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Biochemical Alternation In Fresh Water Fishe Labeo Rohita Exposed To The Sodium Fluoride (NAF).

Kale, M. D. and Muley D. V.
1 Department of Zoology, Elphinstone College, Mumbai, 400 032Maharashtra, INDIA
2 Department of Zoology, Shivaji University, Kolhapur-416 004, Maharashtra, INDIA

Abstract: *Fishes are regarded as an important high grade protein containing food staple of Indian people. Ever increasing water pollution level, especially sodium fluoride (NaF), in inland freshwater reservoir has made significant biochemical changes in the life cycle of fishes. In view of this, the investigations on effects of acute and chronic sodium fluoride toxicity to fish Labeo rohita have been carried out. The changes in glycogen, protein and lipid content of selected tissues like muscle, liver, gill and kidney were examined. The study revealed a highest loss of glycogen, protein and lipid percentage in all tissues as compared to control. .*
Keywords: *Sodium fluoride; Labeo rohita; biochemical; body tissue.*

I. Introduction

Inorganic Fluorides are introduced into the environment as a result of natural emission and anthropogenic sources. Depending on metrological condition and season, gaseous and particulate inorganic fluorides are transported in air and ultimately are deposited on land or open water bodies. Important anthropogenic sources of fluoride to the aquatic environment in clued municipal waste and effluents from fertilized producing plants and aluminum refineries. In water mobility and transport of inorganic fluoride are dependent on PH, water hardness, and the prescience of ion exchange mineral. In water inorganic fluoride remain dissolved in solution under acidic condition, low hardness, and the presence on ion exchange material (Cuker and Shilts 1979; Sahu and Karim 1989.) As a consequence free fluoride levels are generally low (Skjelkvale 1994, Radic and Barlic 1995). Inorganic fluoride are toxic to aquatic organism and may caused adverse biological effect such as change in carbohydrate, lipid, and protein metabolism , reproduction , impairment, reduce embryonic and development life stage, and alternation size and growth.

Sodium fluoride (NaF) is the most common inorganic fluoride to toxic aquatic organism reported by Sanders and Cope (1966). Toxicity studies with fluoride containing different effluent by Woodwiss and Fertwell (1974), Damkaer and Dey (1989), Camargo (1991), Camargo and Tarazona (1991), Samal (1994) reaction to fluoride has been examined in several studies on aquatic animal, chiefly on fishes. If fishes exposed to poisons amount of sodium fluoride (NaF) become apathetic, loss weight, violent movement, increases secretion and wander aimlessly (Neuhold and Singler 1960). Sodium fluoride (NaF) acts as poisons and interupting metabolic process such as glycolysis, lipid and synthesis of protein particularly fishes (Julio A. Camargo, 2003). Significant alternation in protein metabolism on acetylcholinesterase activities and oxygen consumption in fresh water crabes have been described by Reddy and Venugopal (1990). Inorganic fluoride toxicity is negatively correlated to water hardness and positively correlated to temperature (Pimentel and Bulkley 1983). The initial phase of acute inorganic fluoride intoxication in fresh water species such as rinbow trout and carp is characterized by apathetic behavior accompanied by Neuhold and Sigler 1960 and Newhold 1972). In many cases, the surviving young fish had curved spines (Singler and Neuhold 1972).

The present studies was under taken to evaluate the toxic effect on sodium fluoride (NaF) on biochemical changes in different tissue such as gill, liver, kidney and muscle of fresh water carp L rohita.

II. Material And Method

The fresh water fishes L rohita measuring about 6 to 7 cm. in length were collected from state government fish seed rearing center. The collected fish were maintained under laboratory condition at 28-30 oc for 10 days acclimation and were then divided in different group having 10 fishes in each. All the group except control were transferred to separate plastic tub containing different concentration (10 L) sodium fluoride (NaF) grade to determine toxicity LCo and LC_{50} value and fish behavior. Acute toxicity experiment were conducted for 96h and chronic toxicity for 30 days using a static bioassay technique. Toxic medium was changed at an interval of 24h. During experimentation temperature, ph, oxygen contains and hardness of the water determined slandered method by APHA. After acute exposure 96h fishes were sacrificed to obtained gills, liver, kidney and muscle. The pooled samples of the organs were used for estimation of glycogen, total protein and total lipid. Same method was applicable for the chronic exposure for 30 days for estimation of glycogen, total protein and total lipid.

