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Sublethal Effects of Fluoride on some Biochemical Profiles of Freshwater Stinging Catfish, *Heteropneustes fossilis* (Bloch)

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ABSTRACT

Fluoride is a persistent bioaccumulator, which keeps on accumulating in visceral organs through environment. Thus even low concentration can cause harmful consequences to aquatic organisms chronically exposed to it, particularly fish due to their poor ability to metabolize and eliminate xenobiotics as compared to other higher vertebrates. The toxicity of fluoride to freshwater catfish, *Heteropneustes fossilis,* a common edible fish of India was evaluated after chronic exposure to sublethal concentration 77.20 mg F/L (one-fifth of 96 hour LC_{50} value) for a period of three months. Alterations in biochemical constituents such as total protein and lipid were estimated in different tissues such as gill, liver and kidney of the exposed fish and observations were made after 45 and 90 days. The levels of both the biomolecules was found to be decreased significantly in comparison to control after both the durations. The results indicate that fluoride is capable to alter biomolecules in fishes causing great loss in their growth and nutritive value. Such fish when consumed as food lead to the accumulation of toxicant in the soft tissues of human body and can promote 'fluorosis' which is a crippling disease.

***Index Terms*:** Fluoride toxicity, Protein, Lipid, Soft tissues, *Heteropneustes fossilis*

I. INTRODUCTION

Freshwater ecosystems that flow through agricultural areas have high probability of getting contaminated by surface runoff and ground water leaching by a variety of chemicals. Water quality is affected by different types of pollutants that originate from both natural as well as anthropogenic sources and are toxic to the residing organisms (Kopecka *et al*., 2006). Highly effective fertilizers and pesticides are used tremendously, which on entering the aquatic environment bring multiple changes in organisms by altering their metabolism, physiology, growth rate, nutritional value, behavioural pattern etc (Bajpai *et al*., 2009; Bajpai *et al.*, 2010; Al-Otaibi *et al*., 2019). Burden upon aquatic habitat for domestic, industrial and allied waste disposal has increased enormously. The quantum of water is probably not enough to dilute the quantity of wastes that are being dumped into the aquatic habitat. Degradation of habitat is posing great threat to the health and productivity of aquatic animals including fishes (Beg and Ali, 2008; Arner *et al.*, 2009). A major part of the world's food is being supplied from fish source, so it is essential to ensure the health and productivity of fishes (Tripathi & Harsh, 2002).

Among other chemical contaminants, fluoride has recently emerged as one of the major pollutants in ecotoxicological studies (Sharma, 2002). Its concentration is gradually increasing in both ground as well as surface water due to its wide commercial use by several industries and unscientific disposal of fluoride laden wastes into freshwater resources (EPA, 1997; Shankar *et al.*, 2007). Various aquatic organisms particularly fish, due to their poor ability to metabolize and eliminate xenobiotics are vulnerable to fluoride toxicity (Demaute, 1989). Once fluoride enters the food web it is distributed in the ecosystem and gets accumulated mainly in the bones and various soft tissues of fishes (Camargo, 2003).

The accumulation of fluoride may cause disturbance in the metabolic machinery which is interlinked with the structural integrity of tissues and alters the biochemical profile of the exposed organisms (Camargo, 2003; Kumar *et al*., 2007a,b). Since early detection of specific physiological abnormalities provide an indication of exposure prior to manifestation of any gross damage, the

evaluation of biochemical changes in tissue of fish under exposure to toxicant may be used to predict the toxic response of xenobiotics. Therefore, the present study has been designed to study the effect of fluoride on biomolecules such as protein and lipids in catfish, *Heteropneustes fossilis* a common edible fish of India and a cheap source to replenish the total dietary protein requirements among masses.

II. MATERIALS AND METHODS

Healthy specimens of *Heteropneustes fossilis* of average length 18.11±0.18 cm and weight 34.27±0.51 g were procured from the local fresh water resources. They were transported to the laboratory in large plastic containers having fresh water to minimize stress. They were checked for disease as well as injury and rinsed in 0.1% $KMnO_4$ solution to avoid infection. They were acclimated in large steel tank having dechlorinated tap water, with proper aeration for about 20 days.

