

# Alteration in Hepatic Enzyme Activity of *Tilapia mossambica* upon Exposure to Fluoride

Farha Aziz\*, Afshan Zeeshan Wasti and Farah Jabeen

Department of Biochemistry, Jinnah University for Women, Karachi, Pakistan

## ABSTRACT

Fluoride, as the super reactive element fluorine, is found naturally throughout earth's crust. It has been identified as a strong, persistent powerful cumulative toxic agent, commonly distributed in the rivers, lakes, seas of earth. It is highly mobile and biologically active element in aquatic systems. Fish are considered as the excellent and valuable bioindicator of ecosystem pollution. The present study was designed to estimate acute fluoride toxicity on enzyme activity of liver of freshwater fish *Tilapia mossambica*. The major enzymes of Carbohydrate - Protein Metabolic pathways are Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) in association with carbohydrate, protein, lipid in the liver of *Tilapia mossambica*, from Kalri Lake (Keenjhar Lake), Sindh, Pakistan at low amount of fluoride (sub-lethal) was estimated by using UV- Visible Spectrophotometer. Results showed ALP, AST and ALT enzymes present in the liver tissue were significantly changed ( $p < 0.001$ ). Finally, it is concluded that fluoride produces the adverse poisonous effect on liver functioning which may be associated with altered or elevated enzyme activity of protein-carbohydrate metabolism.

### Keywords:

Fluoride, Carbohydrate-Protein Metabolic pathways, Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), *Tilapia mossambica*, Liver, Keenjhar Lake.

### \*Address of Correspondence:

farahtariq2@hotmail.com, farah786star@yahoo.com

### Article info.

Received: April 18, 2018

Accepted: April 23, 2018

**Cite this article:** Aziz F, Wasti AZ, Jabeen F. Alteration in hepatic enzyme activity of *Tilapia mossambica* upon exposure to fluoride. *RADS J. Biol. Res. Appl. Sci.* 2018; 9(1): 30-34.

**Funding Source:** Nil

**Conflict of Interest:** Nil

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## INTRODUCTION

The liver is a chief central metabolic organ, essential for life in vertebrates and invertebrates. The liver plays a main role in the metabolism of carbohydrate, protein, amino acid, and lipid. It acts as a detoxifying organ which may accumulate bio-transform and excretes a variety of toxicants or their by-products<sup>1,2</sup>. Toxicants can produce disorders in the physiology of animal in association with altered enzyme activity. Several studies reported the changes in enzyme activity in the freshwater fish when exposed to various pollutants<sup>3-6</sup>. Fluoride is highly mobile and biologically active element in aquatic systems. Fish are considered as an excellent and valuable bioindicator of ecosystem pollution. A number of cellular and biological

processes including enzymatic reactions, cessation of protein secretion and synthesis, production of reactive oxygen species (ROS), and variation of gene expression can be affected by fluoride<sup>7,8</sup>. Fluoride as an anabolic agent or toxicant promotes cell proliferation and acts as an enzyme inhibitor for lipases, phosphatases and esterases, ATP production cycle and cellular respiration at the sublethal concentrations. Fluoride showed a significant alteration in enzymes activities of Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) in fish gills at the sublethal concentration of fluoride<sup>9,10</sup>.

## MATERIAL AND METHODS

Normal alive *Tilapia mossambica* (weight 90.2g and length 8.7cm) were collected from the Keenjhar Lake, with the help of local fisherman. Fish were divided into two groups under normal condition. Group I was considered as control (non-treated) while group II marked as experimental treated with 1.5 gm NaF / 70 L of water. Each group contains twelve fishes and acclimated in a glass aquarium, filled with tap water. Aquarium was aerated by using air pumps and filters. The Commercial pellet as the fish food was used for both groups (I and II) per a day. Aquarium water was changed after 2 or 3 days. Chemical study of water was performed according to approved procedures of APHA<sup>11</sup>.

