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EFFECT OF ACUTE FLUORIDE TOXICITY ON LIVER ENZYMES IN FRESHWATER FISH, *RITA RITA*

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Abstract: Sodium fluoride, a metal and environmental factor found in freshwater bodies, can alter enzymatic parameters and impair a variety of physiological and metabolic processes in fish. The primary goal of this research was to determine the effect of sodium fluoride toxicity on freshwater fish Rita rita's biochemical content, specifically the levels of liver enzymes like aspartate aminotransferase (AST) and alanine transaminase (ALT). Toxic effects of sodium fluoride on freshwater fish *Rita Rita,* displayed linearly elevated AST and ALP levels. This investigation suggests that serum enzymatic parameters may serve as useful indicators for evaluating biomarkers in sodium fluoride induced eco-toxicological research.

Index Terms - effect, sodium fluoride, liver, enzymes, Rita rita

I. INTRODUCTION

Enzymes control various metabolic processes, which are biochemical macromolecules, hence even if there is a slight variations in surrounding environment would affect the organism. Shivaknmar (2005) reported that the activity of aspartate and alanine amino transferases (AST and ALT), may serve as strategic links between protein and carbohydrate metabolisms. Evaluation of enzyme activities in the tissue and organs of aquatic organs in the diagnosis of the effects of pollutants is one of the emerging areas in toxicological monitoring and remediation programmes (Oluah *et al.,* 2005). Metabolic enzyme can also serve as good indicator of intoxication because of its sensitivity to metallic salts (Boge *et al.,* 1992, and Rajamannar and Manohar , 1998).

Fish growth is affected by physicochemical environment such as temperature, light and water quality in general health problem of fishes (Neelam Singh and Madhu Tripathi , 2015). The fluoride minerals, fluoride-rich minerals in the rocks and soils getting their final way into the water bodies are the main cause of high fluoride contents in water bodies. Anthropogenic sources of fluoride are industries, cosmetics and various products of day to day use (Neuhold and Sigler, 1960). Chitra *et al.,* 1983 and Kumar *et al.,* 2007 observed that fluoride affects the certain biomolecules and enzymes in different tissue of fresh water fishes. Fluoride mainly affects the level of glucose, lipid, protein, cholesterol and glycogen, all of which play an important role for growth, reproduction and survival of fishes. The first major natural source of inorganic fluorides is the weathering of fluoride minerals (CEPA, 1994). Most important inorganic fluoride minerals in the earth's crust are fluorapatite (Ca5(PO4)3F), fluorite (CaF2) and cryolite (Na3ALF6). Volcanoes are the second major natural source through the release of gases with hydrogen fluoride (HF) into the atmosphere (CEPA, 1994). Human activities, such as aluminium smelters, discharges of fluoridated municipal waters, and plants manufacturing brick, ceramics, glass and fluoride chemicals, may cause significant increases in the fluoride concentration of surface waters (Wright and Davison, 1975; Pankhurst *et al.,* 1980).

The purpose of this investigation is to study effect of sodium fluoride on enzyme activities in liver of freshwater fish, *Rita rita*.

II. MATERIALS AND METHODS

The freshwater fishes, *Rita rita* (weight 25-30g) were collected from Bhima river from Solapur district. Fishes were maintained in aerated glass aquarium and acclimatized for two weeks in laboratory conditions. The static bioassay test was performed by using sodium fluoride to determine LC_0, LC_{10} and LC_{50} values. For this investigation fishes were divided into two groups i.e. control group and experimental group. Experimental fishes were exposed to acute concentration of sodium fluoride for 24, 48, 72 and 96 hours. Fishes from both the groups were sacrificed after 24, 48, 72 and 96 hours to observe levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) from liver. Liver tissue homogenized in 5mL physiological saline solution and centrifuge at 3000 rpm for 10 minutes and the supernatant was collected for enzyme analysis. Supernatants were analyzed to determine the enzymes (AST and ALT) levels by using the methods described by Reitman and Frankel (1957).

