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Acute toxic effect of sodium fluoride (NaF) on glycogen, protein and lipid contents in juvenile and adult freshwater carp fish *Labeo rohita*

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ABSTRACT

Fluoride is a naturally occurring material and also as runoff in aquatic ecosystems, resulting in defilement. The concentration varies in lotic and lentic environments, and it is not safe to have potability if it is more than 1.5 mg/l. If it exceeds that level, fluorosis bone adsorption results in humans. The ambient water level of the fluoride poses a threat to all aquatic organisms, especially fish. Humans are linked ecologically to trophic levels, so biomagnification due to bioaccumulation is possible. The toxicity depends on various factors and species-specific and also due to varied hydrographical conditions, the fish *Labeo rohita* is tested with sodium fluoride (NaF) in the laboratory. *In vivo* experimented fish, after exposure to a specific concentration of 20ppm for 7 days, are assayed *in vitro* of certain biochemical parameters viz., glycogen, proteins and lipids. Due to the toxic effect, the total proteins showed decrement and are in the order of Muscle > Liver > Kidney; for Glycogen and lipids liver showed significant reduction compared with the other two tissues.

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1. Introduction

Aquatic ecosystems, encompassing rivers, lakes, streams, and other freshwater bodies, are vital habitats supporting a wide array of organisms; these ecosystems are sensitive to various pollutants, including fluoride, which can harm their health and trophic balance. Fluoride is a naturally occurring mineral in water sources at varying concentrations. While it is generally safe for human consumption at recommended levels (< 1.5mg /l), high concentrations of fluoride (> 1.5mg /l) can pose risks to aquatic organisms and disrupt the delicate ecological dynamics of these ecosystems (Rajinder Kaur *et al*., 2017)

Fluoride can enter aquatic ecosystems from natural sources such as weathering of rocks and minerals, volcanic activity, and leaching from soil (Ali et al., 2016; Koga and Rose-Koga, 2018). Human activities, including industrial processes, agricultural runoff, and discharges from wastewater treatment plants, can also contribute to elevated fluoride levels in water bodies (Paul *et al.,* 201; EFSA, 2013).

Aquatic organisms, including fish, amphibians, invertebrates, and aquatic plants, can be adversely affected by fluoride toxicity; the extent of harm depends on factors like species sensitivity, life stage, exposure duration, and fluoride concentration. Elevated fluoride levels can lead to reduced growth, developmental abnormalities, changes in behaviour, impaired reproduction, increased mortality rates, and alterations in the overall ecological dynamics of the ecosystem (Sandhu *et al.,* 2019). Fluoride has the potential to accumulate in the tissues of aquatic organisms over time. Fish and other organisms that reside higher in the food chain can accumulate higher fluoride levels through consuming contaminated prey or exposure to fluoride-rich water (Piero *et al,* 2014). This bioaccumulation can magnify the toxic effects of fluoride and affect the health of higher trophic levels (Camargo, 2003; Yaseen *et al.,* 2017).

Fish is one of the most common foods in regular diet in many parts of India and is a staple of many other nations' diets. The accumulation of fluoride in fish causes fluoride to enter human bodies. According to studies, a limited quantity of fluoride is necessary to prevent tooth decay, but too much fluoride can have negative effects on the health of all living things, including teeth (Arthur, 1971)[10]. Fluoride toxicity can disrupt the intricate food chains within aquatic ecosystems. The adverse effects on primary producers, such as algae and aquatic plants, can propagate through the system, affecting both herbivorous and carnivorous organisms. Changes in species composition, population sizes, and community interactions can occur, leading to ecological imbalances and reduced biodiversity (Sujatha, *et al*., 2020).

Labeo rohita is the important food fish that belongs to the carp family. There are three important species of Indian major carps (IMC), including *Labeo rohita, Catla catla, and Cirrhinus mrigala.* Due to its attractive flavor, *Labeo rohita* is the most preferred fish on the market. It is the most adaptable and significant species of fish raised in Bangladesh, India, Pakistan, and Myanmar because it can withstand a greater variety of temperatures and all the three are both capture and cultivable fish.

