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Enzymatic Study of Gills of Fish as Good Biomarkers of Environmental State of Fluoride Pressure

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The effect of fluoride on the activity level of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase in gills at two sublethel concentration of fluoride was assessed. Fluoride has long been recognized to influence the activity of various enzymes and send false messages, which are amplified by processes of signal transduction. Results showed that enzymes activities were significantly altered due to which protein carbohydrate metabolism was disturb. Changes in three biomarkers of key enzymes of protein carbohydrate were related to metabolism of fish at both concentrations of fluoride. The carbohydrate concentration initially decreases which later on increases at both concentration with the time. Non-significant depletion of total protein in gills tissue was observed ($p < 05$). It was concluded that fluoride do produce toxic effect on physiology of fish gill, which may be associated with increased ionic permeability.

Key Words: Fluoride, Gill, Enzymes, Protein carbohydrate metabolism.

INTRODUCTION

Fluoride has a tendency to accumulate in organisms, making adverse effects possible even at very low levels of exposure. A measure of a metabolism may be a most sensitive parameter such as enzyme activity or physiological response. Acetylcholinesterase (E.C.3.1.I.7) is regarded as biochemical marker to assess the complex effect of a pollutant like fluoride[1]. Fish are extremely sensitive to many water-borne toxicants, because these affect the gills by increasing the permeability to water and ions of the gill epithelium and by inhibition of the ion exchange activity of the chloride cells[2]. Alteration in enzyme activity of liver and muscle were reported by Chitra *et al.*[3] in *Channa punctatus*, whereas Gupta[4] has observed that fluoride decreased glucose and protein levels in blood and in 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[5-7]. Strochkova *et al.*[8] reported the increased glycogen level in the fish exposed to the higher level of fluoride may be due to disturbance of carbohydrate metabolism.

Hence the present study is aimed to evaluate the toxic potentiality of sublethal concentration of sodium fluoride on key enzymes activity of protein carbohydrate metabolism in tissues of fish like gills. Time-course alterations in gills of *Notopterus notopterus* in enzymes activity with protein carbohydrate were discussed.

EXPERIMENTAL

Collection of fish: Healthy living *Notopterus notopterus* (average weight 53.8 g and standard length 8-13 cm) were collected from Kanjher lake Sindh Pakistan in March 4th, 2008 (temperature 18 ºC, humidity 70 %) in early morning. Fishes were transferred to laboratory under ordinary conditions and maintained under standard laboratory conditions for 20 days. Fishes were placed in glass aquarium containing tap water, size: 36 cm × 18 cm × 15 cm. The aquarium water was changed on alternate days and a fresh dose of F was supplemented after feeding.

Chemical analysis of water was done according to standard methods. The fish were divided into three groups with 15 fishes per group. Group 1 serves as control while group 2 and 3 served as experimental groups. Group 2 were treated with sub-lethal concentration of fluoride 1.5 g (low concentration) and group 3 was treated with lethal concentration of fluoride 3 g (high concentration), all control and treated fishes were feed with commercial pellet once a day. Both control and treated fishes were scarified and tissues were removed (muscles, liver and gills) after 24, 48, 72, 96 h and lastly 45 d. Tissues were blotted and then weighted. Homogenized tissues were centrifuged at 10,000 rpm for 15 min the supernatant was used for quantifying the enzymes by using commercial kits (Randox). UV-Visible spectrophotometer (Jenway) was used to measure the enzyme activity. Three key enzymes alkaline phosphatase (ALP) by *p*-nitro phenol method, glutamate-oxaloacetate transaminase

(GOT, AST) by Kit method and glutamate-pyruvate transaminase (GPT, ALT) by Kit method. Total protein, lipid and glycogen were estimated by standard methods given by Lowry *et al.*[9], Folch *et al.*[10] and Montgomery[11], respectively.