For experimentation, healthy fishes were sorted out and transferred into aquaria measuring 60×40×45 cm having 40 liters of water. They were divided into two groups having 15 fish in each. Group I served as control (without any treatment) and group II was exposed to sublethal concentration of fluoride; 77.20 mg F/L (one fifth of 96 hour LC_{50} value). Sodium fluoride (NaF ER grade) was obtained from Qualigens Fine Chemicals Limited, Mumbai, India. A stock solution of 10 mg F/L was prepared by dissolving weighed amount of toxicant in 500 ml of double distilled water, which was further diluted according to the desired concentration needed for experimentation with chlorine free tap water. The physico-chemical properties of holding water were determined according to APHA *et al.* (2005) methods such as, temperature 27±1.5°C, pH 7.1±0.2, dissolved oxygen 7.5±1.5 mg/L, alkalinity as $CaCO_3$ 225-230 mg/L, hardness as $CaCO_3$ 250-290 mg/L and fluoride 0.1 mg/L. Fishes were fed with dried prawn pieces and the water of aquaria was renewed every alternate day and supplemented with fresh dose of toxicant.

After 45 and 90 days of exposure, fish were taken out from both the experimental and control group and sacrificed for sampling. Gill, liver and kidney were dissected out carefully and subjected for biochemical estimations. Total protein was estimated following Lowry *et al*. (1951) while lipid estimation was done following Annino (1976) methods. The experiment was replicated thrice and the data obtained was subjected to Student's t test with the help of computerized statistical programme.

III. RESULTS AND DISCUSSION

The level of protein and lipid (mg/g) in different tissues in control fish and experimental fish is presented in Table 1 and 2. The result reveals that there was significant depletion in protein and lipid content in tissues like gill, liver and kidney of fish exposed to fluoride after 45 and 90 days in comparison to control group. Decrease in protein content was found to be maximum in gill (21.13%), followed by liver (7.58%) and kidney (3.28%) after 45 days exposure to fluoride. After 90 days, it was found maximum in gill (28.05%), followed by kidney (13.72%) and liver (8.86%) Figure 1, Table 1. A significant decrease in lipid was observed after both the durations. The depletion was found to be maximum in liver (6.71%), followed by gill (6.43%) and kidney (2.21%) after 45 days of exposure. Similarly, it was found maximum in kidney (14.20%), followed by gill (13.56%) and liver (7.15%) Figure 2, Table 2. Present findings indicate that fluoride has induced alterations in the level of macromolecules and their metabolism.

Proteins are the building blocks of tissues and cells. They are required for the growth and development. They also form the structural part of organs, make up hormones and enzymes. A change in the total protein levels could be attributed to various conditions that may cause either abnormal high production of proteins, interference with the production of albumin or globulin proteins or increase the loss or breakdown of proteins. Decrease in the protein level due to toxicity could be a result of massive hepatic necrosis and chronic cirrhosis that significantly destroys liver cells (Achikanu & Ani, 2021).

Decrease caused by fluoride in protein content of gill, liver and kidney in *H. fossilis* as observed in the present study is similar to the observation of Gupta (2003) in *Channa punctatus* after exposure to fluoride. The decrease may be due to blocking of the metabolism of the amino acids, thereby preventing cells from synthesizing protein. Studies have shown that fluoride inhibits protein synthesis (Chinoy *et al.*, 1994) and interferes with amino acid metabolism (Pandit & Narayana, 1940). Another possible reason may be depletion of protein for its conversion to glucose (Srivastava *et al*., 2002) or utilization of protein in the form of mucoprotein which is eliminated in the form of mucus by the fish to combat toxic stress (Kumar *et al*., 2007a). Results of our studies are also in accordance with the findings of Chezhian *et al.*

(2010) who have reported decrease in protein contents in different tissues of fish, *Lates calcarifer* exposed to common mixed effluents of Sipcot Industrial Estate.

