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SODIUM FLUORIDE INDUCED BIOCHEMICAL CHANGES IN THE TISSUES OF FRESH WATER INDIAN MAJOR CARP *CATLA CATLA* (HAMILTON)

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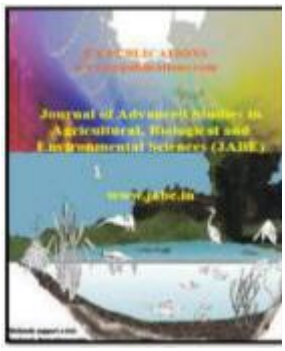

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ABSTRACT

The investigations on effects of acute sodium fluoride (NaF) to fish *Catla catla* has been carried out on fingerling stage, weight 2.5-4.5 gm. The toxicity of fluoride *Catla catla* was assayed after their exposure to sublethal, lethal and above lethal concentrations of NaF for 24 hours. Changes in the biochemical parameters were assayed in gill, brain, muscle, liver and kidney. There was significant decrease in the glycogen content of gill, brain, muscle, liver and kidney in lethal concentrations. There was significant decrease in protein content of muscle, brain and kidney whereas increase in liver. The food Substrates of metabolism in the vital organs of the tissues of the heterotrophic fish are effected due to toxic stress where the enzyme inhibition and accumulation resulted in the ambient situations.

Key words : Biochemical changes, *Catla catla,* glycogen, lethal concentration, protein, sodium flouride, toxicity.

1. Introduction

The careless disposal of various wastes due to anthropogenic activity into the surface water is getting polluted. The aquatic environment is severely affected by different types of chemicals which are toxic to the inhabiting organisms (*Kopeca et.al. 2006*). Pollution of aquatic ecosystem by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and important aquatic biota (*Kumari* and *Rajakumari, 1997*). There are few reports available on the toxic effect of industrial effluent and chemical pollutants on protein, lipid and glycogen of these fishes (*Kumar* and *Gopal, 2011*). Rout et.al 2013 studied the lead acetate *Clarias batrachus* and reported the behaviour of the fish was altered ultimately resulted in death. Fishes are regarded as an important high protein containing food staple of Indian people. Increasing water pollution level especially with sodium fluoride in inland fresh water reservoir has made significant biochemical changes in the life cycle of fishes.

The toxic effects of elevated fluoride on various aquatic species are well documented (*Gikunju 1992, Dwivedi 1997*). Its harmful impact on human and livestock and plants (*Mariappan et.al 2000*). Fluoride also affects vertebrates in their morphological and behavioural parameters (*Tripathi A, et.al 2004*) and cellular architecture (*Gupta 2003*). Fluoride has also been shown to cause many biochemical changes and metabolic disturbances in mammals, rats, rabbits and human beings *Sheshi et.al. 1989, Chinoy et.al. 1994, Chitra et.al. (1983)* found that Fluoride alters enzyme activity in liver and muscle of *Channa punctatus.* Gupta (2003) reported that Fluoride decreased glucose and protein levels in the blood and muscles of these fish. Hence the

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present study is attempted when the fingerlings were exposed to sublethal and above the lethal concentrations of NaF and biochemical changes were assayed in different tissues gill, brain, muscle, liver, kidney.

2. Materials and Methods

The fresh water fish *Labeo rohita* (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 $\pm$ 1^{o}C 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^{o}C - 816 micro ohms/em, pH at 28^{0}C - 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 $\pm$ 2^{o}C.

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.

3. Estimation of total proteins and glycogen

The fish *Catla catla* 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^{o}C in the laboratory for 10 days. Such acclimatized fish was exposed to 24 h to < LC_{50} (29.785 mg/l), LC_{50} (297.85 mg/l) and > LC_{50} (301.22 mg/l) sodium flouride concentrations. 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 1000C 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)*.

4. Results and discussion

There is variation in the distribution of proteins in different tissues because of metabolic calibre. Biochemical changes were observed in the glycogen and protein in different tissues viz. gill, brain, muscle, liver and kidney. In the lyotrophic series the decrement of protein exposed to sublethal concentration is in the

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order of muscle > brain > kidney > gill. The decrement in the fish exposed to lethal concentration (i.e. 297.85 mg/l) is in the order of muscle > brain > gill > kidney and in the tissues exposed to above lethal concentration (301.22) the decrement in the order of brain > gill > kidney > muscle. There is increase in liver exposed to sublethal (29.785) and above lethal (301.22) concentrations. The results are represented in table 1.

