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Sodium fluoride induce alterations in glycogen metabolism in freshwater catfish, *Clarias batrachus* (Linn.)

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Abstract
Effect of fluoride toxicity on glycogen metabolism in fresh water catfish *Clarias batrachus* was evaluated after their exposure to two sublethal concentrations of fluoride (35 mg F ion/L and 70 mg F ion/L) for 60 days and 90 days. Alterations in glycogen content in muscle, liver, and testis tissues were recorded. The insignificant decreased level of glycogen content was recorded in muscle and liver tissues at lower concentration of F in both durations. However its elevated level was recorded at higher concentration of F in both tissues at both durations. The duration and concentration dependent depleted glycogen level was recorded significant (P<0.01) in testis tissues at both durations and concentrations but it was found highly significant (P<0.001) at higher concentration of F after 90 days.

Keywords: fluoride toxicity, glycogen, metabolism, soft tissues, muscle, liver, testis, catfish (*Clarias batrachus*)

Introduction
Fluoride (F) is naturally occurring compound found in the earths crust which enters the ground and surface water through natural and anthropogenic activities. Anthropogenic sources include atmospheric deposition of emissions from coal-fired power plants, glass and ceramic industries and agricultural fertilizers mainly phosphate fertilizers (Madhavan and Subramaniun, 2001) [1]. Fluoride toxicity is a global issue now becoming more widely recognized. Aquatic life is continuously exposed to high concentrations of Fluoride in surface waters and harmful effects ensue when Fluoride enters the food chain (Camargo, 2003) [2]. Fluoride tends to be accumulated in exoskeletons, soft tissues and bones of fish (Chowdhury *et al.*, 2018) [3] and other aquatic animals. The F ion acts as enzymatic poisons, inhibiting enzyme activity and interrupting metabolic processes, such as glycolysis and protein synthesis (Barbier *et al*., 2010) [4]. The toxic effects of elevated concentration of Fluoride on the aquatic animals are well documented by Kumar *et al.* (2010) [5]. Narwaria and Saksena (2012) [6] Saxena *et al.* (2001) [7] and Tripathi *et al.* (2004 and 2006) [8, 9]. Several other workers earlier reported that F affect metabolism of biomolecules in mammals including rabbit (Shashi *et al.* (1992) [10]. mice (Chinoy and Sequeiria,1989) [11] and guinea pigs (Chinoy *et al*.,1997).[12] However, studies on F ions toxicity to fish are relatively limited (Chitra and Rao,1980; Chitra *et al.*,1983; Gupta, 2003 and Kumar, 2005; Kumar *et al*., 2007) [13 -17].
Glycogen is the chief reserve food material stored mainly in liver and muscles and provides energy for metabolic activities. Thus present study was planned to evaluate and understand the effect of F ion on glycogen metabolism and its turnover in muscles, liver and testis tissues under exposure to F stress in freshwater catfish *Clarias batrachus*.

Materials and Methods
Healthy living specimens of *Clarias batrachus* (weight 50±5 g, length 16±5 cm) were collected from local freshwater resourses in Lucknow and maintained under standard laboratory conditions for 15 days. The experiment was conducted in glass aquaria measuring 60 x 40 x 45 cm. In the experiment fish were divided into three groups with 20 fish per group. Group I serve as control while the group II and III serve as exposed groups containing

sublethal concentrations of F, 35 mg F / L and 70 mg F / L of water respectively. The source of Fluoride was NaF (ER grade) obtained from Qualigens Fine Chemicals Ltd., Mumbai, India. The stock solution of NaF containing 10 g F ions / L was prepared by dissolving 22.11 g of NaF / L of distilled water.

The experiment was conducted upto 90 days , during which fish were fed with goat liver once in a day and the water of the aquaria was renewed on alternate days. After 60 days of experimentation, 10 fish from each group were sacrificed for sampling. Its muscle, liver and testis tissues were dissected out and pooled for glycogen estimation. Glycogen was estimated by standard methods given by Montgomery (1957) [18]. The same procedure was repeated after 90 days of experimentation. Estimated glycogen content data were presented in mean and standard error and stastically analysed significant by using student t test.

Results

After exposure catfish, *Clarias batrachus* to sublethal concentrations of F ions for 60 and 90 days, alterations in glycogen content in muscle, liver and testis were recorded.

Muscle glycogen: The insignificant decreased glycogen contents were recorded in both durations at lower concentration of F ions. However its level increased significantly ($P<0.01$) in higher concentration of F ions in both durations (Table 1, Figure 1).

Table 1: Level of glycogen content (mg/g wet tissues) in muscle, liver and testis tissues of *C. batrachus* after exposure to NaF.