The present study was aimed to assess the toxic action of sodium fluoride on certain biochemical alterations viz., glycogen, total proteins and lipids in tissues of liver, kidney, and muscle of different age groups of a freshwater carp *Labeo rohita.*

2. Materials and Methods

The fresh water fishes *Labeo rohita* measuring about 4 to 6 cm (2 months of age) and 10 to 12 cm (6 months of age) in length were collected from fish seed rearing center. The collected fish were acclimatized under laboratory condition at 28-30°C, pH is 8.2 slight alkalinity, Dissolved oxygen 8 – 10 ppm for 7 days. During the period of acclimatization, the water was changed daily and the fish were fed rice bran and groundnut oil cake. Then the fish were divided into different groups having a batch of 10 fish in each. Group I contains 2-month age (4 to 6 cm length) controls, Group II contains 2 months' age group fish about 4 to 6 cm in length as a batch experimental, Group III contains 6 months' age group fish about 10 to 12 cm in length controls and Group IV contains 6 months' age group fish about 10 to 12 cm in length experimental. All are maintained in separate 10 lt. plastic containers. The test chemical sodium fluoride was supplied by BDH Chemical division Bombay. The fish of batch II and IV are exposed to 20 ppm sodium fluoride (NaF) and acute toxicity experiment was conducted for 72hrs by Finney (1971) probit analysis and the LC_{50} value is 220.347mg/l. After acute exposure the fishes for 7 days were sacrificed and the tissues liver, kidney and muscle are collected for the estimation of selected biochemical parameters, viz., glycogen, total proteins and lipids which are estimated by the method of Carroll *et al*., (1956), Lowry *et al*., (1951), Folch *et al*., (1957) respectively.

Table 1. Changes in Glycogen content in different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Tissue	Groups			
	Group I (Juvenile control)	Group II (Juvenile experimental)	Group III (Adult control)	Group IV (Adult experimental)
Liver	13.78 ± 0.054	7.84 ± 0.362	17.23 ± 0.852	10.45 ± 0.523
		-43.11		-39.43
Kidney	9.67 ± 0.158	5.84 ± 0.362	11.23 ± 0.545	7.04 ± 0.382
		-39.3		-37.31
Muscle	13.78 ± 0.054	7.86 ± 0.158	13.68 ± 0.251	9.591 ± 0.436
		-42.96		-29.89

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± SD, Values are significant at P < 0.05

Table 2. Changes in total protein content different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Tissue	Groups			
	Group I (Juvenile control)	Group II (Juvenile experimental)	Group III (Adult control)	Group IV (Adult experimental)
Liver	18.63 ± 0.521	10.53 ± 0.862	23.87 ± 0.342	16.645 ± 0.366
		-43.47		-30.28
Kidney	12.37 ± 0.452	8.04 ± 0.023	17.54 ± 0.126	8.657 ± 0.365
		-35		-50.68
Muscle	19.75 ± 0.283	12.146 ± 0.648	25.35 ± 0.142	14.759 ± 0.681
		-38.53		41.83

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± S. D. values are significant at P<0.05

Table 3. Changes in total Lipid content different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Tissue	Groups			
	Group I (Juvenile control)	Group II (Juvenile experimental)	Group III (Adult control)	Group IV (Adult experimental)
Liver	9.68 ± 0.232	4.74 ± 0.478	12.85 ± 0.442	9.54 ± 0.123
		-51.03		-25.75
Kidney	7.56 ± 0.642	3.42 ± 0.143	10.67 ± 0.468	6.36 ± 0.842
		-54.76		-40.39
Muscle	8.67 ± 0.478	3.97 ± 0.372	9.46 ± 0.213	5.78 ± 0.632
		-54.2		38.9

