Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.2, Issue, 1, pp. 055-060, January, 2011

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF HEAVY METAL NICKEL ON AMINOTRANSFERASE ACTIVITIES IN LIVER TISSUE OF *Cirrhinus mrigala* (HAM.)

Parthiban, P and Muniyan, M*.

Department of Zoology, Annamalai University, Annamalainagar-608002 Tamilnadu, India

ARTICLE INFO

Article History:
Received 17th September, 2010
Received in revised form
25th October, 2010
Accepted 11th December, 2010
Published online 6th January, 2011

Key words:
Nickel, AST, ALT,
Liver, *Cirrhinus mrigala*

ABSTRACT

The contamination of water by metal compounds is an environmental problem. The present study is aimed to investigate biochemical studies in the liver tissue of fresh water fish *Cirrhinus mrigala* exposed to sublethal concentration of nickel. In the present study, the activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the liver tissue were observed. During the sublethal concentration of nickel, the Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were increased in the liver tissue. These results indicate the concentration of nickel damage the liver tissue of *Cirrhinus mrigala.*

INTRODUCTION

Contamination of aquatic ecosystems (lakes, rivers, streams, etc.) with heavy metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment. The main sources of heavy metals in aquatic ecosystems are of the anthropogenic type. Metals after entering the water may precipitate or adsorb on the surface of solids, remain soluble or suspended in it or may be taken up by fauna and flora. One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms. Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects (Palaniappan and Karthikeyan, 2009). Nickel is ubiquitous traces metal and occurs in soil, water, air, and in the biosphere. It is emitted into the environment from both natural and man-made sources. Once released to the environment, nickel readily forms complexes with many ligands, making it more mobile than most heavy metals. The primary sources of nickel emissions into the ambient air are the combustion of coal and oil for heat or power generation, nickel mining, steel manufacture, and miscellaneous sources, such as cement manufacturing. It is also used extensively in electroplating as nickel sulphate and nickel hydroxide is used in nickel–cadmium batteries (Nanda and Behera, 1996). Metal mining, smelting, refining, and processing, along with fuel combustion, and waste incineration activities release significant amounts of nickel (Ni) into freshwater habitats through atmospheric deposition and in liquid effluents and leachates

***Corresponding author:** *drmuniyan@gmail.com*

(Chau and Kulikovsky-Cordeiro, 1995). The nickel is an essential element at low concentrations for many organisms, it is toxic at higher concentrations (Clark and Keasling, 2002). Exposure to nickel may lead to various adverse health effects, such as nickel allergy, contact dermatitis, and organ system-toxicity. The accumulation of heavy metals in water suggests that fish may serve as a useful indicator for contamination metals in aquatic systems, because they respond with great sensitivity to changes in the aquatic environment (Aas *et al*., 2001; Mondon *et al*., 2001). Fish residing in polluted freshwater systems are exposed to *Ni*, primarily, through the ingestion of contaminated food and sediments (Dallinger and Kautzky, 1985). Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes 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 the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al.,* 2004). Liver is one of the most multifaceted and target organ as it is the chief metabolic and detoxification center in higher animal (Bhattacharya and Mukherjee, 1976). It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin. All toxins pass through liver at some point or the other; the liver may manifest the highest toxin concentrations and also the most clearly discernible structural and functional impacts. A limited number of laboratory studies have been investigated the nickel effect on fresh water fish (*Tjalve et al*., 1988; *Sreedevi et al*., 1992). Hence, the present investigation has been carried out to the study the effect on nickel on Aminotransferase activities in the liver tissue of freshwater fish *Cirrhinus mrigala.*

MATERIALS AND METHODS

Experimental fish: The majar carp, *Cirrhinus mrigala* were collected from the fish farm located of Pinnalur, Cuddalore District, 15 Km away from the University campus. These fish were brought to the laboratory and transferred to the rectangular fibre glass tanks of 500 liters capacity containing chlorine free aerated well water. Prior to the start of the experiment, the fish were acclimatized to the food and laboratory conditions with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days.

Experimental design: Fish were divided into two equal groups each comprising of 20 fishes. Each group was kept in separate plastic tanks. The first group was kept as control; which was the maintained in water containing normal water without any treatment. The second groups of fish were exposed to a sub-lethal concentration of Nickel chloride (3.75ppm). Both the groups of fish were 30 days. The Solutions were renewed once in 24 hours of exposure period. After the 30 days of experiment, the fish from the respective experimental and control group was sacrificed. The liver tissue was isolated from the fish and used for the estimation of aspartate aminitransferase (AST) and alanine amino transferase (ALT) activity.

