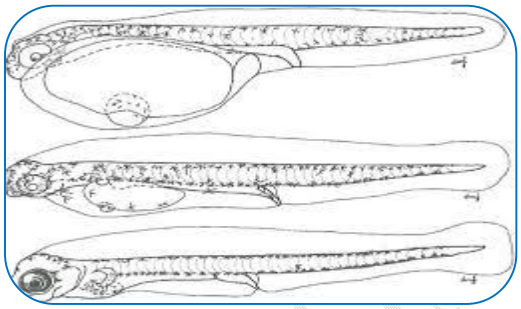
REVIEW OF RESEARCH
IMPACT FACTOR : 5.7631(UIF) UGC APPROVED JOURNAL NO. 48514 ISSN: 2249-894X
VOLUME - 8 | ISSUE - 7 | APRIL - 2019

SODIUM FLUORIDE INDUCED HISTOCHEMICAL CHANGES IN THE OESOPHAGUS OF THE FRESHWATER FISH *TILAPIA MOSSAMBICA* DURING WINTER SEASON.



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ABSTRACT :

The present investigation deals with the effect of acute treatment of sodium fluoride on the histochemical components of the oesophagus from freshwater fish, Tilapia mossambica (Oreochromis mossambicus). This freshwater fish acute toxicity for 24, 48, 72 and 96 hrs. exposure of LC_0 value 35.0 ppm and LC_{50} value was 65.0 ppm for 96 hrs. There observed increased depletion of neutral mucosubstances and glycogen and highly increased in acidic mucosubstuances, sulfomucins in oesophageal mucous cells of mucosa ,connective tissue and muscle fibres .The histochemical changes indicate sodium fluoride is toxic to growth and survival of the species .It is essential to know sodium fluoride is hazardous pollutant. It is essential to make awareness about water pollution.

KEYWORDS : *Sodium Fluoride, Tilapia mossambica, Oesophagus, Histochemistry , Winter.*

INTRODUCTION

Fluoride is one of the true metal which is toxic at higher concentration. Fluoride not only enter, 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. Fluoride is one of the components, which are toxic at high concentrations. The toxic effects of elevated fluoride on various aquatic specis, human, live stock and plants are well documented. (Gikunju, 1992 ; Dwivedi, et al., 1997; Mariappan et al., 2000; Camargo, 2003) MC Clurg (1984) studied the acute, toxicity conventional 96 hrs LC_{50} technique to the estuarine prawn penaeus indicus. He has stated that fluoride generally confined to the skeletal tissue. Where as the mercury and cadmium generally deposits in skeletal as well as non skeletal tissue. Bhatt et al., 2013 have studied alleviation of fluoride toxicity by melatonin in reproductive function of male rat. They have stated that the pineal harmone melatonin is advantageous to trounce fluoride induced reproductive toxicity in male rats. Rout et al., 2013 studied determine the LC_{50} concentration after exposing the fish, clarius batarachus to the lead acetate they have stated that the toxicity affect the behaviour of the fish and ultimately resulted the death of certain fish.

The main objective of the present work is to understand the impact 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 orgun, the oesophagus. In present investigation histochemical changes have done carried out to understand the extent of toxicity of sodium fluoride on the important organ, the oesophagus.

MATERIAL 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 K.M. 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. Everyday, they was changed twice and excreta and debries, 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 65 ppm. For the determination of LC_{50} 96 hrs. the standard method described by Finney, 1971 was used. For acute toxicity tests for histochemical study fishes were exposed to LC_0 concentration for 96 hrs. (35 ppm) and LC_{50} concentration for 96 hrs. (65ppm) 24, 48, 72 and 96 hrs. and control group.

At the end of each exposure time fishes were dissected. oesophagus were removed and fixed in carnoy's fluid for histochemistry for 2-3 hrs. and washing was done for 24 hrs after fixation. The tissue were dehydrated using different alcohol grades and cleared in xyelene. Then the tissue were transferred for cold embedding followed by hot embedding at 58^0C 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 neutral mucosubstances and glycogen described by MC Manus (1946) and Hotchkiss, (1948). Alcian Blue (AB) at PH 1.0 technique was used for detection of acidic mucosubstances (Sulfomucins) 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) & mentioned in table 1 for winter season.

