International Research Journal of Environment Sciences ISSN 2319–1414
Vol. **2(8),** 24-27, August **(2013)** *Int. Res. J. Environment Sci.*

Effect of Fluoride Exposure on Key Enzymes Activity of Protein - Carbohydrate Metabolism in Gills of Fresh Water Fish *Tilapia mossambica*, Keenjhar Lake, Thatta, Sindh, Pakistan

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Available online at: www.isca.in
Received 18th June 2013, revised 30th June 2013, accepted 17th August 2013

Abstract

The effect of fluoride on the activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) in gills of fresh water fish Tilapia mossambica, collected from Keenjhar Lake, Thatta, Sindh, Pakistan at sub-lethal concentration of fluoride was measured. Fluoride is a very common non-metallic trace element found in earth's crust. It is abundant in our environment but is rarely found in elementary state in nature. Fluoride is known as an inhibitor of various enzymes like lipases, phosphatases and esterases. It disrupts fatty acid oxidation and also inhibits the enzyme activity of acyl-Co-A synthetase, involved in lipid metabolism. Results showed that enzyme activities were significantly altered upon exposure to fluoride due to which protein-carbohydrate metabolism was disturb. Changes in three biomarkers, key enzymes of protein-carbohydrate were related to metabolism of fish at sub-lethal concentration of fluoride. The carbohydrate concentration initially increases and later decreases with the time while a significant depletion of total protein and lipids in gills tissue were observed ($p < 0.001$). It was concluded that fluoride produce toxic effect on physiology of fish gill, which may be associated with increased ionic permeability to gill surface.

Keywords: Fluoride, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), protein-carbohydrate metabolism, gills.

Introduction

Fluoride is a permanent bio accumulator and potent toxic element to living organism. Prolonged lethal exposure to fluoride (F^-) may cause local tissue disorders, known as fluorosis. Under the Canadian Environmental Protection Act in 1993, inorganic fluorides were found to be poisonous or lethal to aquatic and terrestrial ecosystems due to their possible long-term harmful effects[1].

Fluoride has anaffinity to be permanently accumulated in exoskeleton of invertebrates, especially in bone, tooth, and scales of fishes. Factors affecting accumulation are species, strain, fluoride concentration, temperature, the duration of exposure, water hardness and decreases with increasing intra specific body size and water content of calcium and chloride[2]. Those parts of the fish directly in contact with the water, such as scales, fins and gills, have the highest fluoride contents due to ionic permeabilty[3]. The chloride and calcium content of the water may also affect fluoride toxicity[4]. Fish are extremely sensitive to many water-borne toxicants, because these affect the gills by increasing the permeability to water and other ions of the gill epithelium and by inhibition of the ion exchange activity of the chloride cells[5]. Alteration in enzyme activity of liver and muscle were reported by Chitra *et al.*[6] in *Channa punctatus*, whereas Gupta[7] has shown fluoride decreased glucose and protein levels in blood and muscles of *Channa punctatus* fish. The increased cholesterol content observed in muscle, liver and testis of the fish exposed to fluoride is reported by several workers, who have observed fluoride induced cholesterol production in animals[8-10]. Strochkova etal.[11] reported the high glycogen level in the experimental fish under fluoride stress may be due to disturbance of carbohydrate metabolism.

This present work is aimed to study the effect of fluoride on the on key enzymes activity of protein - carbohydrate metabolism in gills of fresh water fish *Tilapia massambica*from Keenjhar Lake, Thatta, Sindh, Pakistan at different exposure periods. As gills are the respiratory organs of the fish and the site for gaseous exchange and any morphological abnormality will lead to improper functioning of many organs/system of the fish.

Material and Methods

Fish collection: Healthy living *Tilapia mossambica* (average weight 94.5g and standard length 9cm) were collected from the Fish Farming Area of Keenjhar Lake, Sindh in March 2012 showed in figure 1 and 2. Temperature of lake was 30°C. Humidity was 70%. Fish were caught with the help of local fisherman by using fishing nets and motor boat shown in figure 3.

