International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Sodium Fluoride (NaF) Induce Biochemical and Histological Alternation in Liver and Kidney of Fresh Water Fish Cirrhinus Mrigala

Milind Kale

Department of Zoology, Govt. Vidarbha Institute of Science and Humanities (Autonomous) Amravati, Maharashtra, India 444604
Email: *milindrck19[at]gmail.com*

Abstract: ***A wide range of environmental and genetic factors cause fish to respond differently to given levels of fluorides, but they do display characteristic fluoride intoxication signs. Some of the variation can also be explained by postulating a sodiuum - fluoride excretion mechanism over the epithelial tissues. Such a mechanism would explain variations in toxicity correlated with different concentrations and the survival of natural populations of fish at sodium fluoride concentrations which are lethal under laboratory conditions. Ever increasing water pollution level, especially sodium fluoride (NaF), in natural and inland freshwater reservoir condition has made significant biochemical and histological changes in the fishes. In view of this, the investigations on effects of acute and chronic concentration of sodium fluoride to fish Cirrhinus mrigala have been carried out. The biochemical changes in glycogen, protein and lipid content of selected tissues like liver and kidney were examined. The histological alteration was studied in liver and kidney. The study revealed a highest loss of glycogen, protein and lipid percentage in selected organ and results of the light microscopic studies showed that the histological changes were evident in the liver and kidney of fish exposed to LC_0 (910ppm) and LC_{50} (935ppm) concentration of sodium fluoride. Liver showed clear congestion, infiltration of mononuclear lymphocyte around the vena centralis as compared to control in liver. In kidney tubular epithelium showed necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of the cells. Lumens of the tubules were found invariably dilated.***

Keywords: Sodium fluoride, biochemical, histological, body tissue liver and kidney

1. Introduction

Inorganic Fluorides are introduced into the environment as a result of natural emission and anthropogenic sources. Depending on metrological condition and season, gaseous and particulate inorganic fluorides are transported in air and ultimately are deposited on land or open water bodies. Important anthropogenic sources of fluoride to the aquatic environment include municipal waste and effluents from fertilized producing industry and aluminium refineries. In water mobility and transport of inorganic fluoride are dependent on pH, water hardness, and the prescience of ion exchange mineral. In water inorganic fluoride remain dissolved in solution under acidic condition, low hardness, and the presence on ion exchange material (Cocker and Shilts 1979; Sahu and Karim 1989.) As a consequence free fluoride levels are generally low (Skjelkvale 1994, Radic and Barlic 1995). Inorganic fluoride are toxic to aquatic organism and may cause adverse biological effect such as change in carbohydrate, lipid, and protein metabolism, reproduction, impairment, reduce embryonic and development life stage, and alternation size and growth. Sodium fluoride (NaF) is the most common inorganic fluoride to toxic aquatic organism reported by Sanders and Cope (1966). Toxicity studies with fluoride containing different effluent by Woodwiss and Fertwell (1974), Damkaer and Dey (1989), Camargo (1991), Camargo and Tarazona (1991), Samal (1994) reaction to fluoride has been examined in several studies on aquatic animal, chiefly on fishes. If fishes exposed to poisons amount of sodium fluoride (NaF) become apathetic, loss weight, violent movement, increases secretion and wander aimlessly (Neuhold and Singler 1960). Sodium fluoride (NaF) acts as poisons and interrupting metabolic process such as glycolysis, lipid and synthesis of protein particularly fishes (Julio A. Camargo, 2003). Significant alternation in protein metabolism on acetylcholinesterase activities and oxygen consumption in fresh water crabes have been described by Reddy and Venugopal (1990). Inorganic fluoride toxicity is negatively correlated to water hardness and positively correlated to temperature (Pimentel and Bulkley 1983). The initial phase of acute inorganic fluoride intoxication in fresh water species such as rinbow trout and carp is characterized by apathetic behavior accompanied by Neuhold and Sigler 1960 and Newhold 1972). In many cases, the surviving young fish had curved spines (Singler and Neuhold 1972). The present studies was under taken to evaluate the toxic effect on sodium fluoride (NaF) on biochemical changes in different tissue such as gill, liver, kidney and muscle of fresh water carp C. mrigala.