III. RESULTS AND DISCUSSION

Table No. 1. AST enzyme content in liver of freshwater fish, *Rita rita* after acute exposure to sodium fluoride

Exposure time in Hr.	Control	LC_0	LC_{10}	LC_{50}
24	29.43±0.085	29.50±0.040(0.23)	30.43±0.023(3.39)*	30.62±0.038(4.04)*
48	31.52±0.021	31.71±0.015(0.60)	32.88±0.021(4.31)*	33.06±0.051(4.88)*
72	32.67±0.093	32.71±0.017(0.12)	33.24±0.023(1.74)	34.80±0.032(6.51)**
96	34.00±0.020	35.05±0.028(3.08)*	36.50±0.010(7.35)**	37.15±0.026(9.26)***

Values are significant at * = P <0.05, ** = P <0.01, *** = P< 0.001

Bracket values indicates percent change when compared with control.

The amount of an enzyme AST in liver in respective control group was 29.43±0.085 U/l, in acute treatment after 24 hour it was 29.50±0.040 U/l in LC_0 group, 30.43±0.023 U/l in LC_{10} group and 30.62±0.038 U/l in LC_{50} group. The amount of an enzyme AST in liver in respective control group was 31.52±0.021 U/l, in acute treatment after 48 hour it was 31.71±0.015 U/l in LC_0 group, 32.88±0.021U/l in LC_{10} group and 33.06±0.051 U/l in LC_{50} group. The amount of an enzyme AST in liver in respective control group was 32.67±0.093 U/l, in acute treatment after 72 hour it was 32.71±0.017 U/l in LC_0 group, 33.24±0.023 U/l in LC_{10} group and 34.80±0.032 U/l in LC_{50} group. The amount of an enzyme AST in liver in respective control group was 34.00±0.020 U/l, in acute treatment after 96 hour it was 36.05±0.028 U/l in LC_0 group, 36.50±0.010 U/l in LC_{10} group and 37.15±0.026 U/l in LC_{50} group.

In acute treatment when compared with control after 24 hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme AST content and it was 0.23% , 3.39% ($P<0.05$) and 4.04% ($P<0.05$) respectively. In acute treatment when compared with control after 48 hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme AST content and it was 0.60% , 4.31% ($P<0.05$) and 4.88% ($P<0.05$) respectively. In acute treatment when compared with control after 72 hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme AST content and it was 0.12%, 1.74% and 6.51% ($P<0.01$) respectively. Similarly, in acute treatment when compared with control after 96hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme AST content and it was 3.08% ($P<0.05$), , 7.35% ($P<0.01$) and 9.26% ($P<0.001$) respectively.

Table No. 2. ALT enzyme content in liver freshwater fish, *Rita rita* after acute exposure to sodium fluoride

Exposure time in Hr.	Control	LC_0	LC_{10}	LC_{50}
24	30.12±0.015	31.54±0.072 (1.39)	31.82±0.020 (2.29)*	31.94±0.055(2.72)*
48	30.26±0.055	31.88±0.055(1.02)	32.04±0.017(2.57)*	33.44±0.023(3.89)*
72	30.36±0.018	30.07±0.046(1.15)	32.91±0.010(2.86)*	35.26±0.021(4.15)**
96	30.43±0.020	33.05±0.023(2.04)*	35.08±0.010(6.17)**	37.80±0.055(7.78)***

Values are significant at * = P <0.05, ** = P <0.01, *** = P< 0.001

Bracket values indicates percent change when compared with control.