### Study of biochemical constituents and enzymatic assays

Fishes of both groups (control and treated) were dissected and livers of all fishes were removed and cleaned with water. A glass homogenizer was used to homogenized tissues in cold solution of saline (0.89% NaCl) and centrifuged in a refrigerated laboratory centrifuge. A clear supernatant was used for Alkaline phosphatase (ALP) estimation by p-nitro phenol method However Aspartate transaminase (AST), Alanine transaminase (ALT) were measured by Randox Kit methods. Estimation of glucose was done by GOP-PAP method and protein by Lowry *et al.* method<sup>12,13</sup>. Estimation of total lipid by sulphophospho-vanilline (SPV)

method<sup>14</sup>. Data were represented as Mean  $\pm$  S.E.M. A paired student t-test at 95% confidence interval of the difference was applied to find out the level of significance. P value < 0.05 was considered significant.

## RESULTS AND DISCUSSION

The effect of fluoride on important and essential enzymes of Carbohydrate - Protein metabolism were measured in the liver of *Tilapia mossambica*, at 1st, 2nd, 3rd and 4th week upon exposure to fluoride. Aminotransferases are important group of enzymes participating in the shifting of the amino group from alpha-amino acids to the alpha-keto acid without the liberation of ammonia. The alpha-keto acid enters into the citric acid cycle for the release of energy. In this process alpha-keto acids changed into amino acids might be used in protein synthesis and take part in carbohydrate-protein metabolism regulation<sup>10, 15, 16</sup>. Alkaline phosphatase (ALP) removing phosphate groups from nucleotides, proteins, and alkaloids, a process called dephosphorylation. The results showed significant increase in the enzymatic action of Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate aminotransferase (AST) (Table 1, Fig. 1) in treated liver under fluoride when compare to control fish ( $p < 0.001$ ) due to utilization of glucose and amino acid in carbohydrate-protein metabolism (Table 2)<sup>10,17-21</sup>. High levels of AST, ALT, ALP upon long-term exposure (4<sup>th</sup> week) to fluoride might be due to altered liver function or liver dysfunction under stress<sup>22, 23</sup>.

**Table 1: Enzymatic activity of AST, ALT, ALP in the liver of *Tilapia mossambica* at 1st, 2nd, 3rd and 4th week upon exposure to fluoride (1.5g/70L).**

Biochemical parameter (U/L)	Control Fish	Treated Fish 1 <sup>st</sup> week	Treated Fish 2 <sup>nd</sup> week	Treated Fish 3 <sup>rd</sup> week	Treated Fish 4 <sup>th</sup> week
AST	633.65 $\pm$ 0.077	823.54 $\pm$ 0.009	876.42 $\pm$ 0.013	856.14 $\pm$ 0.008	734.12 $\pm$ 0.051
ALT	755.6 $\pm$ 0.068	890.4 $\pm$ 0.073	974.6 $\pm$ 0.005	1005.6 $\pm$ 0.009	798.34 $\pm$ 0.034
ALP	308.4 $\pm$ 0.090	677.13 $\pm$ 0.007	790.85 $\pm$ 0.007	859.26 $\pm$ 0.007	467.47 $\pm$ 0.074

Values expressed as Mean  $\pm$  S.E.M; \*: Significant at  $P < 0.05$ ; \*\*: Highly significant at  $P < 0.01$ ; \*\*\*, Very highly significant ( $p < 0.001$ ) compared with control

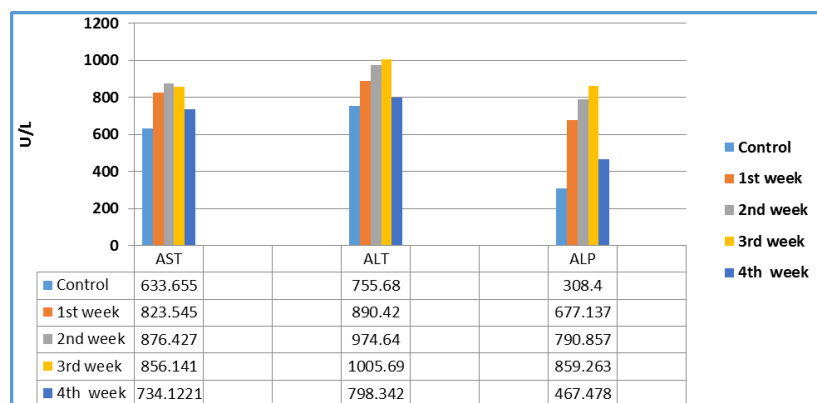


Fig. 1: Fluoride Effect (1.5g/70L) on the activity of ASP, ALT, ALP enzymes in the liver of *Tilapia mossambica* at 1st, 2nd, 3rd and 4th week.

