in the tissues of animals [Casilla *et al*., 1983].

Sharma [1999] has reported that similar pattern of increase in AST and ALT in the liver tissue of *Channa Batrachus* exposed to pesticides. Mary chandravathy and Reddy (1991) have reported that the elevation of AST and ALT in the gill and brain tissues of *Anabas scandens* exposed to lead nitrate. Usha and Raj (1993) have reported the increase in the lever of AST and ALT in the animals exposed to vanadium. Mukhopadhyay *et al*., (1982) have observed that an increase level of AST and ALT activities in the liver tissue of *Clarius batrachus* exposed to carbofuron. Similar results observed by Ganguli *et al*., (1997). They reported that level of these enzyme increase in the gill, liver and kidney tissues of *Anabas testudineus* exposed to lindene, and furandan

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