The experiment was conducted for acute and chronic, during the fish dose not provided food. The aquarium water was changed on after 24h, and fresh dose of the sodium fluoride (NaF) was given. Total protein lipid and glycogen were estimated by standard method by Lowry et al., Folch et al., and De Zawn A and Zandi D.I. respectively.

III. Results And Discussion

Biochemical changes were observed in the glycogen, protein and lipid content in different tissue of L rohita after acute and chronic exposure to sodium fluoride (NaF). .

After acute (96h) and chronic (30 days) of exposure of L rohita to sub-lethal and lethal (LCo-LC_{50}) concentration of the total protein content in muscle, liver, gill and kidney were decreased significantly ($p<0.001$) in both groups of esposed fish in comparison to the control group (Table 1and 2). The glycogen content was significantly decreased in gill and kidney at sub-lethal (LCo) concentration of sodium fluoride, but the decreased was not significant in lethal concentration (LC_{50}) of sodium fluoride. At higher sodium fluoride concentration however glycogen increased significantly in liver and muscle and decreased in gill and kidney Table 3 and 4). The lipid content in liver, gill, muscle and kidney was also significantly decreased in both group as compared to the control group (Table 5 and 6).

The decreases caused by sodium fluoride (NaF) in protein content of muscle, liver, gill, and kidney as observed here is similar to the observation of (Gupta R. 2003). This decreased may due to blocking of the metabolism of amino acid there by preventing cells from synthesizing protein. In fact study has shown that sodium fluoride (NaF) inhibit protein synthesis and interferes with amino acid metabolism (Pandit CG, Narayana RD, 1940). Another possible reason may be depletion of protein for its utilization in conversion to glucose (Sirvastava N, Kaushik N, Gupta P. 2002).

The glycogen content in the fresh water fishes L rohita and C mrigala exposed to tne sodium fluoride (NaF)concentration in sub-lethal (LCo) decreased significantly in liver, muscle, gill, and kidney while in lethal concentration (LCo) of sodium fluoride exposed (NaF) the percentage of glycogen increases significantly was found in the tissue of liver and muscle and decreases in the tissue of gill and kidney. The percentage of glycogen decreases significantly in sub-lethal concentration of all tissue due to enhanced conversion of glycogen to glucose to meet and increased energy requirement under stress condition and increased in higher concentration of sodium fluoride in liver and muscle due to locomotary movement of fish during the experiment. The increased glycogen level in liver and muscle in lethal concentration due to disturbance of carbohydrate metabolism as it has been observed to effect enzyme involved in glycogen turnover at higher sodium fluoride concentration (Strochkova LS, Zhvoronkov AA in 1983). Several other studies have revealed that sodium fluoride inhibit glycolytic enzyme (Camargo JA 2003).

The total lipid decrease in liver, gill, muscle, and kidney of sodium fluoride (NaF) exposed L rohita in sub-lethal (945 ppm.) and lethal concentration in (960 ppm.). The decreased percentage of lipid due to inhabitation of lipid synthesis by sodium fluoride as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic functions. Sodium fluoride is well known as an inhibitor of various enzymes like lipase, phosphatase, and esterases. The interference of sodium fluoride was also observed in fatty acid oxidation and inhibits the enzyme acyl-co-A synthesis (Batenburg JJ, Vanden Bergh SG. 1972). Thus decreased lipid content in various tissue may be due to the inhibition of thes enzyme. Total lipid decreased in muscle, liver, and testis of the fluoride exposed catfish was observed by Sashi et al. in rabbits in 1989.

From the result obtained here, it is cleared that sodium fluoride (NaF) interferes with various metabolic activities and biochemical changes are observed in the level of protein, glycogen, lipid in exposed fishes L rohita.

IV. Tables

Table No. 1 Changes in glycogen content in different tissue of Labeo rohita after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute Exposure	
		LC_0 910ppm	LC_{50} 935ppm
Gill	11.98 ± 0.865	8.53** ± 0.745	7.49*** ± 0.579
Liver	15.40 ± 1.072	8.82*** ± 0.964	6.57*** ± 0.932
Kidney	10.49 ± 1.102	8.28* ± 0.974	7.29** ± 0.777
Muscle	11.42 ± 0.834	7.32** ± 0.675	5.45*** ± 0.606

(Value expressed in mg/100mg wet tissue; ±: SD) $P<0.05$ *, $P< 0.01$**, $P< 0.001$***

Table No. 2: Changes in glycogen content in different tissue of Labeo rohita after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		46.75ppm	93.50ppm
Gill	12.27 ± 0.606	8.43*** ± 0.420	6.76*** ± 0.511
Liver	15.55 ± 0.640	8.40** ± 0.921	6.14*** ± 0.603
Kidney	9.42 ± 1.423	7.74** ± 0.847	6.35** ± 1.221
Muscle	11.80 ± 0.799	7.06* ± 0.861	5.21*** ± 0.870

Value expressed in mg/100mg wet tissue; ±: SD) P<0.05 *, P< 0.01**, P< 0.001***

Table No.3 Changes in protein content in different tissue of **Labeo rohita** after acute exposure to sodium fluoride (96 hrs).

