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Biochemical alteration in Fresh Water Carp *Cirrhinus mrigala* (Hamilton) exposed to the Sodium Fluoride

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ABSTRACT: The investigations on the effects of sodium fluoride to fish *Cirrhinus mrigala* has been carried out on fingerling stage of having weight. 2.5 to 4.5g. Biochemical parameters total proteins and glycogen are assayed in gill, brain, muscle, liver and kidney after exposing the fish for 24 hours to sub-lethal, lethal and above lethal concentrations of sodium fluoride. There was significant decrease of protein in gill, brain, muscle and kidney in sub-lethal concentration exposure, there is significant increase in liver. In lethal concentration there is significant decrease in gill, brain, muscle and kidney in the above lethal concentration there is decrease in muscle, kidney and brain. Whereas the study resulted a highest loss of glycogen in the fish exposed to all the three concentrations of fluoride in the tissue gill, muscle and brain. But there is increase in the glycogen content of liver compared to control. The decrease of protein and glycogen in different tissues as proteolysis and glycogenolysis were discussed in the light of metabolic stress caused due to exposure to the toxicant, fluoride.

Key words: Biochemical changes, *Cirrhinus mrigala*, sodium fluoride, protein, glycogen.

INTRODUCTION

Pollution of the aquatic environment is the serious and rapidly growing problem. Increasing amount of industrial agricultural and commercial chemicals were discharged in to the aquatic environment have led to various deleterious effects on the aquatic organisms (Vander Oost et. al., 2003). Inorganic fluorides are transported in air and ultimately are deposited on land or open water bodies. They are also introduced into the environment due to anthropogenic sources of fluoride to aquatic environment include municipal waste and effluents from fertilizer producing plants and alluminium refineries. The toxic effects of elevated fluoride on various aquatic species are well documented including fish. (Gikunju, 1992; Dwivedi, 1997). Inorganic fluorides are toxic to the aquatic organisms and may cause adverse biological effects such as change in carbohydrate, lipid and protein metabolism, reproduction, reduced embryonic development and alteration in size and growth on human, livestock and plants (Mariappan et. al., 2000, Giri et. al., 2013). Fishes are regarded as an important high grade protein containing food staple of Indian people. Important anthropogenic sources of fluoride in the aquatic environment made significant bio chemical changes in the life cycle of fishes. Fluoride affects vertebrates in their hematological parameters (Saxena et. al., 2001). Sodium fluoride is the most common inorganic fluoride toxic to aquatic organisms reported by Sanders and Cope, (1966).

Toxicity study with fluoride containing different effluents (Camargo and Tarazona, 1991, Samal, 1994), Reaction to fluoride has been reported in studies on aquatic animals chiefly on fishes. Under the Canadian Environmental Protection Act in 1993 inorganic fluorides were found to be poisonous are lethal to aquatic and terrestrial eco systems due to their possible long term harmful effects (CEPA, 1993). Significant alteration in protein metabolism on acetyl cholinesterase activities and oxygen consumption in fresh water crabs have been described by Reddy and Venugopal (1990). Fishes exposed to poisonous amount of sodium fluoride become apathetic, loose weight, violent movement, increases secretion and vander aimlessely (John and William, 2011). Sodium fluoride acts as poison and interrupting metabolic process such as glycolysis, lipid and synthesis of protein particularly in fishes (Camargo J.A, 2003). Fluoride is the permanent bio accumulator and potent toxic

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element to living organisms. Fluoride has affinity to be permanently accumulated in exoskeleton of vertebrates in bon, teeth, scales of fishes. In many cases the surviving young fish had curved spine (Singler and Neuhold, 1972). Those parts of the fish directly in contact with the water such as scales, fins and gills have the highest fluoride content due to ionic permeability (Aziz, 2012). Alteration of enzyme activity of liver and muscle were reported by Chitra et. al., (1983). Barbier et. al. (2012) has sketched a number of cellular processes in which fluoride can have negative effects.

The present study was undertaken to evaluate the toxic effects of Sodium fluoride on certain bio molecules in tissues such as gill, brain, muscle, liver and kidney in fresh water carp in *Cirrhinus mrigala* (Hamilton).