Estimation of biochemical studies in the liver tissue: The activity of AST and ALT was determined by adopting the method of King (1965). 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 supernatant 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 and Statistical significance was evaluated by using “T” test.

RESULTS

***AST and ALT Level in the liver tissue*:** The activity of AST in liver tissue of normal fish was 66.25±1.12 µmoles of pyruvate formed / mg of protein/hr). In the sublethal concentration of nickel, the level of AST enzyme was 124.18±1.72 µmoles of pyruvate formed / mg of protein/hr. Similarly, in the normal liver tissue, the ALT activity was 46.50±0.87 µmoles of pyruvate formed / mg of protein/hr. In sublethal exposure of nickel the level of ALT in the liver tissue was 58.15 ± 0.65 µ moles of pyruvate format / mg of protein/ hr. In the permit group of sublethal exposure of nickel, the AST and ALT levels were increased when compared with their control. The increased levels were significant at 5% levels of student 'T' test.

Table 1. The activity of Asparate aminotransferase (AST) and Alanine aminotransferase (ALT) in the liver tissue of *Cirrhinus mrigala* exposed to nickel.

Parameters	Control	Nickel Exposure of 30 days
Aspartate aminotransferase (AST) (µmoles of pyruvate formed / mg of protein/hr)	66.25±1.12	124.18±1.72 (87.44) 28.22*
Alanine aminotransferase(ALT) (µmoles of pyruvate formed / mg of protein/hr)	46.50±0.87	58.15±0.65 49.78 (10.72)*

Values are expressed as mean ± SE of six observations; Values in parenthesis are the change percent over control; *Indicate the significant at P<0.05. of 'T' test

DISCUSSION

The AST and ALT are the enzymes of amino acid metabolism that link amino acids to intermediates of pathways involved in energy generation particularly the TCA cycle. It is thus expected that increased energy demand, as a result of stress, will result in mobilization of potential energy sources including amino acid resulting in the activation or induction of enzymes of aminoacid metabolism (Masola *et al.,* 2008). AST and ALT are the enzyme s frequently used in diagnosis of damage caused by pollutants in various tissue (De La Torre *et al.,* 2000). The transaminases GOT and GPT (entering the blood after the cell necrosis of certain organs) can be used to indicate the tissue damage of the liver and kidney (Nemcso and Boross, 1982). Alterations in the activity of alanine and aspartate transminase enzymes will be reflected on the energy yielding TCA cycle and nitrogen metabolism. They also influence the gluconeogenic process and any change in the transaminase activity can be correlated with the protein and carbohydrate metabolism and thereby help in analysing the metabolic shifts (Beyer *et al.,* 1996).

In the present study, the level of AST and ALT activity increased in liver tissue *Cirrhinus mrigala* exposed to sub lethal concentration of Nickel chloride for 30 days. The result may be due to necrosis of hepatocyte which causes increase in the permeability of cell membrane resulting in the damage of tissues. Similar results were reported by Hwang and Wang, (2001) and suggested that the level of AST and ALT activities were increased due to heavy metals in toxication on liver. The activity of AST and ALT can be used to indicate the tissue damage of liver (Nemsoc and Boross, 1982). Alteration in the activity of AST and ALT will be reflected nitrogen metabolism on the energy yielding TCA cycle (Beyer *et al*., 1996). The transaminases GOT and GPT (entering the blood after the cell necrosis of organs) can be used to indicate the tissue damage of the liver and kidney (Nemcso and Boross, 1982). Alterations in the activity of alanine and aspartate transminase enzymes will be reflected on the energy yielding TCA cycle and nitrogen metabolism. They also influence the gluconeogenic process and any change in the transaminase activity can be correlated with the protein and carbohydrate metabolism and thereby help in analysing the metabolic shifts in the teleost fish *anabas*

testiudineus treatment with thiouracil (Beyer *et al.,* 1996). The similar increased activities of AST and ALT enzymes were reported, (Padmaja Nair and Oommen, 1998).