2. Material and Method

The fresh water fishe C. mrigala measuring about 6 to 7 cm. in length were collected from state government fish seed rearing centre. The collected fish were maintained under laboratory condition at 28 - 30^o C for 10 days acclimation and were then divided in different group having 10 fishes in each. All the group except control were transferred to separate glass aquarium containing different concentration (10 L) sodium fluoride (NaF) grade to determine toxicity LC_o and LC_{50} value and fish behaviour. Acute toxicity experiment were conducted for 96hrs and chronic toxicity for 30 days using a static bioassay technique. Toxic medium was changed at an interval of 24hrs. During experimentation temperature, pH, oxygen contains and hardness of the water determined. After acute exposure 96hrs fishes were sacrificed to obtained liver and kidney. The pooled samples of the organs were used for histological and estimation of glycogen, protein and lipid. Same method was applicable for the chronic exposure for 30 days. The experiment was conducted for acute and chronic,

during the fish and provided food in adequate amount. The aquarium water was changed on after 24h, and fresh dose of the sodium fluoride (NaF) was given. Total protein lipid and glycogen were estimated by standard method by Lowry et al., Folch et al., and De Zawn A and Zandi D. I. respectively.

Normal histological appearance of any organ reflects normal physiological condition of any animal during toxicological and pathological studies. The variation in the histology is used for the evolution of physiological state of the animals. Therefore liver and kidney were dissected out simultaneously for biochemical and histological purpose and cut into pieces and fixed in Bouins fixative. The tissues were processed for wax sectioning. The sections were cut at 5.0 µm and stained with hematoxylin and eosin. The observations were made under Olympus Microscope.

3. Results and Discussion

Glycogen: Biochemical changes were observed in the glycogen, protein and lipid content in different tissue of C. mrigala after acute exposure to sodium fluoride (NaF). The changes in glycogen content in different tissue such as liver and kidney of the fish *C. mrigala* after exposed to sodium fluoride for acute doses, 935ppm and 960ppm for 96 hrs are given in table 1. The glycogen content in liver was most significantly decrease ($p < 0.05$ to $p < 0.001$) in experimental fishes compared to kidney. (Given in Table no.1)

Table 1: Changes in glycogen content in different tissues of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 hrs).

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Liver	14.00 + 0.322	8.60*** + 0.838	5.76*** + 1.012
Kidney	10.95 + 0.582	8.11** + 0.468	5.74** + 0.617

(Values expressed in mg/100mg wet tissue); Each value is the mean of five observations.
± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***

Protein: There was significant decrease in the protein content of all the tissues analysed after acute exposure to sodium fluoride compared to control. Maximum decline in protein content than liver and kidney in a progressive manner, after chronic exposure as compared to acute.

The protein content in different tissues of *Cirrhinus mrigala* was in the order of liver > kidney. (Table 2)

Table 2: Changes in protein content in different tissues of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 hrs).

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Liver	22.37 + 0.32	15.17* + 0.24	10.59***+ 0.26
Kidney	16.86 + 019	12.22* + 0.56	10.14** + 032

(Values expressed in mg/100mg wet tissue); Each value is the mean of five observations.
± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***)

Lipid: After acute (96 h) and chronic (30 days) exposure to sodium fluoride, the lipid content was found decreased in different tissues of freshwater fish *C. mrigala* as compared to control. Kidney showed maximum decline in lipid content followed by liver after acute and chronic exposure. Lipid content in different tissues of the freshwater fish *Cirrhinus mrigala* was in the order of liver > kidney. (Table 3)

Table 3:

Tissue	Control	Acute Exposure	
		LC_0 (935ppm)	LC_{50} (960ppm)
Liver	8.02 + 0.426	6.19* + 0.580	4.33** + 0.812
Kidney	4.98 + 0.481	3.31** + 0.535	2.45*** + 0.627

Changes in lipid content in different tissues of *Cirrhinus mrigala* after acute exposure to sodium fluoride (96 h).
(Values expressed in mg/100mg wet tissue); Each value is the mean of five observations.
± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***)

Liver:
The normal histological structure and changes induced by sodium fluoride in liver of *Labeo rohita* at acute and chronic concentrations are shown in plate I, fig.1 to 5.