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± S. D. values are significant at P<0.05

3. Results and Discussion

The alteration in glycogen, protein and lipid were determined in the control as well as experimented fishes after exposure to sodium fluoride for seven days at 20ppm. The significant changes were observed in the experimental fish. The carbohydrate content in different tissues of *Labeo rohita* was in the order of liver > muscle > kidney (Fig. 1). The protein content from all the tissues decreased significantly (Fig. 2). Muscle showed the greatest loss of protein as compared to all other tissues (Fig. 3). The rate of decrement is more in Group II fishes than Group IV, Juveniles are effected more than adults.

It's worth noting that fluoride concentrations found in most water sources, such as tap water, are typically well below levels that would cause acute toxicity to aquatic organisms. However, in areas where fluoride concentrations are

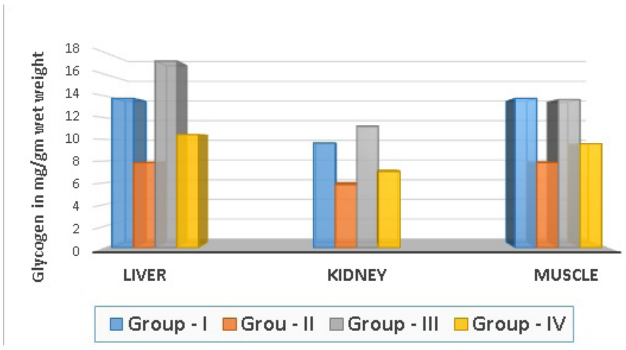

Fig. 1. Changes in Glycogen content in different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Results are the mean values of five observations and the standard deviation, significant at p.0.05 level; "T" test

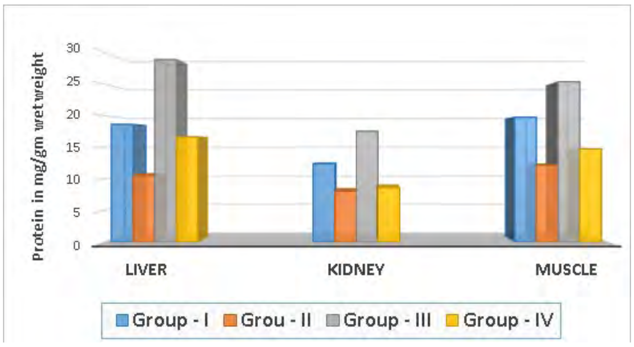

Fig. 2. Changes in total protein content in different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Results are the mean values of five observations and the standard deviation, significant at p.0.05 level; "T" test

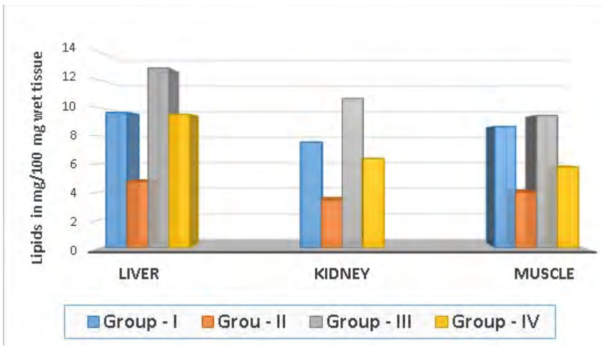

Fig. 3. Changes in total Lipid content in different tissues of *Labeo rohita* after acute exposure to sodium fluoride

Results are the mean values of five observations and the standard deviation, significant at p.0.05 level; "T" test naturally elevated or due to anthropogenic sources, the potential act of toxic action increases. Proper monitoring and management of fluoride levels are crucial to protect the health of the aquatic ecosystems, High fluoride concentrations can adversely affect the fish health. Fish may exhibit symptoms such as reduced growth, developmental abnormalities, changes in behavior, and increased mortality rates. Fluoride can also accumulate in fish tissues over a time period, potentially affecting the entire aquatic food chain.

