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0IMPACT OF SODIUM FLUORIDE (NaF) ON DIGESTIVE ENZYME OF FRESH WATER FISH CIRRHINUS MRIGALA

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Abstract:

The natural and manmade contamination of aquatic environment has become a serious problem for the sustainability of fresh water fisheries sector in India.. Ever increasing water pollution level, especially sodium fluoride (NaF), in natural and inland freshwater reservoir condition has made significant biochemical changes in the fishes. Their toxicity can be assessed by using enzymes as biomarker. These enzymes protect the living organisms from the oxidative stress caused by the sodium fluoride. Therefore, the effect of NaF on the digestive enzyme activity in the tissues (intestine) of *Cirrhinus mrigala* was investigated. In view of this, the investigations on effects of acute and chronic sodium fluoride toxicity to fish *Cirrhinus mrigala* have been carried out. The changes in amylase, protease and lipase enzymes of intestine were examined. The study revealed the activity of these enzymes in fish decreased significantly as compared to control.

Keywords: Sodium fluoride; biochemical; Amylase, Protease, Lipase, Intestine Fish

Introduction

The literature on the influence of fluoride on enzyme systems is overwhelming. Both activating and inhibiting effects of the fluoride ions on enzymes are described. The fluoride ions may exert a direct action on enzyme but, more frequently, the effect is indirect by complexing with metal of enzymes. Work of Hodge and Smith (1965), Taves (1970), Wiseman (1970), USEPA (1980), SOU (1981) suggest that, low fluoride concentrations (about 10µmol F/lit, i.e. 0.18 mg F/lit) in serum will stabilize and activate several isolated as well as membrane bound enzyme systems. Higher concentrations of fluoride (at least 0.3 mg F/liter) in serum will inhibit many enzymes. A pyrophosphatase is inhibited by about 5%, at 0.4 mg F/lit, a level that is higher than that found in plasma of an individual with a skeletal fluoride content of 6000 mg/kg and exposure to drinking water levels of 19 mg F/lit. (Ericsson *et al.*, 1973). Fluoride acts as an activator for adenyl cyclase (Mornslad and Van Dijken, 1982). Alkaline phosphatase activity may be increased by fluoride (Farley *et al.*, 1983) but changes in serum activity levels of this enzyme and in serum calcium and phosphate have been found to be minimal in pot room workers with skeletal fluorosis (Boillat *et al.*, 1979).

Basically amylase is a carbohydrate splitting enzyme. The enzyme acts on the 1.4 α glucoside linkage of starch and glycogen. It is classified as α-amylase and β-amylase. The α-amylase catalyses the hydrolysis of the α-amylose component of starch and splits bonds near the middle of the amylase or amylopectin molecule, yielding the products. The β-amylase hydrolyzes the same bonds but works from the ends of the chains inward, breaking off maltose segments, one after another. The 1,6 α glucosidic linkage of these branches prevent further action at that point in the amylopectin residue. Further digestion requires the activity of another enzyme, 1-6-α glucosidase in removing the branches.

Sources of amylase in fish are pyloric caecea, pancreas and intestinal mucosa. Presence of enzyme invertase was first reported by Kenyen (1925) in the intestine of blue gill (*Lepomis macrochirus*) and carp (*Cyprinus corpio*). Presence of carbohydrate digesting enzymes have been reported in alimentary canal of different fishes (Phillips *et al.*, 1948; McGeachin and Debnam, 1960; Kitamikado and Tachino, 1961a; Fish, 1962; Ushiyama *et al.*, 1966). The amylase amongst the carbohydrases in cyprinid fishes without stomach has been paid considerable attention (Kenyon, 1925). Chitra *et al.*, (1983) found that fluoride alter enzyme activity in muscle and liver of *Channa punctatus,* while Gupta, (2003) has observed that fluoride decreased glucose and protein level in blood and in muscle of these fish. The author reported entire intestine and intestinal bulb as being responsible for secretion of amylase. The activity in intestine of *Cirrhinus mrigala* was more than that of *Labeo rohita* and *Catla catla*. Agrawal *et al.*, (1975) worked on *Wallago attu, Clarias batrachus* and *Labeo rohita* and reported optimum pH value for carbohydrase between 5 to 7. The authors also reported that carbohydrase activity in herbivorous fish *Labeo rohita* was more than in carnivorous fish, *Wallago attu* and the omnivorous fish *Clarias batrachus.*

The enzyme protease catalyses the partial hydrolysis of proteins peptones and peptides, in fact, almost anything containing the peptide bond however, the rate of action is generally much higher for proteins than for small peptides. The proteases also called proteinases or

proteolytic enzymes occur almost in all cells. The mode of action of this enzyme in gastric juice, trypsin and chymotrypsin in pancreatic juice is well studied in animals, including fishes too. The stomach, intestinal mucosa, pancrease and pyloric caecea are sources of protein digesting enzymes in fish.