Control:
In control, the two lobes of the liver were further divided into several lobules, each of which was made up of several large polyhedral hepatic cells, which appeared like spoke of a wheel, arranged around the central vein. Arrangement of hepatocytes was in muralism duplex fashion. Hepatocytes showed centrally placed nucleus with eosinophilic cytoplasm. The large hepatic cells were observed which indicates the presence of glycogen. Blood cells were evenly distributed all over the sinusoids. The storage of glycogen in the liver parenchyma is a universal feature.

Acute:
The results of the light microscopic studies showed that the morphologic changes were evident in the liver of fish exposed to LC_0 concentration of 910ppm, as compared to control. The cells of liver showed occurrence of a light hypertrophy, dilation of sinusoids and vacuolization of cell cytoplasm. Exposure to sodium fluoride caused a disorganization and dilation of vena centralis, a frequent woven of cell cords and an increase in the infiltration of lymphocytes. A light vacuolization and dilation of sinusoids was still apparent (Fig.2).

An increase in binucleated cells and a light infiltration of lymphocyte were observed at exposure of LC_{50} (935ppm) of sodium fluoride, after 96 hrs. Dilation of sinusoids was observed with reduced blood cells. There were few distinct hepatocytes and necrotic cells showed displaced nucleus at the periphery with basophilic cytoplasm. Few binucleated necrotic cells were also observed near the central vein (Fig.3).

Chronic:
After a chronic dose of $1/20^{th}$ of LC_{50} concentration (46.75 ppm) of sodium fluoride for 30 days, liver showed clear congestion, infiltration of mononuclear lymphocyte around the vena centralis. Sinusoids were dilated and appeared irregular. Sinusoids showed hemorrhage and disconnection

among the cells. Necrotic areas were observed near central vein. Necrotic cells showed varied intensity of vacuoles. Hepatocytes showed swollen nuclei, in pyknotic condition (Fig.4).

Chronic dose of 1/10th of LC_{50} concentration (93.50 ppm) of sodium fluoride for 30 days resulted in severe damage to liver, which can be visualized by necrotic areas. Congestion in the sinusoids was observed as a result of chronic toxicity. Infiltration of mononuclear lymphocyte around the vena centralis was noticed. Dilation in the sinusoids resulted in irregular arrangement of sinusoids. Hepatocytes showed hemorrhage and disconnection among the cells. Necrotic areas were observed near central vein and greatly increased. The necrotic cells showed varied intensity of vacuoles. Hepatocytes showed swollen nuclei in pyknotic condition (Fig.5).

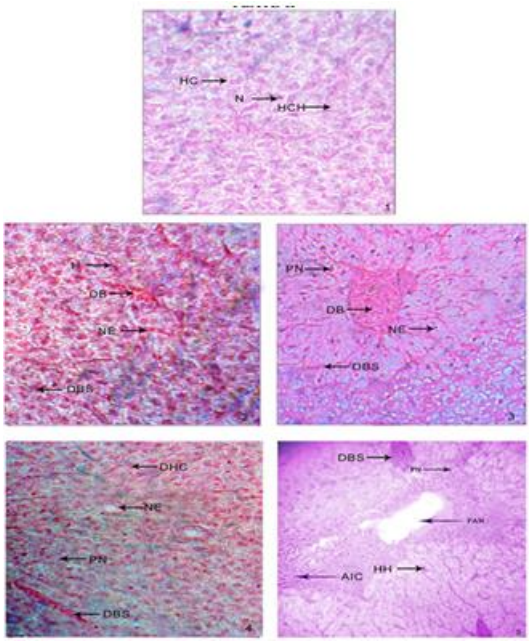


Plate I

Microphotographs of *Cirrhinus mrigala* liver after acute and chronic exposure to sodium fluoride are presented to plate I (Fig.1 to 5).

