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Full Length Research Paper

Biochemical Alternation in Fresh Water Fishes *Labeo rohita* and *Cirrhinus mrigala* exposed to Acute and Chronic Concentration of Sodium Fluoride

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ARTICLE INFORMATION	ABSTRACT
<p>Corresponding Author: M. D Kale</p> <p>Article history: Received: 23-12-2022 Revised: 26-12-2022 Accepted: 28-12-2022 Published: 31-12-2022</p> <p>Key words: Biochemical, Fresh water, Fishes, Chronic, Sodium Fluoride</p>	<p>Static bioassay for acute 96h and chronic 30 days were performed using sub-lethal 945 ppm (LC_{50}) and lethal 960 ppm (LC_{50}) concentration of Sodium Fluoride on the fresh water fishes <i>L rohita</i> and <i>C mrigala</i>. The behavior and mortality rate were recorded. After acute exposed various fish tissue like gill, muscle, liver, and kidney were subjected to biochemical (total protein, glycogen, and lipid) analysis. The result showed marked alternation in experimental fish due to toxicity of Sodium Fluoride compared with control group.</p>

Introduction

Inorganic Fluorides are introduced into the environment as a result of natural emission (eg. Volcanic activity) 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 included 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 level is 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 used in aquatic toxicity studies reported by Sanders and Cope (1966). Toxicity studies with fluoride containing effluent include Woodwiss and Fertwell (1974), Damkaer and Dey (1989), Camargo (1991), Camargo and Tarazona. (1991), Samal (1994). Reaction to fluoride have 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 interrupting 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 crabs have been described by Reddy and Venugopal (1990) under fluoride toxication effect caused by exposure to inorganic fluoride has been observed in aquatic animals (Kalpana et al. 1964, Sigler and Neuhold 1972, Mishra and Mohapatra 1998). 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 Neuhold 1972). In many cases, the surviving young fish had curved spines (Singler and Neuhold 1972). The present study 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* and *C. mrigala*.

Material and Method

The fresh water fishes *L rohita* and *C mrigala* 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 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 aquarium containing different concentration of sodium fluoride (NaF) grade to determine toxicity LCo and LC50 value and fish behavior. During experimentation temperature, ph, oxygen contains and hardness of the water determined slandered method by APHA. After acute exposure fishes were sacrificed to obtained gills, liver, kidney and muscle. The pooled samples of the organ were used for estimation of glycogen, total protein and total lipid. Same method was applicable for the chronic exposure for estimation of glycogen, total protein and total lipid. 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.

Result and Discussion:

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

Total protein:

After acute and chronic exposure of *L rohita* and *C mrigala* to sub-lethal and lethal concentration of fluoride, the total contents in muscle, liver, gill, and kidney were decreased significantly ($p < 0.001$) in both group of exposed fish in comparison to the control group (Table 1). (value expressed in mg/100mg wet tissue).

Table 1. Changes in protein content in different tissue of *L rohita* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0 C50		Control	Chronic dose LC0 C50	
Gill	17.73	12.43	9.73	15.52	11.16	7.27
Liver	21.90	16.85	13.83	17.00	12.90	8.13
Kidney	15.37	9.77	7.19	13.54	10.29	7.09
Muscle	23.45	15.22	10.02	18.43	11.22	7.73

Table 2. Changes in protein content in different tissue of *C mrigala* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0 C50		Control	Chronic dose LC0 C50	
Gill	20.10	13.75	10.61	16.60	11.46	7.71
Liver	22.37	17.17	10.59	17.87	13.62	8.31
Kidney	16.86	11.21	7.09	15.32	10.18	6.98
Muscle	24.30	14.06	8.96	20.85	11.26	6.57

Table 3. Changes in glycogen content in different tissue of *L rohita* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0 C50		Control	Chronic dose LC0 C50	
Gill	14.23	11.09	7.99	12.30	9.45	6.55
Liver	18.45	11.60	13.35	15.65	10.23	12.08
Kidney	10.65	7.64	5.29	8.20	6.01	4.85
Muscle	16.70	11.47	12.65	11.95	8.36	9.61

Table 4. Changes in glycogen content in different tissue *C mrigala* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0 C50		Control	Chronic dose LC0 C50	
Gill	29.33	23.44	19.70	24.62	18.73	13.98
Liver	85.33	78.44	72.49	79.89	72.64	66.79
Kidney	36.00	31.14	26.56	31.30	27.45	23.50
Muscle	22.32	17.54	12.65	18.61	14.26	10.05

Table:5. Changes in lipid content in different tissue *L rohita* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0		Control	Chronic dose LC0	
		LC50	LC50		LC50	LC50
Gill	11.95	9.36	5.71	9.45	6.43	3.57
Liver	11.64	8.66	5.65	9.24	6.26	3.72
Kidney	8.54	6.31	3.42	6.14	4.13	2.15
Muscle	9.10	6.42	3.67	7.35	5.29	2.50

Table:6 Changes in lipid content in different tissue *C mrigala* after acute and chronic exposure to sodium fluoride (NaF).

Tissue	Control	Acute dose LC0		Control	Chronic dose LC0	
		LC50	LC50		LC50	LC50
Gill	13.55	10.50	7.52	11.54	8.65	6.64
Liver	14.65	9.97	6.93	10.02	7.93	5.04
Kidney	8.64	7.49	3.85	7.62	5.61	2.61
Muscle	10.43	7.49	4.47	8.85	6.50	3.82

After acute (96h) and chronic (30 days) of exposure of *L rohita* and *C mrigala* to sub-lethal and lethal (LCo-LC50) concentration of the total protein content in muscle, liver, gill and kidney were decreased significantly ($p < 0.001$) in both groups of exposed fish in comparison to the control group (Table 1 and Table 1.1). The glycogen content was significantly in gill and kidney at sub-lethal (LCo) concentration of sodium fluoride, but the decreased was not significant in lethal concentration (LC50) of sodium fluoride. At higher sodium fluoride concentration however glycogen increased significantly in liver and muscle and decreased in gill and kidney Table 2 and 2.1. The lipid content in liver, gill, muscle and kidney was also significantly decreased in both group as compare to the control group (Table 3 and 3.1).

Conclusion

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 the sodium fluoride (NaF) concentration in sub-lethal (LCo) decreased significantly in liver, muscle, gill, and kidney while in lethal concentration (LC0) 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 locomotory 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* and *C mrigala* in sub-lethal (945 ppm.) and lethal concentration in (960 ppm.). The decrease 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 function. Sodium fluoride is well known as an inhibitor of various enzyme like lipase, phosphatase, and esterases. The interference of sodium fluoride was also observed in fatty acid oxidation and inhibit 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 these 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* and *C mrigala*.

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