RESULTS AND DISCUSSION

The key enzymes of protein-carbohydrate metabolism activities like alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured in gills, while fish fed with protein diets at every 24 h. The results are presented in Figs 1-3, showed that changes occur in the enzymes activity of tissues under studied. Initially ALP, ALT and AST activities in gills, were increased after 24 h as compared to control fish whereas after 72 h, ALP significantly decreases at 3 g/ 70 L ($p < 0.001$)[12]. This is supported by the facts that when an organ is directly exposed to toxicants, enzymes activity may be increased or decreased due to active sites being either denatured or distorted. Since some enzymes catalyze, some steps in the metabolism of carbohydrate and proteins they are present in most tissues and their increased or decreased may be sufficient to provide information of diagnostic value[8] (Figs. 1-3). Aspartate aminotransferase enzymes activity in gills at both doses of fluoride were significantly ($p < 0.001$) decreased with the time of exposure (Figs. 1-3) as compared to non treated fish. While a significant decrease in the ALT ($p < 0.001$) was observed in the gills at high dose of fluoride as compared to control one and at low exposure of time. These alteration in enzymes activities may be related with the gills morphology and physiology, affected by fluoride and fluoride in the environment possibly associated with structural damage to the gills epithelium as observed in this study and other reported work[13]. These structural damages could merely be the reflections of generalized stress responses which can results in the failure of gill cellular osmoregulation[2,12]. The specific activities of enzymes of carbohydrate and protein metabolism were consistently depressed after long-term exposure to low oxygen, suggesting a decreased capacity for carbohydrate metabolism in this organ. The physiological study of fish gills develop a comprehensive picture of enzymatic activity of protein carbohydrate metabolism in gills tissues from a single fish species subjected to two sub lethal dose of fluoride concentration. The measurements of enzymes of ALT, ALP and AST in gills showed variation in protein carbohydrate metabolism due to which carbohydrate concentration found to be increased ($p < 0.001$) (Tables 1 and 2) which may be due to the decreased in oxidation of glucose while decreased in protein concentration may be related to produce the energy to overcome the stress for survival of fish. The increased glucose level in the fish exposed to the higher level of fluoride after 24 h may be due the reduction in absorption of oxygen in gills which perturb carbohydrate metabolism and inhibit the oxidation of carbohydrate by which energy is produced, used for the movement of fish. These results related with other studies[14] who reported that fluoride inhibits many glycolytic enzymes. Consequently the decrease in carbohydrate contents at lower level (1.5 g/70 L) at 24 h was reported (Tables 1 and 2) whereas increase in glucose contents supported by the facts that fluoride toxicity depend on time and higher concentration[8]

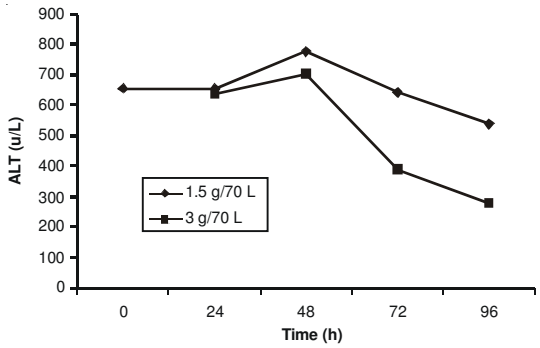

Fig. 1. Alanine aminotransferase enzymatic activity of gills of *N. notopterus* under fluoridation

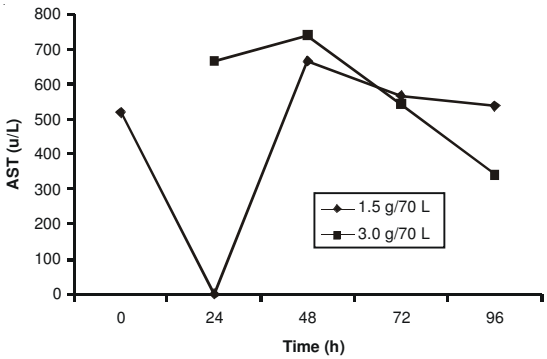

Fig. 2. Aspartate aminotransferase enzymatic activity of gills of *N. notopterus* under fluoridation

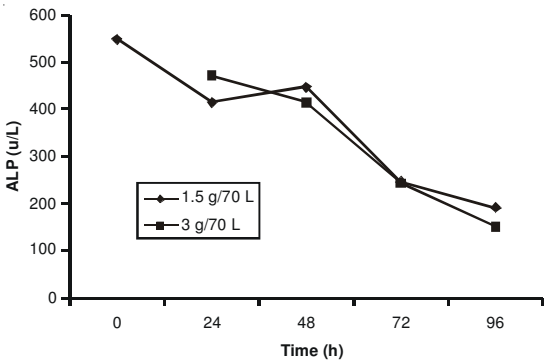

Fig. 3. Alkaline phosphatase enzymatic activity of gills of *N. notopterus* under fluoridation

but it is evident that accumulation of fluoride in the tissues suppress the oxygen absorption by which energy demand of fish cannot meet by oxidation of carbohydrate therefore concentration of carbohydrate increases with the time due to non-utilization of glucose[15]. The decrease in protein content of gills ($p < 0.05$) in fish under studied caused by fluoride as observed here is similar to the observation of Gupta *et al.*[4] on *Channa punctatus* after exposure to fluoride for 90 days. This decrease in protein may be due to inhibition of metabolism of amino acids thereby preventing cells from synthesizing protein or depletion may be of its utilization in energy production. The total lipid ($p < 0.001$) decrease in gills is similar to earlier work reported by Kumar *et al.*[14] that the in fluoride-exposed catfish the decrease may be due to inhibition of lipid synthesis by fluoride as well as increased utilization of stored lipids as a source of energy to conduct regular metabolic functions. Fluoride is well-known as an inhibitor of various enzymes