The amount of an enzyme ALT in liver in respective control group was 30.12±0.015 U/l, in acute treatment after 24 hour it was 31.54±0.072 U/l in LC_0 group, 31.82±0.020 U/l in LC_{10} group and 31.94±0.055 U/l in LC_{50} group. The amount of an enzyme ALT in liver in respective control group was 30.26±0.055 U/l, in acute treatment after 48 hour it was 31.88±0.055 U/l in LC_0 group, 32.04±0.017 U/l in LC_{10} group and 33.44±0.023 U/l in LC_{50} group. The amount of an enzyme ALT in liver in respective control group was 30.36±0.018 U/l, in acute treatment after 72 hour it was 30.07±0.046 U/l in LC_0 group, 32.91±0.010 U/l in LC_{10} group and 35.26±0.021 U/l in LC_{50} group. The amount of an enzyme ALT in liver in respective control group was 30.43±0.020 U/l, in acute treatment after 96 hour it was 33.05±0.023 U/l in LC_0 group, 35.08±0.010 U/l in LC_{10} group and 37.80±0.055 U/l in LC_{50} group.

In acute treatment when compared with control after 24 hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme ALT content and it was 1.39% , 2.29% ($P<0.05$) and 2.72% ($P<0.05$) respectively. In acute treatment when compared with control after 48 hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme ALT content and it was 1.02%, 2.57% ($P<0.05$) and 3.89% ($P<0.05$) respectively. In acute treatment when compared with control after 72 hour exposure to LC_0, LC_{10} and LC^{50} group there was significant increase in the an enzyme ALT content and it was 1.15%, 2.86% ($P<0.05$) and 4.15% ($P<0.01$) respectively. Similarly, in acute treatment when compared with control after 96hour exposure to LC_0, LC_{10} and LC_{50} group there was significant increase in the an enzyme ALT content and it was 2.04 % ($P<0.05$), 6.17% ($P<0.01$), 7.78% ($P<0.001$) respectively.

When freshwater organisms exposed to high concentration of fluoride its harmful effects on alteration in carbohydrate, lipid and protein metabolism are commonly observed. Dausset *et al.*, (1987); Gikunju *et. al*, (1992) have reported that fluoride increase the cholesterol level in liver, muscle, testis of fishes. Aziz *et al.*, 2013 have found that fluoride increased alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) level in gills of fresh water fish . Jha.A.N. (2004) reported that DNA and cytogenetic alterations in aquatic organisms impaired enzyme function or general metabolism, abnormal development, cytotoxicity, immunotoxicity, reduced survival, growth and reproduction potency. Alamine amino transferase (ALT) and aspartate amino transferase (AST) are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes and can be assessed within a shorter time (Balint *et al.*, 1997). In this study, there were increased values of AST and ALT activity in liver . This elevated in the liver in order to cope with the increasing energy demand during stress condition. The increasing energy demand is to fulfill the demand through amino-acids.

Yildirim *et al.*, (2006) exposed Oreochromis niloticus to deltamithrin for four days and observed increase in enzyme activities (AST and ALT) in the gill, liver and kidney and assumed that the observed (enzyme elevation) is intended to increase the role of proteins in the energy production during toxicant stress. Das *et al.*, (2004) showed that there was an elevation in activity level of AST, ALT and ATP of Indian major carps exposed to nitrite toxicity and suggested that the elevation of the transferases is as a result of the diversion of the alphamino acids in the TCA cycle as keto acids to augument energy production.

In the present investigation reported that changes in enzyme activity were observed with all concentrations and exposure period. ALT and AST concentrations were significantly higher in the experimental group, compared with the control group. Elevated levels of liver AST and ALT enzymes after acute exposure of sodium fluoride causes liver disorders. These enzymes which are the biomarkers of acute liver injury and can also be used as a diagnostic method to determine liver cell necrosis.

The present results show that sodium fluoride induced alterations in liver AST and ALT c and they point to disrupt activity enzymes has shown significant elevation in liver after lethal exposure (Tables 1 to 2). Awasthi *et al.,* (1984) proposed that stress conditions in general induce elevation in the transamination pathway. A progressive increase was noticed in the activities of ALT, AST with increase in time and dose concentration. It can be concluded that sodium fluoride causes considerable alterations in enzymes activities in liver tissue damage in freshwater fish, *Rita rita*.

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