Kenyon (1925) reported peptic activity in stomach tissues of carp and bluegill. Babkin and Bowie (1928), Obestey (1934), Johnson, (1937), Togasawa *et al.*,(1959), Croston, (1961), Kitamikado and Tachina, (1961), Chepik, (1966), Morishita *et al.*, (1966) reported proteases in alimentary tract of different fishes. Digestion of proteins in stomachless teleosts is a matter of considerable physiological importance. Since in such fishes both pepsin and hydrochloric acid have been reported to be absent (Vonk, 1927;. The peptic activity of intestinal extracts of stomachless fishes has also been reported by some workers (Spandorf, 1954). Dhage (1968) reported more protein digestion along the proximal and distal parts of intestine of three major Indian species of carps, *Cirrhinus mrigala, Catla catla and Labeo rohita*. Agrawal *et al.*, (1975) reported optimum pH for trypsin between 6.8 and 7.8 for *Wallago attu, Clarias batrachus* and *Labeo rohita*.

The enzyme lipases catalyze the hydrolysis of esters and lipids into the individual acids and alcohols. Lipases acts on the lipids. These enzymes yield the separate components of both fats and oils for further metabolic transformations. Pancreatic lipase is fairly typical, playing an important role in animal digestive tracts. It is activated by calcium ions. Since the substrates are ordinarily not water soluble, the enzymatic action is accelerated by emulsifying agent which helps to disperse the non polar substrate molecules.

In teleosts, there are two known sources of lipases, pyloric caecea and intestinal mucosa. Lipase has been reported from the intestinal mucosa of fish (Babkin and Bowie, 1928). Lipase activity in different fishes (Kitamikado and Tachino, 1961;; Chpik, 1966; Morshita *et al.*, 1966) has also been reported. Baldwin (1967) reported lipolytic enzyme in liver which has no action on the ester. Dhage (1968) worked on digestive enzymes in three species of the major carps and reported lipase activity in intestinal bulb and proximal part of intestine and maximum activity of lipase in liver extracts. Agrawal *et al.*, (1975) worked on digestive enzymes of *Wallago attu, Clarias batrachus and Labeo rohita*. Some of the authors reported enhanced lipase activity in alkaline medium (Tripathi *et al.*, 2005; Shivkumar *et al*. 2006; Satyaparameshwar *et al.*, 2006; Loganathan *et al.*, 2006;).

Material and Method

After acute (96 h) and chronic (30 days) exposures, the alive fishes were sacrificed, the intestine was quickly removed, blotted and weighed. The pooled and weighed tissues of intestine were homogenized to make the tissue concentration of 100 mg/ml using 0.91% chilled NaCl. The homogenate was centrifuged at 3000 rpm for 10 minutes. Aliquots of supernatant were used as enzyme source.

Amylase:

The activity of enzyme was determined using 3, 5, dinitrosalicylic acid (DNSA) (Bernfield, 1955), the free aldehyed group formed due to enzymetic action on substrate reduces DNSA reagent, which was measured spectrophotometerically at 540 nm (Ishaaya and Swirski, 1970).

Protease:

The activity of protease was measured according to Euguchi and Zwamota, (1982).

Lipase:

The lipase activity was measured according to Hayashi and Tappel, (1970).

Result and Discussion

1) Amylase:

Changes in the activity of amylase in intestine of fish *Cirrhinus mrigala,* was observed when exposed to sodium fluoride in acute (96 hrs) and chronic (30 days) concentration, as compared to control.

Control of Acute:

Specific amylase activity in the intestine of fresh water fish *Cirrhinus mrigala* was 4.464 mg maltose/gm protein/h.

Experimental:

Changes in the activity of amylase in intestine of fresh water fish *Cirrhinus mrigala* exposed to sodium fluoride for 96 h (acute) is shown in Table 1,

The decrease in activity of amylase in intestine after exposure to sodium fluoride for 96 h (acute) was observed. A significant ($p<0.05$) decrease in amylase activity, (32.35%) at LC_0 concentration and (41.96 %) at LC_{50} concentration was observed.

In present investigation, significant decrease in amylase activity of fish *Cirrhinus mrigala* was observed at higher concentration of fluoride than lower one as compared to control.

Table No. 1

Changes in Amylase activity in intestine of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Intestine	4.464 ± 0.048	3.032** ± 0.023	2.391*** ± 0.033

(Enzyme activity in mg maltose/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

Control of Chronic:

The normal activity of amylase in the intestine of fish *Cirrhinus mrigala* for chronic exposure was found to be 4.473 mg maltose/gm protein/hrs.

Experimental:

The changes in amylase activity in the intestine of *Cirrhinus mrigala* due to chronic exposure to sodium fluoride (30days) at the concentration of $1/20^{th}$ (48 ppm) and $1/10^{th}$ (96 ppm) are shown in Table 18. The amylase activity in the intestine of fish was significantly (p < 0.05 to 0.001) decreased, (10.50%) at $1/20^{th}$ and (38.57%) at $1/10^{th}$ concentration of sodium fluoride as compared to control (Table 2).