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 elevated level of AST and ALT indicated that the feeding of amino acids into the TCA cycle occurs in order to cope up the energy crisis during toxicity (Philip *et al*., 1995). The significant increase of these enzymes 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. Mukhopadhyay *et al*., (1982) have observed that an increased 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 enzymes increased in the gill, liver and kidney tissues of *Anabas testudineus* exposed to lindene, and furandan. Karan *et al.* (1998) reported that increased activities of AST and ALT have been observed in gill tissue of Cyprinus carpio exposed to copper sulphate.. The increased activities of AST and ALT in fish may thereby enhanced transamination for the channeling of free amino acid into TCA cycle and /or to favour glucogenesis (Jurss and Bastrop, 1995). Similar increased AST and ALT have been found under different forms of cadmium in heart gill and liver tissues of *Mugil cephalus* (Hilmy *et al.,* 1985). MacInnes et al., (1977) reported that increased activities of AST and ALT which may therefore increased in order to counter the energy crisis during stress. They GOT and GPT activity were increased in heart, liver and kidney of *Cirrhinus mrigala* when exposed to sublethal concentration of cypermethrin (Sivakumari *et al.,* 1997). Bakthavathsalam (1980) has studied the transaminase activity in six selected tissues of gill, liver, brain, kidney, intestine and muscle of *Anabas testudineus* exposed to Lindane, Disyston and Furadan for different periods. The present study concludes that the sublethal concentration of nickel break down transaminase activities in the liver tissue of *Cirrhinus mrigala.* The alteration in these enzymes suggests an increased participation of protein in the energy metabolism in response to an increased energy demand to cope with stress situation.

Acknowledgement

The authors are thankful to the Professor and Head, Department of Zoology, Annamalai university for providing necessary facilities to carry out the work successfully.

REFERENCES

Aas, E., Beyer, J., Jonsson, G., Reichert, W.L. and Andersen, O.K. 2001. Evidence of uptake, biotransformation and DNA binding of polyaromatic hydrocarbons in Atlantic cod and corkwing wrasse caught in the vicinity of an aluminum works. *Marine Environmental Research*, 52: 213–229.

Bakthavathasalam, R. 1980. Toxicity and Physiological impact of three selected pesticides on air breathing fish, Anabas testudineas (Bloch.) Ph.D. Thesis Annamalai University.

Battacharya, S. and Mukherjee, M. 1976. Ultrastructural changes in hepatocytes of juvenile rainbow trout and mature brown trout exposed to copper and zinc. *Environ. Toxicol. Chem.,* 2: 353-368.

Beger, J., M. Sandvik, K. Hylland, E. Field, E. Eggas, E. Aas, J.U Skare and A. Goksoyr, 1996. Contaminant accumulation and biomarker responses in flounder *(Plattichthys flesus L.)* and atlantic Cod (*Cadus morhua* L.) exposed by caging to polluted sediments in sorfjorden. *Norway Aquat. Toxicol.,* 36 : 75 . 98.

Casilla, E., Meyers, M. and Ames, W. 1983. Relationship of serum chemistry values to liver and kidney histopathology in English sole *(Parophyrys vetalus)* after acute exposure to carbon tetrachloride. *Aquat Toxicol* 3: 61-78.

Chau, Y.K. and Kulikovsky-Cordeiro, O.T.R. 1995. Occurence of nickel in the Canadian environment. *Environ. Rev.* 3, 95–120.

Clark D S, Keasling J D, 2002. Nickel accumulation and nickel oxalate precipitation by *Aspergillus niger*. *Applied Microbiology and Biotechnology*, 59: 382–388.

Dallinger, R. and Kautzky, H. 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): a field study. *Oecologia,* 67: 82–89.

Dautremepuits, C., Paris-palacios, S., Betoulle, S. and Vernet, G. 2004 Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. *Comp Biochem Physiol.,* 137: 325-33.

De La Torre, F.R., Salibian, A. and Ferrari, L. 2000. Biomarker assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. *Environ. Pollut*., 109:277-282.

De La Torre, F.R., Salibian, A. and Ferrari, L. 2000. Biomarker assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. *Environ. Pollut*., 109:277-282.

Ganguli, G., Medda, S.K., Sarkar, C. and Basu, T.K. 1997. Acute toxic effect of sublethal phosphomidon exposure on *Anabas testudineus Ind. J. Fish*., 44:211-215.

Hilmy, A.M., Shabana, M.B. and Daabees, A.Y. 1985. Effect of cadmium toxicity upon the in vitro and in vivo activity of protein and five enzymes in blood serum and tissue homogenates of Mugil cephalus. *Comp. Biochem. Physiol*., 81:145-153.