Tissue	Control	Acute Exposure	
		LC_0 910ppm	LC_{50} 935ppm
Gill	19.81 ± 0.415	14.85** ± 0.258	11.67*** ± 0.909
Liver	24.47 ± 0.664	17.38** ± 1.02	13.62** ± 0.468
Kidney	16.52 ± 1.450	10.76** ± 0.721	8.87** ± 0.854
Muscle	27.89 ±0.947	20.34 *** ±0.856	18.87***± 0.957

Value expressed in mg/100mg wet tissue; ±: SD) P<0.05 *, P< 0.01**, P< 0.001***

Table No. 4 Changes in protein content in different tissue of Labeo rohita after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		LC_0 46.75ppm	LC_{50} 93.50ppm
Gill	18.64 + 0.703	12.96** ± 0.886	10.52*** + 0.667
Liver	24.01 + 1.073	15.73** ± 1.070	12.86*** + 0.883
Kidney	17.30 + 1.450	10.53*** ± 1.352	8.39*** + 0.958
Muscle	27.44 + 0.947	19.58** ±0.856	16.07*** + 0.957

Value expressed in mg/100mg wet tissue; ±: SD) P<0.05 *, P< 0.01**, P< 0.001***

Table No. 5 Changes in lipid content in different tissue of Labeo rohita after acute exposure to sodium fluoride (96 hrs).

Tissue	Control	Acute Exposure	
		LC_0 910ppm	LC_{50} 935ppm
Gill	5.94 ± 0.935	4.67*± 0.643	3.89** ± 0.552
Liver	8.54 ± 0.651	5.74** ± 1.138	4.94*** ± 0.578
Kidney	3.66 ± 0.605	2.12 *** ± 0.664	1.51*** ± 0.143
Muscle	7.63 ± 0.567	5.60* ± 0.948	4.91** ± 0.765

Value expressed in mg/100mg wet tissue; ±: SD) P<0.05 *, P< 0.01**, P< 0.001***

Table No. 6 Changes in lipid content in different tissue of Labeo rohita after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		LC_0 46.75ppm	LC_{50} 93.50ppm
Gill	6.87 ± 0.799	5.04* ± 0.857	4.63** ± 0.942
Liver	8.09 ± 0.843	5.23** ± 0.727	4.12*** ± 0.557

Kidney	3.99 ± 0.608	2.44*** ± 0.639	1.92*** ± 0.071
Muscle	7.12 ± 1.00	5.02* ± 0.237	4.27** ± 0.441

Value expressed in mg/100mg wet tissue; ±: SD) P<0.05 *, P< 0.01**, P< 0.001***

V. Conclusion

Toxic effects of inorganic sodium fluoride (NaF) change the normal physiological function of the experimental organism. Biochemical changes were observed in glycogen, protein and lipid content in various tissues of experimental fish. Glycogen is the prime sources of energy showed decreasing order after acute and chronic exposure to sodium fluoride concentrations. Decrease in glycogen at acute concentration was more in gill and liver. Gill may be affected as they are first organ to come in contact with toxicant, while liver may be affected as it is prime detoxifying organ. Influx of glycogen to meet the demand created due to stress may be responsible for increasing glycogen while increased glycolysis may result in decrease glycogen level. The depletion of glycogen level in the specific tissues indicates the possibility of active glycogenolysis, subsequent hypoxia that increases carbohydrate consumption.

In present investigation, depletion of protein was to be found at both acute and chronic concentration of sodium fluoride in different tissues in experimental fish. This decrease may be due to proteolytic activity or anaerobic conditions, rapid utilization of body protein under stress condition as well as sodium fluoride interrupt the metabolic process of protein synthesis in fishes.

Lipids serves as the reserve energy sources, which decreases significant when exposed the experimental fish to both acute and chronic concentration of sodium fluoride. The decrease may be due to inhibition of lipid synthesis by fluoride as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic functions under the stress of toxicant.

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