Table 1
Physicochemical parameter of water used par the experimentation.

Season	Temperature 0^C	PH	No. (Ma/L)	Total Hardness (Ma/L)
Winter	25-26 ^{0C}	7.5 – 7.7	4.4- 4.5	135-140

RESULT

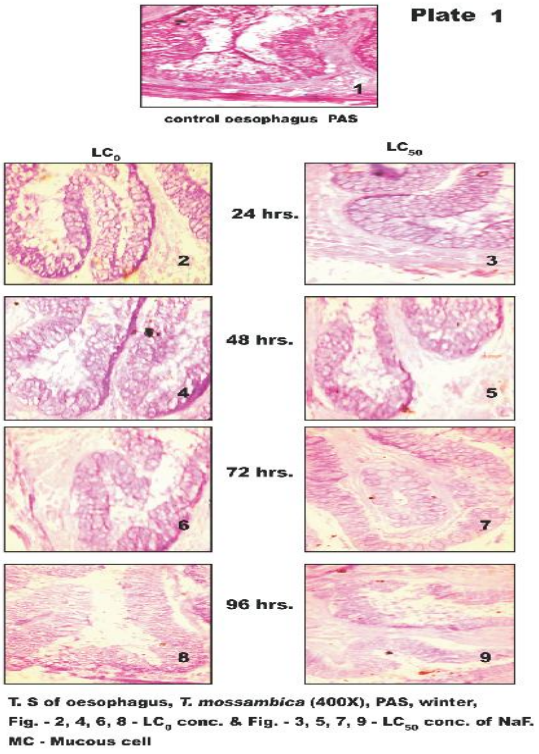
In the PAS staining mucous cells of the mucosa of esophagus showed moderate pink staining indicating intense amount of neutral mucoustances. The connective tissue and musale fibres showed modereate staining indicating moderate amount of neutral mucosubstances and glycogen in control (plate 1 fig.1). After 24 hrs of acute exposure to LC_0 concentration of sodium fluoride oesophagal cells showed decreased neutral mucosubstance & the connective tissue showed less concentration of neutral mucosubstances and glucogen (plate 1 fig.2). After 24 hrs. of exposure to LC_{50} concentration of sodium fluoride mucous cells showed more decreased in neutral mucosubstance. The connective tissue showed decreased amount of neutral mucosabstances and glycogen. (Plate 1 fig.3). After 48 hrs. of exposure to LC_0 concentration of sodium fluoride mucous cells of the mycosed showed slightly decreased neutral mucosbstance. The connective tissue showed less amount of neutral mucosubstances & glycogen. (Plate 1 fig.4). After 48 hrs. of exposure to LC_{50} concentration of sodium fluoride mucous cells of esophageal mucosa showed further decreased in neutral mucosubstance. The connective tissue showed very lesss amount of neutral mucosubstance, and glucogen. (plate 1 fig.5). After 72 hrs of exposure to LC_0 concentrating of sodium fluoride esophageal mucous cells showed reduced neutral mucosubstances. The connective tissue showed less amount of neutral mucosubstances & glucogen (plate 1 fig.6). After 72 hrs. of exposure to LC_{50} concentratinn of sodium fluoride esophageal mucous cells showed highly decreased neutral mucosubstances. The connective tissue showed very less amount of neutral mucosubstances & glucogen. (Plate 1 no.7). After 96 hrs. of exposure to LC_0 concentration of sodium fluoride further decreased in neutral mucosubstances was observed in the mucous cells of the

oesophaged mucosa. The connective tissue showed reduced amount of neutral mucosubstances and glucogen (plate 1 fig.8). After 96 hrs. of exposure to LC_{50} concentration of sodium fluoride esophageal mucus showed highly reduced neutral mucosubstance. The connective tissue showed lower concentration of neutral mucosubstances, and glycogen. (plate 1 fig.9).