Under condition of stress, many organisms mobilize proteins as an energy source through the oxidation of amino acids. Decreased protein level observed in the study may also be attributed to stress mediated mobilization of these compounds to fulfill an increased demands for energy by the fish to cope with the environmental stress after exposure to toxicants (Jenkins & Smith, 2003). On the other hand, Saxena and Mani (1985) have suggested that decline in protein content may be related to decreased food intake, increase energy cost of homeostasis, tissue repair and detoxification mechanism during stress. Reduced feeding intensity, after fluoride exposure in fishes, has also been observed in different studies in our laboratory (Gupta, 2003; Kumar *et al*., 2007a; Bajpai *et al*., 2009; Bajpai *et al.*, 2010). The decrease in the protein content may also be attributed either to the increase turnover rate of protein interference of the toxicant at certain site during protein synthesis or due to the increase proteolytic activity and decreased protein synthesis (Verma & Chand, 2007).

Lipids are the best energy producers of the body next to carbohydrates. Lipids also play important role in metabolic activities of animals because they are used as energy source and are involved in the building of cellular components. They are stored form of metabolites and provide energy when an organism faces adverse condition (Jezierska *et al*., 1982). The decrease in the lipid constituent in tissues suggests that the lipid have been channelized to meet the metabolic demand for extra energy needed to reduce the toxic stress (Gijare & Tantarpale, 2014). Significant depletion of lipid in gill, liver and kidney of the exposed fish in the study may be associated with the inhibition of lipid synthesis by fluoride or comparatively more utilization of stored lipids as instant energy source to withstand stress situation. It may also be due to liver dysfunction or mobilization of glycerol or inhibition of oxidative phosphorylation. Johnson & Lardy (1950) have reported *in vitro* inhibition of oxidation of fatty acids by fluoride. Zebrowski *et al.* (1964) have also reported that fluoride reduced catabolism of fatty acids in rats. Singh *et al.* (1985) have reported decreased lipid content in liver of rabbits treated with fluoride.

Fluoride is also known to inhibit the enzyme acyl-CoA synthesis which is involved in fatty acid oxidation. Thus reduction of lipid in gill, liver and kidney may be due to the enzymatic inhibitory action

of fluoride. Present finding is supported by the observations of Shashi *et al*. (1989) who have reported decreased lipid in rabbits and suggested that it might be due to the inhibition of lipase by fluoride. Similar views have been suggested by Kumar *et al.* (2007b) after sublethal exposure of *Clarias batrachus* to fluoride. According to them, reduction in lipid after fluoride exposure may be due to the inactivation of hormones that regulate lipid biosynthesis or inactivation of enzymes involved in lipid metabolism. Stalin & Das (2012) also reported a decrease in the lipid content in liver tissue of *Cirrhina mrigala*, exposed to fenthion.

IV. CONCLUSIONS

Thus, from the present findings it can be concluded that fluoride affects protein and lipid metabolism in fish and causes its depletion in gill, liver and kidney most probably by inactivating enzymes and hormones which regulate its synthesis on one hand and on the other by increasing its utilization in cell repair, tissue reorganization and to meet high energy demand during stressful situation caused by fluoride exposure.

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Figure 1: Effect of fluoride on total protein content (mg/g) in different tissues of *Heteropneustes fossilis* after 45 and 90 days.