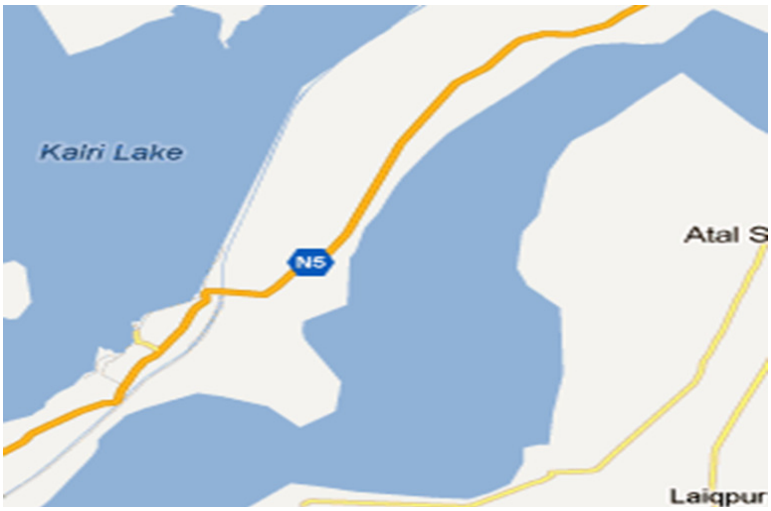

Figure-1
Site map of Keenjhar Lake, Thatta, Sindh, Pakistan

Figure-2
Fish collection area at Keenjhar Lake, Thatta, Sindh, Pakistan

Figure-3
Fish were caught with the help of professional local fisherman

Fish Acclimation: Fish were transported to laboratory under ordinary conditions. Fish were dividing in two groups, each contain ten fish and placed in a fiber glass aquarium, size: 36cm x 18cm x 15cm containing tap water. Air pumps and filters were used to aerating the aquarium water by circulating it. All control and treated fish were feed with commercial pellet once a day. Water in aquaria was changed after two days. Chemical analysis of water was done according to standard methods. Group 1 serves as non-treated while group II served as experimental group. Group II was treated with sub-lethal concentration of fluoride (1.5gm NaF / 70 L of water).

Biochemical Analysis: Both control and treated fish were dissected and gills of non-treated and treated were removed and homogenized in a glass homogenizer (Kontex, USA) in cold saline (0.89% NaCl) solution. Homogenized tissues were centrifuged in a refrigerated cold centrifuge (Bencthtop centrifuge, Hettich) to get clear supernatant and used for the quantitative determination of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Enzymatic assays were analyzed by UV- Visible Spectrophotometer (Jenway, 6315) with standardized Randox Kits, (UK). Three key enzymes alkaline phosphatase (ALP) by p-nitro phenol method, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) by Kit methods. Estimation of glucose by GOP-PAP method [12]. Estimation of total protein by Lowry *etal,* method[13]. Estimation of total lipids by sulpho-phospho-vanilline (SPV) method[14].

Statistical Analysis: The results are represented as Mean ± S.E.M. The reported data was statistically analyzed by paired student t-test at 95% confidence interval of the difference to determine the level of significance. P values ≤ 0.05 were considered significant.

Results and Discussion

The activities of key enzymes of protein-carbohydrate metabolism like alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured in gills, at 1st, 2nd, 3rd and 4th week. The results are presented in table 1 showed that changes occur in the enzymes activity of tissues under studied. ALP, ALT and AST activities in gills, were significantly increased after 1st, 2nd, 3rd and 4th week as compared to control fish ($p < 0.001$). These alterations in enzymes activities may be associated with the gills structure and functioning, affected by fluoride in the environment. Structural damage to the gills epithelium results in the failure of gill cellular osmoregulation[5, 15]as revealed in this present study and other reported work.