Parameters	Exposure Time (Days)	Experimental setup		
		Group I (Control)	Group II (35 mg F/L)	Group III (70mg F/L)
Muscle glycogen (mg/g wet tissue)	60	0.87 ± 0.16	0.72 ± 0.13	1.72 ±0.14*
	90	0.85 ± 0.12	0.52 ±0.10	1.85±0.10*
Liver glycogen (mg/g wet tissue)	60	20.82 ± 1.01	18.24 ± 0.68	25.92 ± 0.93*
	90	20.95 ±1.02	18.12 ± 1.20	28.02 ±1.10*
Testis glycogen (mg/g wet tissue)	60	9.12 ± 0.85	6.02 ± 0.83*	5.60± 0.62*
	90	9.25 ± 0.70	4.85 ± 0.52*	3.02± 0.54**

(Values are mean ± SE, n= 6, Compared with control *$P<0.01$, **$P<0.001$)

Liver glycogen: The liver glycogen content was also decreased insignificantly in both durations at lower concentration of F ions and increased significantly ($P<0.01$) at higher concentration of F ions in both durations (Table 1, Figure 2).

Testis glycogen: Duration and concentration dependent depleted glycogen content in testis tissues were recorded significantly ($P<0.01$) in both groups and durations but it was recorded highly significant ($P<0.001$) at higher concentration of F ions after 90 days exposure (Table 1, Figure 3)