TABLE-1
PHYSIOLOGICAL PROCESSES OF GILLS OF *N. notopterus* LIKE GLUCOSE, PROTEIN AND LIPID ACTIVITY (mg/g) UNDER 1.5 g/70 L NaF

	Control	24 h	48 h	72 h	96 h
Glucose	67.1698 ± 0.978981 N = 10 SD = 3.095809 V = 9.584035	36.9809 ± 0.532913 N = 10 SD = 1.685219 V = 2.839964 p = < 0.001	69.8376 ± 0.257586 N = 10 SD = 0.814559 V = 0.663506 p = < 0.001	80.6638 ± 0.211503 N = 10 SD = 0.668831 V = 0.447335 p = < 0.001	141.12 N = 10 SD = 0.23401 V = 0.25 p < 0.001
Protein	4.2698 ± 0.149715 SD = 0.473441 V = 0.224147	4.5875 ± 0.055383 SD = 0.175136 V = 0.030673 p = 0.225122	3.5876 ± 0.10237 SD = 0.10237 V = 0.10237 p = < 0.001	4.9685 ± 0.211503 SD = 0.160985 V = 0.025916 p = < 0.001	2.91 ± 0.04521 SD = 0.03125 V = .0251 p = 0.001
Lipid	716.7078 ± 1.339381 N = 10 SD = 4.235495 V = 17.93941	486.1168 ± 0.86897 N = 10 SD = 2.747924 V = 7.551086 p = < 0.001	422.811 ± 0.372019 N = 10 SD = 1.176428 V = 1.383982 p = < 0.001	503.396 ± 0.464919 N = 10 SD = 1.470204 V = 2.161499 p = < 0.001	342.02 ± 0.4521 N = 10 SD = 0.956 V = 3.1.213 p < 0.001

TABLE-2
PHYSIOLOGICAL PROCESSES OF GILLS OF *N. notopterus* LIKE GLUCOSE, PROTEIN AND LIPID ACTIVITY (mg/g) UNDER 3.0 g/70 L NaF

	Control	24 h	48 h	72 h	96 h
Glucose	67.1698±0.978981 N = 10 SD = 3.095809 V = 9.584035	63.5933 ± 0.596031 N = 10 SD = 1.884814 V = 3.552525 p = < 0.001	77.1837 ± 0.479108 N = 10 SD = 1.515072 V = 2.295443 p = < 0.001	114.4201 ± 0.866977 N = 10 SD = 0.866977 V = 7.516489 p = < 0.001	121.12 N = 10 SD = 0.07801 V = 8.25 p < 0.001
Protein	4.2698±0.149715 SD = 0.473441 V = 0.224147 716.7078±1.339381	3.5636 ± 0.596031 N = 10 SD = 0.16742 V = 0.16742 p = < 0.001	4.4317 ± 0.053438 N = 10 SD = 0.168985 V = 0.028556 p = < 0.001	4.6518 ± 0.067628 N = 10 SD = 0.213859 V = 0.045736 p = < 0.001	3.21 ± 0.04521 N = 10 SD = 0.03125 V = .0251 p = 0.001
Lipid	N = 10 SD = 4.235495 V = 17.93941 67.1698±0.978981 N = 10	430.5586 N = 10 SD = 0.315225 V = 0.996829 p = < 0.001	419.187 ± 0.473828 N = 10 SD = 1.498375 V = 2.245126 p = < 0.001	367.0293 ± 0.826614 N = 10 SD = 0.826614 V = 6.832905 p = < 0.001	342.02 ± 0.8231 N = 10 SD = 0.085 V = 5.213 p < 0.001

like lipases, phosphatases and esterases. It interferes with fatty acid oxidation[1] and also inhibits the enzyme acyl-Co-Asynthetase involved in fatty acid oxidation[14]. Thus decreased lipid content in various tissues may be due to the inhibition of these enzymes. These measurements allowed us to address that the enzymatic study is a good biomarkers of environmental state and satisfy the criterion of being statistically significant after accounting for multiple comparisons (Tables 1 and 2). Moreover the specific activities of enzymes of protein carbohydrate metabolism were consistently depressed after long-term exposure to fluoride concentration, suggesting a decreased capability for carbohydrate metabolism in this tissue.

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