Transaminases play important role in carbohydrate - protein metabolism involved in the inter conversion of amino acid to α–ketoacid then enters into citric acid cycle and ultimately produce effect on metabolic pathways. During this process alpha keto acids converted into amino acids may be consumed in the protein synthesis, thus regulating the carbohydrate and protein metabolisms[16,17]. Alkaline phosphatase (ALP) belongs to hydrolase enzyme carried dephosphorylation, removing phosphate groups from various types of molecules, including nucleotides, proteins, and alkaloids. Elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity shows the utilization of amino acid in protein synthesis. Fluoride may cause increase in the activities of Alanine and Aspartate transferases as reported earlier[18] in Wiston rats. A similar increase in the activities of AST/GOT and ALT/GPT in the liver and the kidney of mice was also reported[19] and in humans in a common areas of fluorosis[20]. Similar results were also found on the liver and muscle of fresh water fish*Channa punctatus* exposed to sodium fluoride 6 and in fresh water Crab, *Barytelphusa guerini*[21]. These findings revealsignificant support to the present study.

The quantitative measurements of enzymes ALP, AST and ALT, in gills tissue showed alteration in protein carbohydrate metabolism due to which carbohydrate concentration found to be increased ($p < 0.001$) in table 2 which may be due to the reduced oxidation of glucose while decreased in protein concentration may be related to produce the energy demand to overcome the stress for survival of fish [22]. This is supported by the facts that when an organ is directly exposed to toxicants, enzymes activity may be increased or decreased due to denaturation of active sites[3]. Subsequently specific enzymes catalyze some stages in the metabolism of carbohydrate and proteins in most tissues and their increased or decreased may be sufficient to provide information of fish health[23]. Decreased lipid content in several tissues may be due to the inhibition of enzyme acyl co-A synthetase activity. These valuable measurements allowed us to state that the enzymatic study is excellent biomarkers of environmental condition and fulfill the criterion of being statistically significant after accounting for multiple assessments tables 1 and 2. Furthermore the specific activities of enzymes of protein-carbohydrate metabolism were consistently increased after long-term exposure to fluoride concentration, revealed an increased ability for carbohydrate metabolism in this tissue.

Table-1
Effect of fluoride (1.5g/70L) on enzyme activity of ALP, AST and ALT in gills of fresh water fish *Tilapia mossambica* at 1st, 2nd, 3rd and 4th; Temp 30ºC, pH= 7.8

Enzyme Activity(U/L)	Control	1st week	2nd week	3rd week	4th week
ALP	356.058±0.006	907.6±0.101	890.662±0.046	867.797±0.084	823.765±0.05
AST	601.408±0.070	905.332±0.142	875.506±0.086	873.688±0.051	865.284±0.01
ALT	883.4±0.106	966.6±0.063	948.3±0.148	935.1±0.064	928.453±0.03

Values expressed as Mean ± S.E.M; represent highly significant ($p<0.001$) compared with control

Table-2
Effect of fluoride (1.5g/70L) on Glucose, Protein, Lipid and in gills of fresh water fish *Tilapia massombica* at 1st, 2nd, 3rd and 4th week; temp. 30ºC, pH= 7.8

Biochemical constituents(mg/g)	Control	1st week	2nd week	3rd week	4th week
Glucose	46.013±0.005	48.137±0.005	47.343±0.006	44.323±0.010	41.763±0.003
Protein	5.195±0.038	4.321±0.005	3.414±0.003	3.108±0.046	2.98±0.026
Lipid	647.166±0.009	484.13±0.012	462.412±0.098	430.425±0.126	398±0.056

Values expressed as Mean ± S.E.M; represent highly significant ($p<0.001$) compared with control

Conclusion

Fluoride is very mobile inorganic pollutant and its presence in environment is directly poisonous to aquatic life, stored in the various tissues, at different concentrations where absorption rates exceed emission rates. This study clearly reveal that permeability in gills tissue due to fluoride exposure result alteration in enzymes activities which may cause detritus changes with disturb tissue metabolism and structure at respiratory level that finally suppress the absorption of oxygen in gills tissue results in death of fish at long period of exposure time.

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