Fluoride primarily act on for poison the enzymes and thus prevents their activity. The various essential metabolic processes are disturbed with in the cells/tissues/organs. Some such metabolic processes like glycolysis etc., are essential for maintaining normal physiology of fishes (Barbier *et al.*, 2010). Significant alteration in the carbohydrate metabolism including the decreased glycogen content and reduced activities of enzymes involved in the glucose metabolism (glycolysis) of sub lethal fluoride exposure in freshwater fish *Channa punctata* was reported by Adhikari *et al*., (2018).

Sarkar *et al*., (2017) reported in the fish *Channa punctata* due to Sublethal exposure caused alternations in the activity of enzymes involved in glycogen synthesis, glycogenolysis, and gluconeogenesis, indicating disruptions in carbohydrate metabolism.

The effects of fluoride exposure on carbohydrate metabolism and oxidative stress in the gill tissues of *Cyprinus carpio* (common carp) indicated disturbances in carbohydrate metabolism too, including reduced glycogen content and also altered enzyme activities involved in glucose metabolism by Kaur *et al*., (2020) as in the present study.

Alterations in protein metabolism, as decrement, disrupted amino acid profiles, and also changes in enzyme activities that are related to protein synthesis and finally degradation reported in fresh water fish *Oreochromis mossambicus* exposed to fluoride toxicity by Magesh and Manju (Magesh and Manju, 2017), and also by the decreased protein content of muscle, liver and kidney observed in the fish exposed to sodium fluoride by Gupta (2003) as of the present study. Alterations in protein content, changes in amino acid profiles, and disruptions in enzyme activities associated with protein synthesis and degradation were observed in a common carp *Cyprinus carpio* with the effects of sublethal fluoride exposure (Wijayawardena *et al.,* 2016).

The acute effects of sodium fluoride on protein metabolism in the common carp demonstrated changes in protein metabolism, proteolysis including alterations in protein content qualitatively, modifications in amino acid profiles, and disruptions in the enzyme activities involved in protein synthesis and degradation (Alarcon *et al*., 2014) Zebrafish (*Danio rerio*) exposed to chronic fluoride toxicity, it is observed that alterations in protein expression patterns, indicating potential disruptions in protein metabolism (Watanabe *et al.,* 2020).

Impact of fluoride exposure on lipid peroxidation and the antioxidative system in the freshwater fish *Channa punctata* revealed increased lipid peroxidation levels and disruptions in antioxidant enzyme activities, indicating potential disturbances in lipid metabolism (Hasan *et al*., 2016). Effects of sublethal fluoride exposure on the antioxidant defense system and lipid peroxidation in the liver of common carp indicated alterations in antioxidant enzyme activities and increased lipid peroxidation levels, suggesting potential disruptions in lipid metabolism (Wijayawardena *et al.,* 2017).

Sodium fluoride (NaF) interferes with various metabolic activities and biochemical changes are observed in the level of protein, glycogen, and lipid content in experimental fish *L rohita* (Kale, 2020).

4. Conclusion

Fluoride toxicity poses a significant risk to aquatic ecosystems, potentially impacting the health and balance of these vital habitats. Elevated fluoride concentrations can have adverse effects on various organisms, disrupt food chains, impair ecosystem functions, and reduce biodiversity. Proper management, regulation, and monitoring of fluoride levels all are crucial to mitigate the potential harm and ensure the long-term sustainability of freshwater ecosystems. 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 Carbohydrate, protein and lipid content in experimental fish *L rohita*. The changes are more pronounced in juvenile experimental fishes compared to adult fishes, it is concluded that juvenile *L rohita* fishes are more sensitive to fluoride toxicity. Toxicity values are different and juveniles are sensitive than adults. But Quantitatively adults have more biochemical aspects such as glycogen, proteins and lipids.

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