Fig.1: Section passing through liver of fish *Cirrhinus mrigala* from control group (10 X).

Fig.2: Effect of 935 ppm Sodium Fluoride on liver of *C. mrigala* after 96 h exposure (40 X). Fig.3: Effect of 960 ppm Sodium Fluoride on liver of *C. mrigala* after 96 h exposure (40 X). Fig.4: Effect of 48.00 ppm Sodium Fluoride on liver of *C. mrigala* after 30 days exposure (40 X). Fig.5: Effect of 96.00 ppm Sodium Fluoride on liver of *C. mrigala* after 30 days exposure (40 X).

HC – Hepatocytes; N – Nucleus; VDBV – Vacuolar degeneration of blood vessel; HH – Hypertropic hepatocytes; PC – Pyknotic nucleus; NE – Necrosis; FAN - Focal area of necrosis; DB – Dilated blood vessel

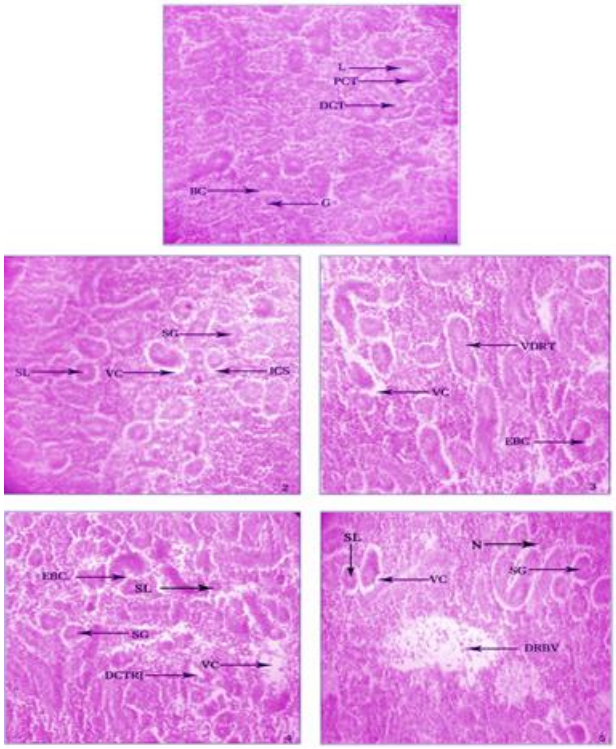
DHC – Degenerating hepatocytes; F - Fibrosis; DBS - Degenerated blood sinus; AIC - Aggregation of nflammatory cell

Kidney: The normal histological structure and changes induced by sodium fluoride in kidney of *Labeo rohita* at acute and chronic concentrations are shown in plate II, fig.1 to 5.

Control: In control group, each nepheric tubule begins to form a swollen blind end, called renal capsule. It contains a tuft of capillaries with afferent and efferent arteries so that the glomerulus is formed. The neck region, proximal convoluted tubule, distal convoluted tubule and collecting tubules are provided with a single layer of the epithelial cells. The proximal tubule showed normal distribution with the presence of the clear brush border, formation of mucus - layer, lining in the lumen. Distal tubules ends in collecting duct showed well developed epithelium (Fig.1).

Acute: The changes in tubular epithelium could be observed after (910 ppm) LC_0 exposure of sodium fluoride in *Labeo rohita* kidney. Tubular epithelium showed necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of the cells. Lumens of the tubules were found invariably dilated. Interstatium was markedly infiltrated with mononuclear cells (Fig.2). The histopathological changes in kidney at (935ppm) LC_{50} concentration of sodium fluoride were severe as compared to LC_0 exposure. Bowman's capsule showed irregular arrangement of tuft. Necrotic cells were also observed in Bowman's capsule. Proximal and distal tubular epithelium showed necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of the cells. The renal tubules exhibit shrinking of lumen and vacuolation of cytoplasm. Brush border of proximal and distal tubules were disturbed (Fig.3).