Table No. 2

Changes in Amylase activity in intestine of *Cirrhinus mrigala* after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		1 / 20^{th} of LC_{50} (48.00ppm)	1 / 10^{th} of LC_{50} (96.00ppm)
Intestine	4.591 ± 0.049	3.185** ± 0.044	2.748*** ± 0.020

(Enzyme activity in mg maltose/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

2) Protease:

The effect of sodium fluoride on protease activity in intestine of *Cirrhinus mrigala,* after acute exposure, is given in Table 3.

Control of Acute:

Specific activity of protease in *Cirrhinus mrigala* was found to be 1.707 mg tyrosin/gm protein/h in intestine.

A significant decrease in protease activity in intestine due to toxicity of sodium fluoride was observed (Table 3)

Experimental:

Changes in protease activity in intestine of fish *Cirrhinus mrigala* due to exposure to sodium fluoride for 96h was found to decrease significantly. There was 20.27 % decrease at LC_0 (935ppm) and 38.96% at LC_{50} (960ppm) concentrations, as compared to control (table 3).

In general, the activity of protease decrease in higher concentration of sodium fluoride than lower as compared to control was observed.

Table No. 3

Changes in Protease activity in intestine of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Intestine	1.707 ± 0.035	1.067* ± 0.016	0.880* ± 0.014

(Enzyme activity in mg tyrosin/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

Control of Chronic:

The effect of sodium fluoride on protease activity in intestine of *Cirrhinus mrigala*, after chronic (30 days) exposure is given in Table 4.

Experimental:

Due to exposure to sodium fluoride toxicant, a significant decrease in protease activity in intestine was observed. This decrease was 14.44% at 1/20th (48.00 ppm) and 37.56% at 1/10th (96.00 ppm) concentration of LC_{50}.

In general, protease activity was decreased in the intestine. The change in activity was significant at higher concentration (960ppm) of sodium fluoride than lower (935) as compared to control.

Table No. 4

Changes in Protease activity in intestine of *Cirrhinus mrigala* after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		1 / 20th of LC_{50} (48.00ppm)	1 / 10th of LC_{50} (96.00ppm)
Intestine	3.831 ± 0.36	2.342** ± 0.32	1.422*** ± 0.43

(Enzyme activity in mg tyrosin/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

3) Lipase:

Changes in lipase activity in intestine of *Cirrhinus mrigala*, after acute exposure to sodium fluoride is shown in Table 5, **Control of Acute:**

The normal specific activity of lipase in intestine of *Cirrhinus mrigala* was 1.411 mg palmatic acid/ gm protein/h.

Experimental:

After acute (96 h) exposure of sodium fluoride concentrations, there was a significant decrease in lipase activity in intestine of freshwater fish *C. mrigala*. The decrease was 13.61% at LC_0 (935ppm) and 33.03% at LC_{50} (960ppm) concentration of sodium fluoride.

In general, the activity of lipase decrease in higher concentration of sodium fluoride than lower as compared to control was observed.

Table No. 5

Changes in Lipase activity in intestine of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Intestine	3.411 ± 0.29	1.291** ± 0.04	0.745** ± 0.03

(Enzyme activity in mg palmatic acid/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

Control of Chronic:

The normal specific activity of lipase in intestine of *Cirrhinus mrigala* was found to be 1.521gm palmatic acid/gm protein/h.

Experimental:

A significant decrease in lipase activity was observed, it was 26.50% at 1/20th (48.00 ppm) and 41.10% at 1/10th (96.00ppm) of LC_{50} concentration.

In general, lipase activity in intestine was decreased significantly at higher concentration of sodium fluoride (Table 6).

Table No. 6

Changes in Lipase activity in intestine of *Cirrhinus mrigala* after chronic exposure to sodium fluoride (30 days)

Tissue	Control	Chronic Exposure	
		1 / 20th of LC_{50} (48.00ppm)	1 / 10th of LC_{50} (96.00ppm)
Intestine	0.721 ± 0.08	0.518** ± 0.02	0.236*** ± 0.04

(Enzyme activity in mg palmatic acid/gm protein/hr) Each value is the mean of five observations.

± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***,

Conclusion

Effect of sodium fluoride on the activity of digestive enzymes in intestine of fishes was observed in decreasing order to acute and chronic concentrations in present studies. The decrease amylase activity in the intestine of both experimental fish may be due to sodium fluoride is more at higher concentrations and at the lowest concentrations. The enzyme activity showed significant decrease as compared to control. Protease activity in the intestine decreased due to acute and chronic exposures of sodium fluoride particularly at higher concentrations. The decrease in activity was more at higher concentrations (l/20th of LC_{50}) and less at lower concentrations (1/10th of LC_{50}) as compared to control. Lipase activity decreased in intestine of fishes exposed to acute and chronic concentrations of sodium fluoride. Decrease in lipase activity was less in intestine as compared to control. The decrease in activity was more marked in fishes exposed to sodium fluoride due to inhibition of amylase, protease and lipase activity. It is postulated that there may be denaturation of enzyme protein due to toxicity of sodium fluoride by alteration in protein metabolism on acetylcholinesterase activity.

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