Hwang, D.F. and Wang, L.C. 2001. Effect of taurine on toxicity of cadmium in rats. *Toxicology*. 167 : 173-180.

Jurss, K. and Bastrop, R. 1995. Amino acid metamolism in fish. In 'Biochemistry and Molecular biology of fishes: Analytical techniques' (P.W. Hochachka and T.P. Mommsen, Eds.). 3: pp 159-189. Elsevier, Amsterdam.

Karan, V., Vitrorovic, S., Tutundzvic, V. and Poleksic, V. 1998. Functional emzyme activity and gill histology of carp after copper exposure and recovery. *Ecotoxicology and Environmental safety,* 40:49-55.

Khandelwal, S., Shukla, L.J. and Shanker, R. 2002. Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat. *Ind. J.Exp. Biol.* 40: 564-570.

King, J. 1965. In: Practical clinical enzymology var Nortant, D. Company. London. 106-107.

Masola, M., Chibi, M., Kandare, E., Naik, Y.S. and Zaranyika, M.F. 2008. Potential marker enzyme and metal- metal interactions in *Helisoma duryi* and *Lymnaea natalensis* exposed to cadmium. *Ecology and Environmental safety*, 70:79-87

Mondon, J A, Duda, S. and Nowak, B F, 2001. Histological, growth and 7-ethoxyresorufin *O*-deethylase (EROD) activity responsesof greenback flounder *Rhombosolea tapirina* to contaminated marine sediment and diet. *Aquatic Toxicology*, 54:231–247.

MucInnes, J.R., Thurberg, F.P., Greig, R.A. and Gould, E. 1977. Long-term cadmium stressin the cunner *Tautogolabrus adspersus.* Fish B-NOAA. 75:199-203.

Mukhopadhyay, P.K., A.P. Mukherjee and P.V. Dehadrai, 1982. Certain biochemical responses in the air-breathing catfish clarias batrachus exposed to sublethal carbofuran. *Toxicology,* 23: 337-345.

Nanda, P. and Behera, M.K. 1996. Nickel induced changes in some haemato biochemical parameters of cat fish *Heteropneustes fossilis* (Bloch.), *Environ. Ecol*., 14 : 82–85.

Nemscok, J. and Boross, T. 1982. Effect of sublethal concentration of phenol on some enzyme activities and blood sugar level of carp *Cyprinus corpio. Environ. Monit Assess.,* 14: 377-383.

Padmaja Nair, N and Oommen, O.V. 1998. Effect of TRH and TSH on hepatic intermediarg metabolism in normal and thiouraciltreatedteleost, *Anabas testudineas* (Bloch.) *Indian .J. Exp. Biol*., 36: 470 - 475.

Palaniappan, PL. and Karthikeyan, S. 2009. Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of reshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. *Journal of Environmental,* 21: 229–236.

Philip, G.H., Reddy, M.P. and Sridevi, G. 1995. Cypermethrin induced in vivo alterations in the carbohydrate metabolism of freshwater fish,

Labeo rohita. Ecotoxicol. Environ. Saf., 31: 173-178.

Rana, S.V.S., Rekja, S. and Seema, V. 1996. Protective effects of few antioxidants in liver function in rats treated with cadmium and mercury. *Ind.J.Exp. Biol.,* 34: 177-179.

Sharma, B. 1999. Effect of carbaryl on some biochemical constituents of the blood and liver of *c. puntatus*, a fresh water teleost. *J. Toxicol. Sci*., 24:157-164.

Sivakumari, K., Manavalaramanujam, R., Ramesh, M. and Lakshmi, R. 1997. Cypermethirin toxicity sublethal effects on enzyme activities in a freshwater fish, *cyprinus carpio* var. Communis *J. Environ. Biol*., 18 (2): 121 – 125.

Tjalve, H., Gottofrey, J. and Borg, K., 1988. Bioaccumulation, distribution and retention of 63Ni2+ in the brown trout (*Salmo trutta*). *Wat. Res*., 22, 1129–1136.

Torres, P., Tort, L. and Fish, R. 1987. Acute toxicity of copper to mediterranear dog fish. *Comp Biochem Physiol.,* 86: 169-171.

Tulasi, S.J., Reddy, P.U.M. and Ramano Rao, J.V. 1992. Effects of lead on the spawning potential of the fresh water fish, *Anabas testudineus*. *Bull. Environ. Contam. Toxicol.,* 43: 858-863.

****