Chronic: The exposure of fish *C. mrigala* to 1/20th of LC_{50} concentration (46.75 ppm) of sodium fluoride for 30days showed thick lining of Bowman's capsule. Epithelial cells in the Bowman's capsule showed necrosis. Proximal and distal tubular epithelium showed shrunken lumen, vacuolated cytoplasm and disintegration with chronic necrotic changes. Few epithelial cells in the proximal and distal tubules showed intensely stained nucleus. Lumen of the tubules was shrunken with complete destruction of brush border (Fig.4). The exposure to 1/10th of LC_{50} concentration (93.50 ppm) of sodium fluoride for 30 days was more intense than 1/20th does. A thick lining of Bowman's capsule, shrunken glomerulus, increased capsular space with swelling, sloughing off the epithelium of the capsule cells with necrotic changes were observed. Proximal and distal tubules showed intensely stained nucleus. The renal tubule exhibit shrunken lumen and vacuolated cytoplasm with complete destruction of brush border (Fig.5).



Microphotographs of *Cirrhinus mrigala* kidney after acute and chronic exposure to sodium fluoride are presented to plate III (Fig.1 to 5).

Fig.1: Section passing through kidney of fish *Cirrhinus mrigala* from control group (40 X).

Fig.2: Effect of 935 ppm Sodium Fluoride on kidney of *Cirrhinus mrigala* after 96 h exposure (40 X).

Fig.3: Effect of 960 ppm Sodium Fluoride on kidney of *Cirrhinus mrigala* after 96 h exposure (40 X).

Fig.4: Effect of 48.00 ppm Sodium Fluoride on kidney of *Cirrhinus mrigala* after 30 days exposure (40 X).

Fig.5: Effect of 96.00 ppm Sodium Fluoride on kidney of *Cirrhinus mrigala* after 30 days exposure (40 X).

PCT - Proximal tubule; DCT - Distal tubule; BC - Bowman's capsule; G - Glomerulus; ICS - Increased cellular space; L - Lumen of tubule; SG - Shrunken Glomerulus; VC - Vacuolated cytoplasm; N - Necrosis; PN - Pyknotic nucleus; SL - Shrunken lumen; DNC - Degenerative necrotic changes; DRBV - Dilated blood vessel; VDRT - Vacuolated degenerating renal tubule; EBC - Edema in Bowmans capsule

4. Conclusion

Toxic effects of inorganic sodium fluoride (NaF) change the normal physiological function and histological alteration of the experimental organism. Biochemical changes were observed in glycogen, protein and lipid content in various tissues of experimental fish in acute concentration of sodium fluoride. Glycogen is the prime sources of energy showed decreasing order after acute exposure to sodium fluoride concentrations. Decrease in glycogen at acute and chronic concentration was more in liver as compared to kidney. While liver affected as it is prime detoxifying organ. Influx of glycogen to meet the demand created due to stress responsible for increasing glycogen while increased glycolysis may result in decrease glycogen level. The depletion of glycogen level in the specific tissues indicates the possibility of active glycogenolysis, subsequent hypoxia that increases carbohydrate consumption. In present investigation, depletion of protein was to be found in acute and chronic concentration of sodium fluoride in different tissues due to proteolytic activity in anaerobic conditions, rapid utilization of body protein under stress condition as well as sodium fluoride interrupt the metabolic process of protein synthesis in fishes. Lipids serves as the reserve energy sources, which decreases significant when exposed the experimental fish in acute and chronic concentration of sodium fluoride. The decrease may be due to inhibition of lipid synthesis by fluoride as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic functions under the stress of NaF toxicant. Histopathological changes in cells of liver showed occurrence of a light hypertrophy, dilation of sinusoids and vacuolization of cell cytoplasm. Exposure to sodium fluoride caused a disorganization and dilation of vena centralis, a frequent woven of cell cords and an increase in the infiltration of lymphocytes. A light vacuolization and dilation of sinusoids was still apparent. Necrotic areas were observed near central vein and greatly increased. The necrotic cells showed varied intensity of vacuoles. In kidney epithelial cells in the Bowman's capsule showed necrosis. Proximal and distal tubular epithelium showed shrunken lumen, vacuolated cytoplasm and disintegration with chronic necrotic changes. Lumen of the tubules was shrunken with complete destruction of brush border. Liver and kidney of fish exposed to acute and chronic concentration of sodium fluoride shows several drastic histopathological alterations. The result indicates these drastic degenerative changes will finally leads to malfunctioning of that organ. The changes in the cellular architecture cannot be attributed to a single factors and may be dependent on cumulative effects of many factors induce by presence of sodium fluoride in the body tissues of the fish.

