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SODIUM FLUORIDE INDUCED HISTOCHEMICAL CHANGES IN THE LIVER OF FRESHWATER FISH, *TILAPIA MOSSAMBICA (OREOCHROMIS MOSSAMBICUS)*

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ABSTRACT
The present investigation deals with the effect of chronic treatment of sodium fluoride, on the histochemical components of the liver from freshwater fish, *Tilapia mossambica (Oreochromis mossambicus)*.This freshwater fish was exposed to the predetermined 1/10th of LC_{50} concentration (5.4ppm) of sodium fluoride in laboratory conditions for a duration of 10, 20 and 30 days. The histochemical observation revealed that the carbohydrate reserves were severely depleted particularly glycogen as well as increased acidic mucosubstances noticed. This was time dependent to sodium fluoride toxicity. As the fish, *Tilapia mossambica* is largely consumed by people, it is essential to know the effect sodium fluoride on histochemical changes from liver.

INTRODUCTION

The careless disposal of various wastes due to anthropogenic activity, the surface water is getting polluted. The industrial waste is also contributing much through their discharges mostly includes heavy metals and trace metals which are most toxic for aquatic in habitats. The entire world now -a- days facing a major problem of pollution through various sources. All sorts of media are appearing to be polluting day by day, likewise aquatic medium is also getting polluted on large scale. There are so many toxicants polluting the water and hence adversely affecting the inhabiting organisms at cellular as well as molecular levels. The aquatic environment is severely affected by different types of chemicals which are toxic to the inhabiting organisms (Kopeca *et al.*, 2006).

Discharge of effluent into freshwater system deplete the dissolved oxygen content and by interfering with respiratory metabolism cause heavy mortality (Quasim and Siddique, 1960; David and Ray, 1966; Venkataraman, 1966; Hingoroni *et al.*, 1979). Pollution of aquatic ecosystem by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic biota (Kumari and Ramkumari, 1997). Shaikh (2013) analyzed the heavy metals from the fish, *Cirrhina mrigala* from Godavari river .He has stated that these metals accumulate more in gills and muscles damaging the physiology of the fish. Anand Kumar and Tejpal Dahiya (2013) have studied the impact of zinc on liver from the fish, *Cirrihnus mrigala*. They have observed that the liver

levels decreased when exposed to zinc from *C.mrigala*. There are few reports available on the toxic effect of industrial effluent on protein, lipid and glycogen of fishes (Fujiya 1961; Mcleay and Brown, 1974, 1979; Stonner and Livingston, 1978; Saffi, 1980, 1981; Oikari and Nakari, 1985; Kumar and Gopal, 2001). Rout *et al.* (2013) studied determine the LC_{50} concentrations after exposing the fish ,*Clarius batrachus* to the lead acetate they have stated that the toxicity affect the behavior of the fish and ultimately resulted the death of certain fish.

Fluoride not only enters into the aquatic media through various human activities but also from sediment rocks. Human intervention is also equally responsible for increasing the

Fluoride content in aquatic media. The other source of fluoride excess release is through phosphate fertilizer production, aluminum smelting etc. (Environment Canada, 1972). Fluoride is one of the components, which are toxic at high concentrations. The toxic effects of elevated fluoride on various aquatic species, humans, livestock and plants are well documented. (Gikunju, 1992; Dwivedi *et al.*, 1997; Mariappan *et al.*, 2000; Camargo, 2003). Giri *et al.*, (2013) have evaluated the sub acute exposure of sodium fluoride to the male wistar rats and studied the impact on male reproductive system. They have stated that sodium fluoride significantly affects the cytoarchitecture of the spermatozoa and ultimately influences adversely the male reproductive system.

Fishes are considered as index organism for assessment of toxicological studies. Bioassay studies of any toxicant will give

the idea of intensity of pollution of a given component. The trace metal, Fluoride if it is above permissible level, it will deleterious impact on aquatic fauna. However, the information regarding assessment of the toxicity of sodium fluoride and its impact on freshwater fish, *Tilapia mossambica* was not on record .The main objective of the present work is to understand the impact of sodium fluoride on one of the most popular edible freshwater fish,*Tilapia mossambica* from Bheema river, at Takali, Solapur (Maharashtra) on the histochemical changes from important organ, the liver. In present investigation histochemical changes have done carried out to understand the extent of toxicity of sodium fluoride on the important organ, the liver.

MATERIALS AND METHODS

The fishes, *Tilapia mossambica* were collected from Bheema River at Takli (Solapur district). It is located on the south side of the Solapur. It is 28 km away from Solapur city and soon after the fishes were brought to the laboratory. They were maintained in aerated glass aquaria and acclimatized for four weeks to the laboratory conditions. During acclimatization declorinated water was used. Every day, the water was changed twice and excreta and debris were removed. During the period of acclimatization the fishes were fed with commercial fish food every day. Fishes were exposed to natural photoperiod.