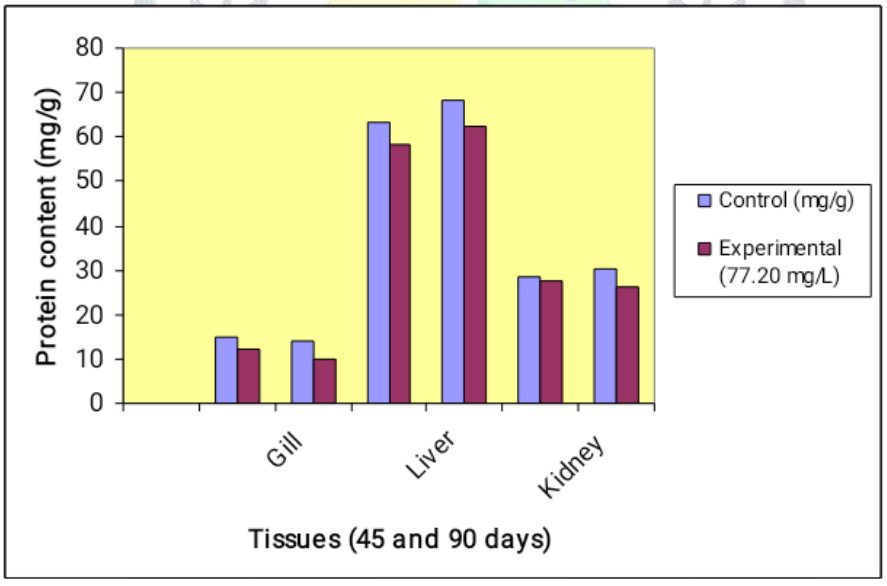

Figure 2: Effect of fluoride on total lipid content (mg/g) in different tissues of *Heteropneustes fossilis* after 45 and 90 days.

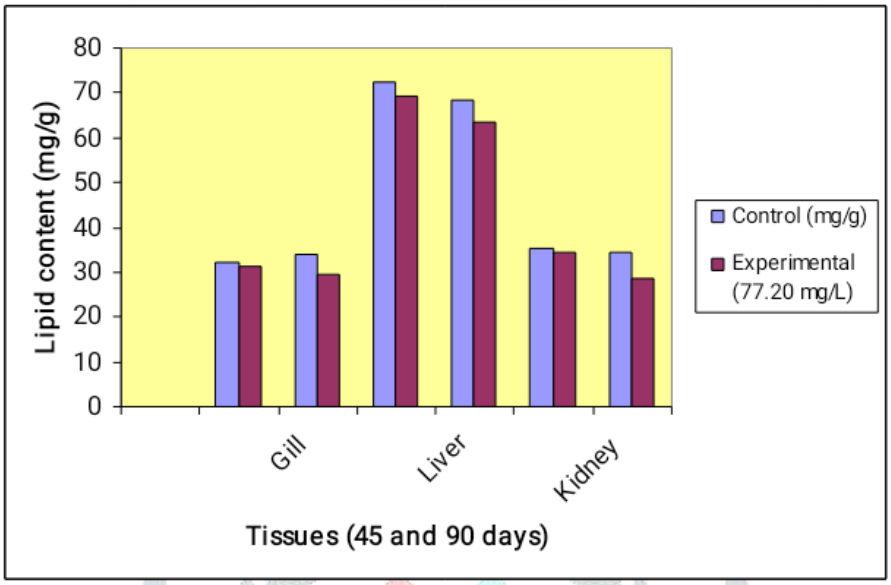

Table 1: Effect of fluoride on total protein content (mg/g wet tissue) in different tissues of *Heteropneustes fossilis* after exposure to different duration.

Tissues	Duration of Exposure (Days)	Control (mg/g)	Experimental (77.20 mg/L)	% Decrease (↓)
Gill	45	14.15±0.12	11.16±0.17***	21.13
	90	14.08±0.23	10.13±0.32***	28.05
Liver	45	63.15±0.18	58.36±0.29***	7.58
	90	68.34±0.24	62.28±0.20***	8.86
Kidney	45	28.31±0.22	27.38±0.21**	3.28
	90	30.23±0.48	26.08±0.54***	13.72

Values are Mean±S.E.M., N= 6 (number of observations per value)
**P<0.01; ***P<0.001

Table 2: Effect of fluoride on total lipid content (mg/g wet tissue) in different tissues of *Heteropneustes fossilis* after exposure to different duration.

Tissues	Duration of Exposure (Days)	Control (mg/g)	Experimental (77.20 mg/L)	% Decrease (↓)
Gill	45	33.26±0.21	31.12±0.25*	6.43
	90	34.14±0.67	29.51±0.20**	13.56
Liver	45	72.20±0.13	69.35±0.29***	6.71
	90	68.39±0.11	63.50±0.22***	7.15
Kidney	45	35.21±0.27	34.43±0.23*	2.21
	90	34.43±0.23	28.45±0.52***	14.20

Values are Mean±S.E.M., N= 6 (number of observations per value)
* P<0.05; ***P<0.001