Acknowledgements

I express our sincere thanks to our guide Prof. D. V. Muley. I also express our deep sense of gratitude Director Prof. Subodh Bhandarkar Govt. Vidarbha Institute of Science and Humanities (Autonomous), Amravati and our HOD Prof. V. S. Zade for providing us the laboratory facilities and the moral support.

References

[1] Cocker, W. B. and W. W. Shilts, (1979): Lacustrine geochemisby around the North shore of Lake Superior, implications for evalution of the effect of acid precipitation. Current research part C, Geological survey of Canada. Paper 79 - 1C.

[2] Sahu, N. K. and M. A. karim, (1989): Fluoride incidence in natural waters in Amrels district Gujarat J. Geaol. Soc. India 33 (5): 450 - 456.

[3] Skjelkvale, B. L., (1994a): factor influencing fluoride concentration in Norwegian lakes. Water Air Soil Pollute 77 (1 - 2): 151 - 167.

[4] Sanders, H. O. and O. B. Cope, (1966): Toxicity to several pesticides to two species of productive ocean regions. Geochem. Int.21 (1): 82 - 86.

[5] Woodiwiss, F. S. and Fertwell, G. (1974) The toxicity of sewage effluents, industrial discharge and

[6] some chemical substance to brown trout (Salmo trutta). In the Trent River authority area. Water pollut. Cont. (G. B.), 73: 396
[7] Damkaer, D. M. and Dey, D. B. (1989) Evidence for fluoride effect on salmon passage at John Day Dam,
[8] Camargo, J. A. (1991) Eco - toxicological analysis of the influence of an industrial effluent on fish population in a regulated stream. Aquacult. and fish. Manage., 22: 509 - 518.
[9] Camargo, J. A. and Tarazona, J. V. (1991) Short term toxicity of fluoride ion (F -) in soft water to rainbow and brown trout. Chemosphere, 22: 605 - 611.
[10] Samal, U. N. (1994) Effect of fluoride on growth of certain freshwater fishes. Environ. Ecol., 12 (1): 218 - 220.
[11] Neuhold, J. M. and Sigler, W. F. (1960) Effect of sodium fluoride on carp and rainbow trout. Trans. Am. Fish. Doc., 89: 358 - 370.
[12] Julio, A. Camargo and Jose, V. Tarazona, (2003): Short term toxicity of fluoride ion (F -) in soft water to rainbow trout and brown trout.
[13] Reddy, S. L. N. and Venugopal, N. B. R. K. (1990) Effect of fluoride on acetylcholinesterase activity and oxygen consumption in a freshwater field crab, Barytelphusa guerini. Bull. Environ. Cont. and Toxicol., Vol.45 (5): 760 - 766.
[14] Pimentel, 16. R., and Bulkley, R. V. (1983) Influence of water hardness of fluoride toxicity to rainbow trout. Environ. Toxicol. Chem., 2: 381 - 386.
[15] Sigler, W. F., and Neuhold, J. M. (1972) Fluoride intoxication in fish: a review. J. Wild. Dis., 8: 252 - 254.
[16] Lowry, O. H., Rosebrough, N. J, Farr, A. B. and Randall, R. J. (1951) Protein measurement with folin - phenol reagent. J. Bio. Chem., 193: 265 - 275.
[17] Dezwaan, A. and D. I Zandee, (1972): The utilization of glycogen and accumulation of some intermediate during anaerobiosis in Mytilus edulis. Comp. Biochem. Physiol.43: 47 - 54