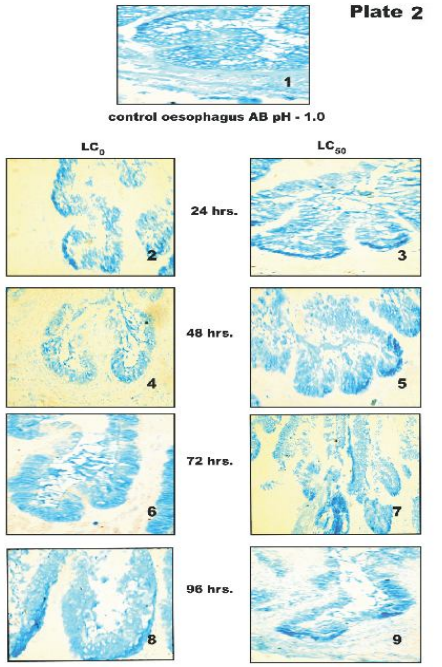
In the AB PH 1 staining technique the mucous cells of the mucosa of osophagus showed faint blue staining indicating moderate amount of acidic mucosubstances, sulfomucins. The connective tissue and muscle fibres also showed very faint blue colour indicating very less amount of acidic mucosubstance, sulfomucins control. (Plate 2 fig1).

After 24 hrs. of exposure to LC_0 concentrating of sodium fluoride mucous cells of osophagus showed slightly increased acidic mucosubstances, sulfomucins. (plate 2 fig.2). After 24 hrs. of exposure to LC_{50} concentration of sodium fluoride mucous cells showed increased acidic mucosubstances, sulfomucins. The connective tissue showed negligible amount of acidic mucosubstances. (plate 2 fig.3). After 48 hrs. of exposure to LC_0 concentration of sodium fluoride mucous cells of oesophaous showed moderate amount of acidic mucosubstances. Acidic mucosubstances increased in connective tissue. (plate 2 fig.4). After 48 hrs. of exposure to LC_{50} concentration of sodium fluoride increased acidic mucosubstances, sulfomucins were observed in the mucous cells of the oesophageal mucosa. The connective tissue showed fair amount of acidic mucosubstances (plate 2 fig.5). After 72 hrs. of exposure to LC_0 concentration of sodium fluoride increased acidic mucosubstances, sulfomucins were observed in the mucous cells of the esophageal mucosa. The connective tissue showed poor amount of acidic mucosubstances. (plate 2 fig.6). After 72 hrs. of exposure to LC_{50} concentration of sodium fluoride osophageal mucous cells showed moderate acidic mucosubstance, sulfomucins. poor AB1 activity was observed in the connective tissue. (plate 2 fig.7) After 96 hrs. of exposure to LC_0 concentrating of sodium fluoride mucous cells from mucosa of esophagus showed increased acidic mucosubstance, sulfomucins. The connective tissue showewd slightly increased in acidic mucosubstance, (plate 2 fig.8). After 96 hrs. of exposure to LC_{50} concentration of sodium fluoride highly increased acidic mucosubstance, sulfomucins were observed in the mucous cells of the esophageal mucosa. The connective tissue showed poor to moderate amount of acidc mucosbstances. (plate 2 fig.9).

Plate 1



T. S of oesophagus, *T. mossambica* (400X), PAS, winter,
Fig. - 2, 4, 6, 8 - LC_0 conc. & Fig. - 3, 5, 7, 9 - LC_{50} conc. of NaF.
MC - Mucous cell



T. S of oesophagus, *T. mossambica* (400X), AB pH - 1.0, winter, Fig. - 2, 4, 6, 8 - LC_0 conc. & Fig. - 3, 5, 7, 9 - LC_{50} conc. of NaF. MC - Mucous cell

Table – 2

Histochemical staining reaction of esophagus in control & sodium fluoride LC_0 & LC_{50} sodium fluoride intoxicated fish, Talpia Mossambic during winter season.