Table 2: Biochemical constituents Protein, Lipid, Cholesterol, Glucose in the liver of *Tilapia massombica* at 1st, 2nd, 3rd and 4th week upon exposure to fluoride (1.5g/70L).

Biochemical parameter (mg/g)	Control Fish	Treated Fish 1 <sup>st</sup> week	Treated Fish 2 <sup>nd</sup> week	Treated Fish 3 <sup>rd</sup> week	Treated Fish 4 <sup>th</sup> week
Protein	6.745±0.089	4.553±0.098	3.497±0.068	3.300±0.067	2.756±0.023
Lipid	86.063±0.018	72.55±0.008	48.4±0.105	52.637±0.056	49.327±0.016
Cholesterol	82.805±0.078	308.612±0.066	177.6±0.077	131.625±0.055	101±0.078
Glucose	63.418±0.084	37.418±0.037	32.495±0.050	12.947±0.008	10.650±0.097

Values expressed as Mean ± S.E.M; \*\*: Highly significant at P<0.01; \*\*\*; Very highly significant (p<0.001) compared with control

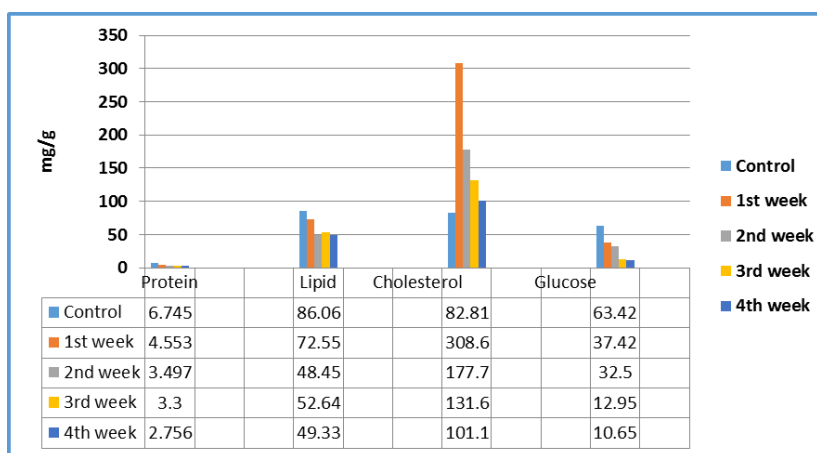


Fig. 2: Fluoride Effect (1.5g/70L) on Protein, Lipid, Cholesterol Glucose in the liver of *Tilapia mossambica* at 1st, 2nd, 3rd and 4th week.

Quantitative measurements of biochemical constituents in the liver of freshwater fish *Tilapia mossambica* at 1st, 2nd, 3rd and 4th week are presented in Table 2 and Fig. 2. Results showed decreased in protein-carbohydrate and lipid contents due to changes in carbohydrate-protein metabolism ( $p < 0.001$ ). This condition might be due to the decrease oxidation of glucose while decreased in protein might be associated with energy production. The whole process is needed to overwhelm the tension for the endurance of fish<sup>24</sup>. Decreased lipid content in fish liver exposed to fluoride may be due to the decrease in enzyme acyl co-A synthetase activity, play an essential role in fatty acid synthesis. An increase in the cholesterol level in the liver due to fluoride dose, measurements of these important clinical biochemical parameters serve as excellent biomarkers of the aquatic environmental condition.

## CONCLUSION

The liver is a fundamental organ of various metabolism and different process in fishes. This study has revealed that the liver of *Tilapia mossambica* upon exposure to fluoride may result in the alteration in the enzyme activities might be due to a defensive mechanism to overcome the lethal stress caused by Fluoride in freshwater fish *Tilapia mossambica*. The present study demonstrates the disorder of biochemical processes occurring in the fish body caused by the fluoride, the mobile persistent toxic agent producing the adverse effect on fish health, growth and number in the freshwater reservoir.

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