MATERIALS AND METHODS

The fresh water fish *Cirrhinus mrigala* (Hamilton) fingerlings of both sexes measuring 4-6 cm, weighing 2500-4500 mg have been used as the test organisms in the present investigation. Healthy and active fish were obtained at Nandivelugu fish form, Guntur district, Andhra Pradesh, India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for 10 days at room temperature 28 ± 1^0C and 12-12 h dark and light cycle. Water was renewed every day during the period of acclimatization, the fish were fed (at libitum) with groundnut oil cake and ricebran. Feeding was stopped one day prior to acute toxicity test. All the precautions recommended by APHA toxicity test of aquatic organisms (*APHA 1998, 2005 and 2012*) were followed. If mortality exceeds 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

Physical and Chemical properties of water used for the present experiments are (in mg/l) : Turbidity - 8 silica units, Electrical conductivity at 28^0C - 816 micro ohms/em, p^H at 28^0C - 8.1, Alkalinity, Phenolpthalene - Nil, Methyle orange as $CaCo_3$ - 232, Non-carbonate hardness as ($MgCo_3$) - Nil, Nitrate nitrogen as (N) - Nil, Sulphate (as So_4) - Trace, Chloride (as Cl) - 40, Fluoride (as F) - 1.8, Iron (as Fe) - Nil, Dissolved Oxygen - 8-10 ppm, Temperature - 28 ± 2^0C.

Sodium fluoride reagent grade was used as a toxicant supplied by LOBA Chemical Company, Bombay. The test solution of sodium fluoride, was prepared by using water as solvent. The water used for acclimatization of the fish and for conducting experiments was the same.

The toxicity experiments are conducted by following the APHA (1998, 2005 and 2012) guidelines and the LC_{50} values are determined by Finneys Probit Analysis (1938, 1971) by also looking into the Fisher and Yates (1938) tables that were given in Roberts and Boyce (1972) for probit values of percent, mortality. The data is further analyzed by regression analysis by Xcel method.

The fish *Cirrhinus mrigala* of size 6 to 8 cm in length and 2500-4500 mg in weight were brought from local fish form and acclimatized at 28 ± 2^0C in the laboratory for 10 days. Such acclimatized fish was exposed to 24 h to < LC_{50} (46.913 mg/l), LC_{50} (469.13 mg/l) and > LC_{50} (515.101 mg/l) sodium fluoride concentrations.

ESTIMATION OF TOTAL PROTEINS AND GLYCOGEN

The surviving fish tissues were taken for estimation of total proteins and glycogen. The total proteins were estimated by the modified method of *Lowry et.al., (1951)*. The animals were sacrificed and fresh tissue was collected from gill, brain, muscle, liver and kidney. 30 mg of each tissue was taken and homogenised in 5% trichloroacitic acid (TCA) and centrifuged at 3000 rpm for 10 minutes. The suspended protein residue was dissolved in 1 ml. of 1 N NAOH. 0.2 ml of the extract was taken into the test tube and the 5 ml of alkaline Copper solution (50 ml of 2% $NaCO_3$ in 0.1 N, NaOH 1 ml of 9.5% $CuSO_4$. $5H_2O$ in 1% Sodium or Potassium tartrate) was added. After 30 minutes, the optical density was measured spectro-photometrically at 540 nm.

The standard graph was plotted by the method of *Lowry et.al, 1951* with Bovine serum albumin supplied by Singer Chemical Company (U.S.A.).

The total glycogen was estimated, employing the method of *Kemp et.al (1954)* 30 mg of each tissue was taken and homogenized in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of TCA and boiled for 15 minutes at 100^0C and then cooled in running water. The solution was made upto 5 ml with TCA to compensate for the evaporation and then centrifuged. From this 2 ml of supernatant was taken into the test tube, 6 ml of concentrated H_2SO_4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose (Analar supplied by B.D.H. Bombay) by the above method. The glucose obtained was converted into glycogen by the multiplication factor 0.98 *(Hawks 1951).*

RESULTS AND DISCUSSION

There is difference in the distribution of proteins as total content and glycogen in tissue as total content i.e., gill, brain, muscle, liver and kidney. The lyotropic series the decrement of protein in *Cirrhinus mrigala* exposed to sub-lethal concentration (i.e., 46.913 mg/l) is in the order brain > muscle > gill < kidney. The decrement in the fish exposed to lethal concentration (469.13 mg/l) is in the order of kidney > gill > brain > muscle and in the tissues exposed to the above lethal concentrations 515.101 is in the order of kidney > muscle > gill > brain. There is increase in liver tissue of fish exposed to sub lethal, lethal and above lethal concentrations. The decrease is more in the fish exposed lethal concentrations. The results are represented in Table – 1.