Sr. No.	Histochemical technique	Tissue / Cells	Control group	Animal Group							
				Experimental Groups							
				LC_0 Fluoride Exposure Period				LC_{50} Fluoride Exposure Period			
				24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
1.	PAS	MC	++++ P	+++ P	++P +P	++P	++P	++P	++P	+P	+P
		CT	++ P	+P	+P	+P	+P	+P	+P	+P	+P
		M	++P	++P		+P	+P	+P	+P	+P	+P
2	ABpH-1	MC	++B	++B -	++B +B	+++ B -	+++ B +B	++ B -	++B -	++ +B -	+++B -
		CT	+B	-	+B	-	+B	-	-	-	
		M	+B			-	+B	-		-	

MC - Mucous Cells
CT - Connective Tissue
M - Musale Fibres
++++ - Very Intense
+++ - Intense
++ - Moderate
+ - Poor.

DISCUSSION

The present investigation on the histochemical alteration evaluates the sodium fluoride stress and toxicity on stomach for acute toxicity of the fish *Tilapia mossambica.* The various mucosubstances are synthesized and temporarily stored in different cells & tissues. Depending upon location, number and type of cell the secreation of neural mucins and acidic mucosubstances is varied. It was observed that ample amount of neutral mucosubstances, glycogen proteins and lipids were present in most of the cells and tissues of controlled group of fish, which were free from the stress of sodium fluoride toxicity.

In our histochemical study it was observed that, when the fish *Tilapia mossambica* were exposed to toxic stress environment of sodium fluoride their secretion of neutral mucosubstances was decreased. Sodium fluoride altered the concentration of neutral mucosubstances, glycogen in the cells and tissues under study. These effect may be due to higher utilization of neutral mucins, to overcome the toxic stress on the cellular metabolism. On the other hand, the toxic effects of sodium fluoride caused enhancement in the concentration of acidic mucosubstances such as weakly and strongly sulfated acidic mucosubstance, sulfamucins in the cells. The quantity and quality of acidic mucosubstances was increased in the targeted cells and tissues. These were dependant upon the type of toxicant as well as time of exposure.

Mucous is an important layer protecting the epithelia either from mechanical injuries or bacterial invasion Humbert et al.,(1984). Mucus cells containing sialo and sulfoglycoproteins lead to an increase in the viscosity of secretion, which is likely to be related to a higher protectuion role Suprasert et al., 1987.

The sulphomucins present in the esophagus would confer high viscosity to the mucus, which aids in trapping small partcle, (Tibbetts,1977). Some authors have suggested that the chemically variable mucus produced by esophageal goblet cells may have a role in digestion (Reifel & Travill 1977). Neutral and acid mucus secreting esophageal cells that occur in Trichomyeterus brasiliensis (Ciro Alberto and Edith Fanta, 2000). Mucosubstances were also described by Oliveira and Silva (1971) in Cichla ocellaris by pignalberi et al., (1973) in P. albicans by Godinho et al ;(1970) in P maculates and by Sis et al ;(1979) in Icturulus Punctatus. Teleostean goblet cells are known to contain carbohydrates (Refiel and Travill 1977) without Characterizing their specific nature. Different mucosubstances found in different regions of the gut are correlated with assorted digestive functions murry et al., (1996).

In the present work, the mucous cells, connective tissue and muscle fibres of oesophagus of *Tilapia mossambicus* showed moderate depletion in neutral mucosubstances, glycogen after exposure of LC_0 concentration and more depletion in LC_{50} concentration of sodium fluoride was quite evidented in the amount & stainability. There observed increased depletion of neutral mucosubstances, and glycogen over time of exposure. The mucous cells, connective tissue and mussle fibres showed highly increase in concentration of acidic mucosubstances, after exposure to LC_0 concentration and great increase in concentration of acidic mucosubstances after exposure to LC_{50} concentration of sodium fluoride. The increase was quite evidented in the amount and stainability. There observed sharply increased acidic mucosubstances over time of exposure. Verma and Chand (1986) observed similar histochemical alteration on Notepterus notopterus due to the toxic effect of mercuric chloride Gopal and Rao (1984) also recorded similar histochemical alteration in the snail Bellamya bengalensis due to toxicity of heavy metal zinc.

ACKNOWLEDGEMENT

Authors are thankful to Principal, Walchand College of Arts and Science, Solapur for providing necessary laboratory facilities for conducting the work.

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