Sodium fluoride was used in the present study for experimentation. The predetermined LC_{50} value $_{of}$ sodium fluoride at 96 hours was found to be 54ppm. For the determination of LC_{50} 96 hrs the standard method described by (Finney, 1971) was used. Acute toxicity tests were conducted and 96 hrs LC_{50} value was established. Feeding was stopped 24 hr before to the chronic toxicity testing .The well acclimatized, healthy fishes weighing 10-15 gm and 5-8 cm in length were selected for experimentation and were divided into four sets each containing 10 fishes. The fish, *Tilapia mossambica (O.mossambicus)* were exposed to $1/10^{th}$ of LC_{50} concentration (5.4ppm) in three sets of sodium fluoride *i.e.* 10 day, 20 day and 30 days and fourth set act as control.

At the end of each exposure time, fishes were dissected to isolate the liver from control and $1\backslash10^{th}$ of LC_{50} concentration of sodium fluoride. They were fixed into carnoy's fixative from the experimental as well as control during summer season. The chronic tests were carried for a duration of 30 days and the liver samples for histochemical fixation samples were drawn at the interval of 10, 20 and 30 days along with control group. The tissues were fixed into carnoy's fixative for 2-3h. The fixation of tissue was followed by washing under running tap water for overnight. The tissues were dehydrated using different alcohol grades and cleared in xylene. Then the tissue were transferred for cold embedding followed by hot embedding at 58ºC for one and one half hrs, respectively .The paraffin block of the tissue were prepared. The trimmed blocks were used for sectioning. The sections were cut at six micron thickness on rotary microtome and various histochemical techniques were employed.

Periodic acid Schiff's (PAS) technique was use for detection of glycogen described by Mc Manus, (1946) and Hotchkiss, (1948). Alcian blue (AB) at pH 1.0 technique was used described by Lev and Spicer (1964).

The physicochemical parameters of the water used for experimentation and for control were analyzed during the toxicity test according to APHA (1980) and mentioned in Table 1 for summer season.

Table 1: Physico-chemical parameter of water used for the experimentation

Season	Temperature ºC	pH	DO (mg/L)	Total hardness (mg/L)
Summer	28-30	7.5-8.0	5.2-5.3	110-120

RESULTS

The hepatocytes were stained intensely magenta colored to PAS staining indicating presence of glycogen in control liver (Plate 1; Fig. 1). After chronic exposure to $1/10^{th\ of}$ LC_{50} concentration on sodium fluoride of 10^{th}day, the hepatocytes showed good amount of glycogen (Plate 1; Fig. 2). After 20^{th} day, the hepatocytes, showed slight decrease in the glycogen (Plate 1; Fig. 3) and at 30^{th} day the hepatic cells showed very less amount of glycogen (Plate 1; Fig. 4).

The hepatic cell of liver showed faint blue staining indicating less amount of acidic mucosubstances in control liver when stained with alcian blue (Plate 2; Fig. 1). After chronic exposure to $1/10^{th}$ of LC_{50} concentration of sodium fluoride on 10^{th} days, the hepatic cells of liver showed slight increase in the acidic mucosubstances, sulfomucins (Plate 2; Fig. 2). While on 20^{th} day liver showed slighted increased acidic mucosubstances (Plate 2; Fig. 3). On 30 day, the hepatic cells of liver showed further increased acidic mucosubstances, sulfomucins (Plate 2; Fig. 4).

DISCUSSION

The histochemical tests revealed the localization of chemical products of cellular activity. The intensity of staining can be used for comparing the protein, lipid, glycogen content present

Table 2: Histochemical observation of mucosubstances in the control liver and $1/10^{th}$ of LC_{50} sodium fluoride intoxicated fish, *Tilapia mossambica* during summer season

Histochemical Techniques	Tissue/Cells	Animal Group Control group	$1/10^{th}$ of LC_{50} experimental group sodium fluoride exposure group Period 10days	20 days	30 days
PAS	HC	+ + + + +P	+ + + + P	+ + + P	+ + P
AB pH – 1	HC	+ B	+ + B	+ + + B	+ + + B

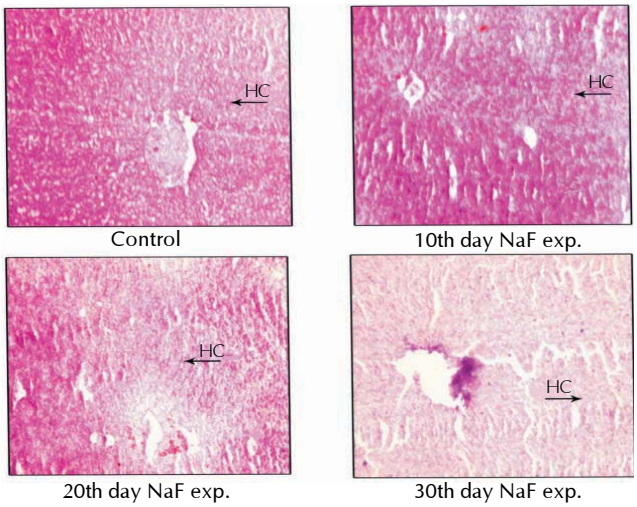

Figure 1: T. s. of liver, *T. mossambica* (400X) PAS, summer, chronic. of control, 10th, 20th and 30th days of exposure to 1/10th of LC_{50} conc. of NaF(HC - Hepatic cell)