Table – 1: The amount of total proteins mg/gm wet weight in tissues of fish *Cirrhinus mrigala* (Hamilton) exposed to <LC_{50}, LC_{50}, > LC_{50} fluoride concentrations

Tissue	Control	Fluoride concentration (mg/l)		
		46.913 Sub lethal	469.13 Lethal	515.101 Above lethal
GILL	68.56 ± 0.022	72.60 ± 0.089	55.40* ± 0.034	61.96 ± 0.019
BRAIN	100.10 ± 0.056	89.30 ± 0.062	90.20 ± 0.016	95.70 ± 0.071
MUSCLE	102.30 ± 0.016	95.70* ± 0.033	95.70 ± 0.016	83.96* ± 0.034
LIVER	102.3 ± 0.016	132.166* ± 0.059	149.76* ± 0.077	115.36 ± 0.077
KIDNEY	105.8 ± 0.276	89.00 ± 0.020	76.53* ± 0.032	79.56 ± 0.031

Results are the mean values of 5 determinations and the Standard Deviation is indicated.
* Significant at P.0.05 level; 'T' Test

The observed variation in protein distribution (Table-1) suggests gradual difference in metabolic calibers of various tissues. The present trend in the tissues is justifiable in the wake of metabolic potential being oriented towards liver as it is central site for the synthesis of proteins and it is the regulating centre of metabolism. In the sub lethal concentration there is decrease in brain, muscle and kidney and significant increase in liver. In lethal concentration there is significant decrease in gill ($P<0.05$), muscle and kidney and a significant ($p<0.05$) increase in liver. In the above lethal concentrations there is decrease in muscle (more significant), kidney, brain whereas an increase in lever which is not significant.

The decrease caused by sodium fluoride in the protein content of muscle, gill, kidney and brain observed in present study are similar to observations made by Gupta (2003). It was also earlier reported by Chinoy et. al., (1994) that fluoride inhibit protein synthesis and interferes with amino acids metabolism (Pandit C.G, Narayana, 1940).The decrease may be due to blocking of the metabolism of amino acids thereby preventing cells from synthesis. Another possible reason may be depletion of protein for its utilization in conversion to gluconeogenesis. (Srivatsava et. al., 2002). The decrease of protein (proteolysis) in muscle tissue of fluoride exposed *Chenna punctata* (Bloch) was reported by Gupta, (2003) and in *Clarias batrachus* (Linn) by Kumar et.

al., (2007). The depletion in the total protein content (proteolysis) which the augmentation and possible utilization of their product for metabolic purposes as reported by Ravinder et.al. (1988). The decreased trend of protein in muscle, gill, brain and kidney may be metabolic utilization of keto acids for the alternative path way of synthesis of glucose directing the free amino acids for the maintenance of osmotic and ionic regulation (Schmidt, 1975) decrease and such protein levels may be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with the disturbed environment created by toxicant (Jenkins et. al., 2003). *Labeo rohita* was exposed to pesticide chemical (fenvalerate) resulted in decreased protein content also in gill, brain, muscle and kidney as reported by Anitha et. al., (2010).

The decrease of protein in brain in lethal and above lethal concentrations leads to impairment of brain function. As a result the brain cannot have the complete control on the organs like gills, muscles, liver and kidney which results in disturbed physiological activity. The gills are the important organs in osmatic pressure regulation and maintenance of acid base balance and ion transport. Once the gills are damaged the osmatic regulation functions could be affected resulting in physiological and histological changes in fish (Haque et. al., 2012). As the result of the physiological changes the enzyme activity in muscles is altered leading to decrement of protein. The increase of protein in the liver is structural damage of liver that leads to suppressed proteolytic enzyme activity (Aziz et. al., 2013) consequent disturbance in metabolism.

The increase of protein content of liver is more in sub lethal and lethal concentration and it is significant in lethal concentration. The fish is trying to synthesize more protein as a source of energy to fight against the toxicant stress. It was reported that on exposure to high fluoride concentration which inhibits growth and induced apoptosis. Jha, (2004) has reported DNA and cytogenetic alterations in aquatic organisms and impaired enzymatic function or general metabolism (Glyconeogenesis) and abnormal development. Fluoride interrupts the signaling pathways which are involved in cell proliferation and apoptosis and then cause the inhibition of protein synthesis and secretion (Barbier et. al., 2010). Another possible reason for its depletion in muscle and gills may be for its utilization in conversion to glucose (Gluconeogenesis) as reported by Srivatsava et.al., (2002).

Glycogen Result

In *Cirrhinus mrigala* when exposed to sub lethal concentration the glycogen decrement is in the order of muscle > gill > brain. When exposed to lethal concentration the decrement was liver > brain > gill > muscle and exposed to above lethal concentration the decrement was kidney > gill > muscle > brain. The decrease is more significant in liver when exposed to lethal dose. The results are represented in Table-2.