Table-1: The amount of total Proteins Mg/Gm wet weight in tissues of Fish Catla catla (Hamilton) Exposed to < LC_{50}, LC_{50} > LC_{50} Fluoride concentrations

Tissue	Control	Fluoride concentration Mg/l		
		29.785	297.85	301.22 .
Gill	101.2	96.9	71.267*	55.13
	±0.607	±0.024	±0.019	±0.549
Brain	93.5	71.7*	65.06*	49.43
	±0.041	±0.039	±0.052	±0.044
Muscle	111.27	81.3*	74.8*	105.13
	±0.090	±0.024	±0.026	±0.083
Liver	106.47	121*	138.93*	84.03
	±0.048	±0.036	±0.029	±0.020
Kidney	121	99*	89.1*	85.8
	±0.010	2±0.0	±0.024	±0.016

Results are the Mean values of five determinations and the Standard Deviation is indicated.

*Significant at P. 0.05 level : 'T' Test

The decrease in muscle of fluoride exposed fish *Channa punctata* (bloch) was reported by (*Gupta 2003*) and in *Clarias batrachus* (Linn), (*Kumar et.al., 2007*). The decreased trend of the protein in muscle, gill, brain and kidney may be due to metabolic utilization of the keto acids for the alternative pathway of synthesis of glucose, directing the free amino acids for the synthesis of proteins / for the maintenance of osmotic and ionic regulation (*Schmidt 1975*). The fish *Catla catla* is under toxicant stress. Under toxicant stress many organisms will mobilize proteins as a source of energy via the oxidation of amino acids. Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfil an increased element for energy by the fish to cope with the environmental condition created by toxicant (*Jenkins et.al. 2003*).

The depletion in the total protein content may be due to augumented proteolysis and possible utilization of their product for metabolic purposes as reported by *Ravinder et.al., (1988).* The decrease is significant at 0.05 level 't' test. The decrease is due to protein ketabolism as an alternative source of energy (*Kabeer et.al., 1981).*

The decrement of protein in brain leads to impairment of brain function. The brain can not have the complete control the other organs like gill, muscle, liver and kidney by which their failure in physiological activity happens. The gills are important organs that fulfill the multiple functions including gas exchange, osmotic pressure regulation, acid base balance and ion transport. All these are related to gill chloride cells (*Malatt* and *Stinson 1990).* Once the gills are damaged the osmotic regulation functions could be affected resulting in physiological and histological changes in fish *Haque et.al., (2012).* As a result of physiological changes the enzyme activity in muscles is altered leading to decrement of protein. The increment in the liver is due to structural damage of liver that leads to suppressed proteolytic enzyme activity (*Aziz et.al., 2013)* and disturbance in metabolism.

The increase in protein content of liver is more in sublethal, lethal and above lethal concentration. The fish is trying to synthesize more protein as a source of energy to fight against the toxicant stress and it might also be due to alteration in enzyme activity (Glyconeogenesis).

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In fact the study has shown that sodium fluoride inhibit protein synthesis and interferes with amino acid metabolism (*Pandit Narayana, 1940*). Another possible reason for its depletion in muscle and gills may be for its utilization in conversion to glucose (Glyconeogenesis) (*Srivastava, Kaushik, Gupta, P. 2002*).

The decrease of protein in the kidney in all the concentrations of NaF is due to altered physiological activity to cope up with the toxicant stress. As a result the loss of ammonotelic nature leads to failure of kidney function.

In *Catla catla* when exposed to sublethal concentration the glycogen decrement was in the order of muscle > gill > liver > brain and kidney, in the lethal concentration the decrement is in the order of brain > kidney > gill > muscle. The decrease is significant in all the tissues. In the fish exposed to above lethal concentration the glycogen decrement is gill > brain > kidney > muscle. The results are represented in Table 2.

Table-2: The amount of total Glycogen Mg/Gm wet weight in tissues of Fish Catla catla (Hamilton)

Exposed to < LC_{50}, LC_{50} > LC_{50} Fluoride concentrations

Tissue	Control	Fluoride concentration Mg/l .		
		29.785	297.85	301.22.
Gill	9.79 ±0.02	5.489 ±0.07	4.351	3.264
Brain	8.702 ±0.03	7.014 ±0.015	2.176* ±0.030	3.263 ±0.0
Muscle	20.908 ±0.0	13.054*	8.705*	6.526
Liver	392.0 ±0.0	245.00 ±0.0	198.15* ±0.08	479.08 ±0.15
Kidney	14.14 ±0.015	16.257 ±0.030	4.354* ±0.015	5.52* ±0.07

Results are the Mean values of five determinations and the Standard Deviation is indicated.