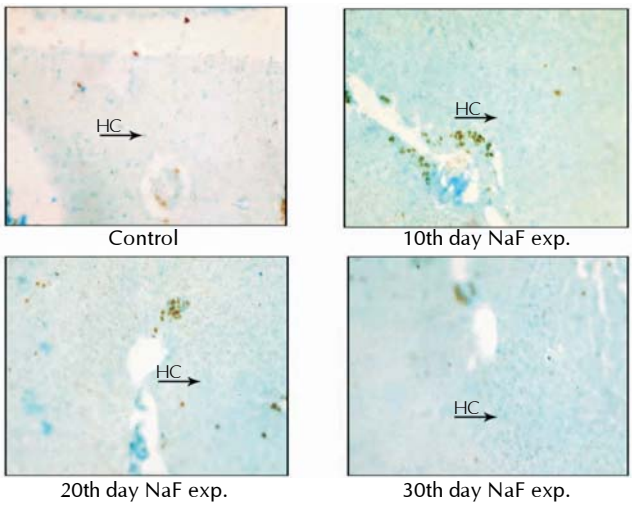

Figure 2: T. s. of liver, *T. mossambica* (400X) ABpH-1.0, summer, chronic. of control, 10th, 20th and 30th days of exposure to 1/10th of LC_{50} conc. of NaF (HC - Hepatic cell)

in the liver cells of the normal and treated fishes with sodium fluoride at different duration. The liver is the primary organ for detoxication of toxicant. Wide varieties of insecticides and several toxins tend to accumulate in high concentration within it and the liver gets damaged (Metlev, *et al.,* 1971). In the present work hepatocytes of the fish, Tilapia *mossambica* showed rich amount of glycogen in control group. Glycogen depletion in liver after exposure of $1/10^{th}$ of LC_{50} was quite evidenced which was reflected by comparing the intensity of staining property. There observed increased depletion of glycogen over time of exposure indicates that the glycogen might have decreased due to stress condition. Aquatic animal generally depend their energy source from glycogen due to intoxication of the trace metal fluoride induced maximum utility of their reserve material to combat adverse condition. Similar depletion of liver glycogen observed after methyl alcohol administration by Eletskii (1965) and reported that the glycogen content in the liver from treated rats exhibited variable changes at different dose levels. Sabry *et al.* (2009) have studied the histochemical alterations induced by the phenol from the fish *Oreochromis aureus* juveniles .They have subjected the sub-lethal doses of LC_{50} for 7 days and studied histochemical changes. They concluded that induction of phenol resulted drastic reduction in overall carbohydrates from various tissues. Depletion of liver glycogen reported in fish after exposure to pollutants (Hanke *et al.,* 1983; Gluth and Hanke, 1985; Anderson *et al.*, 1991). Reddy *et al.* (1991) reported that reduction in glycogen content in the hepatocytes of fish, *Cyprinus carpio* after administration with Fenvalerate. Krapaganapathy *et al.* (1988) reported that the carbohydrate content of liver in fish, *Colisa lalia* showed progressive decrease in staining intensity to PAS when treated at sub-lethal and median lethal concentrations of lindane treatment. They also suggested that the carbohydrates in the liver are utilized to greater extent during the stressful condition. Pathan *et al.,* (2009) showed that paper mill effluent induce reduction in glycogen from liver of freshwater fish, *Rasbora daniconius*.

The results of present investigation conclusively prove that carbohydrate reserves were severely depleted during exposure to $1/10^{th}$ of LC50 concentration of sodium fluoride in the liver of fish, *Tilapia mossambica* PAS reactivities indicate progressive depletion of glycogen in the liver of *Tilapia mossambica* exposed to chronic treatment of sodium fluoride. In the present work hepatocytes of fish, *Tilapia mossambica* showed very small amount of acidic mucosubstances in control group. The hepatocytes (hepatic cell) of *Tilapia mossambica* showed slightly increased acidic mucosubstances in liver after exposure of fishes to $1/10^{th}$ LC50 of sodium fluoride. It was quiet evident that amount and stainability was progressively increased.

Similar, increased acidic mucosubstances of liver observed after exposure to phosphate of edible fish, *Channa striatus* (Bloch) from river Krishna by (Gonjari and Mahadik, 2009). They have further reported that the acidic mucosubstances of liver of treated fish exhibited variable changes at different dose levels. They also stated that sulfomucins reactivites indicate even though these mucins are not normally synthesized and deposited in the liver, they might have been diverted from other sources. Verma and Chand (1986) observed similar histochemical alterations on *Notopterus notopterus* due to the toxic effect of mercuric chloride. In the present studies sub-lethal exposure to concentration of sodium fluoride increased the deposition of these mucosubsances, which might be a protective mechanism. Further experimentations are under way to observe enzymatic manipulations by using histochemical techniques.

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