Table – 2: The amount of total glycogen mg/gm wet weight in tissues of fish *Cirrhinus mrigala* (Hamilton) exposed to <LC_{50}, LC_{50}, > LC_{50} fluoride concentrations

Tissue	Control	Fluoride concentration (mg/l)		
		46.913 Sub lethal	469.13 Lethal	515.101 Above lethal
GILL	10.878 ± 0.015	7.614* ± 0.015	6.526* ± 0	6.526 ± 0
BRAIN	10.880 ± 0.015	8.643 ± 0.082	6.526 ± 0	9.702 ± 0.015
MUSCLE	6.527 ± 0.02	3.264 ± 0.005	4.354* ± 0.015	3.263* ± 0
LIVER	193.779 ± 0.29	253.689* ± 0.122	154.60* ± 0.062	206.00 ± 0.154
KIDNEY	42.466 ± 0.046	36.778* ± 0.015	62.634* ± 0	23.882 ± 0.04

Results are the mean values of 5 determinations and the Standard Deviation is indicated.
* Significant at P.0.05 level; 'T' Test

The percentage of glycogen decreases significantly in sub lethal and lethal concentrations in tissue due to enhanced conversion of glycogen to glucose (glycolysis) to meet the increased energy requirement and stress

condition and increased in liver in higher concentration as the fish is inactive and reduced utilization of energy. The increased glycogen level in liver and muscle in lethal concentration was due to disturbance of carbohydrate metabolism as it was observed to affect enzyme involved in glycogen turnover. (Strochkova, Jhvoronkov, 1983).

Several other studies revealed fluoride inhibit mini glycolytic enzymes (Camargo, 2003, Chitra et.al., 1983) transaminases to play important role in carbohydrate / protein metabolism involved in the inter conversion of amino acid to alpha keto acid and then enters into citric acid cycle and ultimately produce effect on metabolic pathways. Fluoride may cause increase in the activities of transaminases. Similar results were found on the lever, muscle of fresh water fish *Chenna punctatus* (Bloch) by Chitra et.al., (1983) and in fresh water crab *Barytelphusa guerini* (Reddy et. al., 1989).

Histopathelogical changes reported in liver by fluoride toxicity in *Cyprinus scarpio* and *Chenna punctatus* (Bloch) by Haque et.al., (2012) include vacular degeneration and focal necrosis and nuclear pyknosis. The liver glycogen is used as a source of energy by gluconeogenesis and leads to decreased level of glycogen in liver. Histopathelogical changes also contribute to certain levels of decrease of glycogen content. Aquatic animals generally depend on glycogen source for energy due to intoxication of trace metal fluoride for the maximum utility of their reserve food to combat adverse condition.

The present result was supported by Panthan et. al., (2009) i.e., depletion of liver glycogen level in fresh water fish *Rosbora daniconius* exposed to paper mill effluent. Pesticide toxicant exposure causes severe alterations in the tissue bio chemistry of fishes (Srivastava and Singh, 2004). Decrease in the total glycogen content after exposed to mixture of pesticides i.e., monocrotophos and fenvelerate (1:4; M;F) in fish *Labeo rohita* was reported by Tilak et.al., (2001). The depletion of glycogen content in the liver and muscle of *Cirrhinus mrigala* and *Labeo rohita* (Hamilton) when exposed to both sub lethal and lethal concentrations of fenvelerate (toxicant) was reported by Anitha et.al., (2010).

A significant reduction of glycogen content was found in the muscle and testis at the lower concentration (35mg/lit) but increased in all the three tissues in the higher concentration (75mg/lit) when exposed to sodium fluoride as reported by Kumar et.al., (2007). On exposure to technical grade fenvelerate caused changes as decrement in the glycogen content resulting in the disruption of enzymes associated with the carbohydrate metabolism (Helimeyer et.al., 1970). Aziz et.al., (2013) reported that fluoride increased the ALP, ALT and AST levels in the gills of fresh water fish *Tilapia mossambica* increased level of three biomarker enzymes are due to disturbances of carbohydrate and protein metabolism. Tripathi et. al., (2005) found that higher concentration of fluoride inhibit growth of fishes such as weight and length and fingerlings of *Heteropneustis fossilis* as supported by the reports of Aziz et. al., (2013).

CONCLUSION

From the results obtained it is clear that sodium fluoride interferes with various metabolic activities and biochemical changes were observed in the levels of protein and glycogen in *Cirrhinus mrigala* exposed different concentrations of fluoride. Depletion of protein was found in the muscle which curtails the growth of heterotrophic fish. The decrease is due to negative effect of fluoride in cellular process disruption of enzyme activity mostly due to inhibition of protein synthesis. Fluoride disrupts the activity of enzyme by binding it to the functional amino acid groups that encircles the enzyme active center. Because of the decrease of prime bio molecules glycogen and protein in the heterotropic fish, growth is curtailed and the venture of aquaculture is affected. The primary idea of boosting the economy of the farmers when they have culture as the profession. Further research is required on enzymatic analysis at molecular level on fluoride exposed fish during chronic periods. This enables to decrease the toxic effect on fish.

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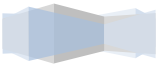

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