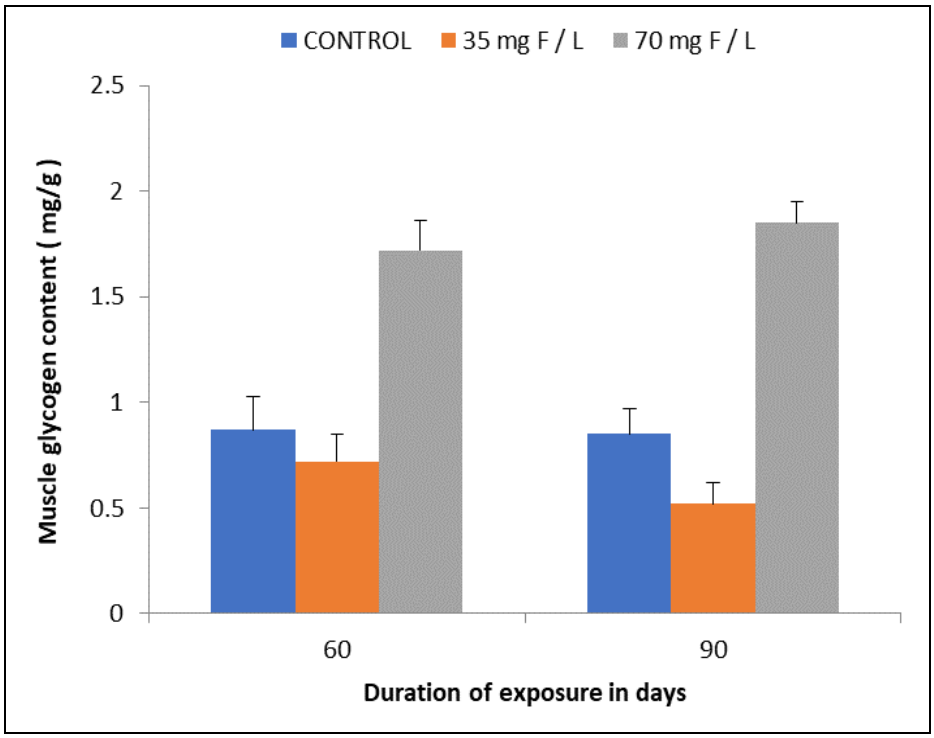

Fig 1: Effect of NaF on muscle tissue glycogen content

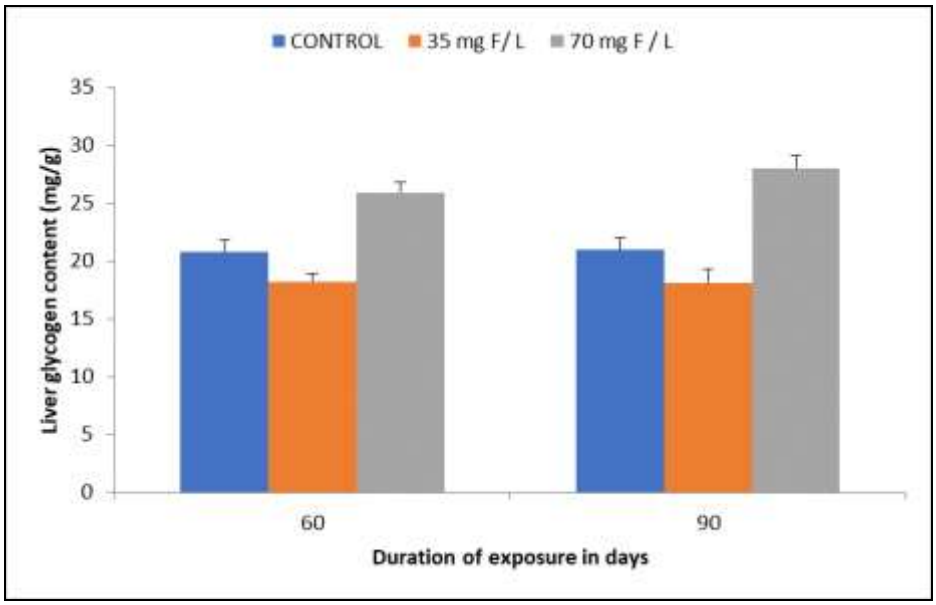

Fig 2: Effect of NaF on liver tissue glycogen content

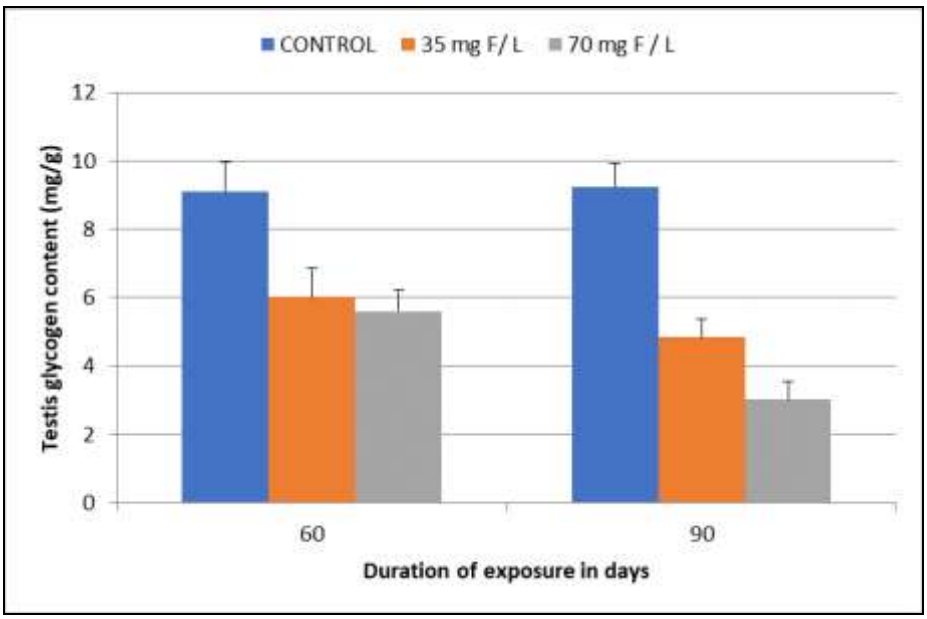

Fig 3: Effect of NaF on testis tissue glycogen content

Discussion

Fluoride is a potent inhibitor of many enzymes and capable of altering several enzymatic activities and cellular metabolism (Den, 1986 and Sharma *et al*., 2008).[19, 20] The insignificant depletion in muscle and liver glycogen content recorded in present investigation in fishes exposed to lower concentrations of NaF might be attributed to effect of Fluoride, it impairs glycolytic enzymes responsible for catabolism of glycogen to glucose during stress and fish uses other biomolecules (protein/ lipid) for energy to maintain normal body functions. Similar observations were earlier reported by Chitra and Rao (1980) [13] in *Channa punctatus* after exposure to NaF. Kasthuri and Chandran (1997) [21] have also made a similar suggestions in their study with *Mystus gulio* exposed to lead.

The elevated glycogen content in muscle and liver tissues at higher concentration of F may be due to disturbance of carbohydrate metabolism as it has been observed that F affect enzymes involved in glycogen turnover (Strochkova and Zhavorankov, 1983) [22]. Several studies have revealed that Fluoride inhibit many glycolytic enzemes (Barbier *et al*., 2010 and Den,1986) [4, 19]. Mendoza-Shulz *et al*. (2009) [23] suggested that millimolar concentrations of Fluoride act as an enzyme inhibitor on phosphatases which play an important role in the ATP production cycle and cellular respiration.

The significant decreased testis glycogen content in both durations and concentrations exposed fishes can be attributed to disturbed glycogen turnover from liver to testis due to inhibition of hormones and enzymes by F ion. Many studies have revealed that Fluoride depressed glycogen turnover (Zebrowski and Suttie, 1966) and Gupta and Poddar, 2014) [24, 25]. Tilak *et al*. (2005) [26] suggested that pesticide inhibited hormones which contributed glycogen synthesis.

Conclusion

On the basis of observations, it is clear that F ions act as enzymatic poisons, inhibiting enzyme activity and ultimately interrputing metabolic processes such as glycogen metabolism in *C. batrachus* that responsible for physiological activities, survival, growth and reproduction.

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