*Significant at P. 0.05 level : 'T' Test

The decrease in liver was observed in sublethal and lethal concentration. There is slight increase in above lethal concentration. The decrease in muscle glycogen may be due to increased muscular activity of fish under toxicant stress and the glycogen was used up in rapid rate leading to decrement in muscle glycogen.

The percentage of glycogen decreases significantly in sublethal concentration of all the tissues due to enhanced conversion of glycogen to glucose to meet enhanced energy requirement under stress condition. There is slight increase in liver glycogen (glycogenolysis) in the above lethal concentration may be due to disturbance of carbohydrate metabolism, as it has been observed, to effect the enzyme activity at higher sodium fluoride concentration. Several studies revealed that sodium fluoride inhibit glycolytic enzyme (*Camargo 2003*).

The liver is the main metabolic organ and plays an important role in the uptake, accumulation, biotransformation and excretion of toxic elements. (*Pedlar et.al., 2002*). Histopathological changes are reported in liver by fluoride toxicity in *Cyprinus carpio* and *Channa punctatus* bloch by *Haque et.al. (2012)* include vacuolar degeneration and focal necrosis and nuclear pyknosis. The liver glycogen is used as a source of energy by gluconeogenesis and leads to decreased levels by glycogen in liver. 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. Depletion of liver glycogen levels in fresh water fish *Rosbora daniconius,* exposed to paper mill effluent by *Panthan et.al. (2009).*

Pesticide toxicant exposure causes severe alterations in the tissue biochemistry of fishes *(Tilak et.al. 2003), (Srivastava* and *Singh 2004).* Decrease into total glycogen after exposure to mixture of pesticide i.e. monocrotophas and fenvalerate (1 : 4; m : f) in fish *Labeo rohita* was reported by *Tilak et.al. (2001).* Increase in

the kidney when exposed to sublethal concentration the fish might be under severe toxicant stress and disturbance in the physiology of excretory organs. Increased glycogen level in liver in above lethal concentration is due to disturbance of carbohydrate metabolism as it was observed to effect the enzymes of glycolytic pathway and the krebs cycle leading to depletion and disturbance in cell membrane potential and ATP depletion (*Schuliga et.al. 2012).*

The depletion of glycogen content in liver and muscle of *C.mrigala* was reported by *Anitha 2010.* The depletion was also observed in fish *Labeo rohita* when exposed to both lethal and sublethal concentration of fenvalerate (*Anitha 2010*) whereas glycogen in muscle remain unchanged in Labeo.

Earlier reports that fluoride can induce many biochemical changes in mammals including rats, rabbits, goats and human beings by (*Chinoy et.al. 1994, Chitra et.al. 1983)*. A significant reduction of glycogen content was found in the muscle and testis at the lower concentration (35 mg/l) but increased in all the three tissues at higher concentration 75 mgF/l when exposed to sodium fluoride (*Kumar et.al. 2007).* Reported by *Helimeyer et.al. 1970* on exposure to technical grade fenvalerate caused changes in the glycogen content resulting in the disruption of enzymes associated with carbohydrate metabolism. (*Aziz et.al. 2013*) reported that fluoride increased the ALP, ALT and AST levels of the gills of fresh water fish Tilapia massambica. Increased level of these three biomarker enzymes are due to disturbances of carbohydrate and protein metabolism. The present reports are in congnisize with the order reports on different fish. The increase or decrease in biomolecules is sufficient to provide information on fish health.

5. Conclusion

When the fingerlings of *Catla catla* was exposed to sublethal and lethal concentrations of fluoride the normal physiological function was effected. Biochemical changes were observed in glycogen, protein content in various tissues of experimental fish. Decrease in muscle glycogen is more in sublethal concentration as it shows rapid swimming activity as a result of toxic stress. Liver glycogen levels were decreased due to altered enzyme activity as the liver is the prime detoxifying organ.

In the present investigation depletion of protein was found which curtails the growth of heterotrophic fish. The decrease may be due to proteolytic activity or anaerobic conditions, rapid utilization of body protein to combat with the stress condition as well as sodium flouride interrupt the metabolic process of protein synthesis in fish. Because of the decrease in these prime biomolecules in heterotrophic fish, growth is curtailed and the venture of aquaculture is affected. Further research is required on enzyme analysis of fluoride exposed fish for chronic periods and study on cellular level changes in fresh water fish.

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