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Sodium Fluoride Alter Architecture of Intestine and Kidney of Fresh Water Fishes *Labeo rohita*

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Abstract: ***The present study was conducted to investigate the histological structures of the intestine and kidneys of fresh water fish Labeo rohita after short (96 hrs) and long term exposure (30 days) in sodium fluoride (NaF) (46.75ppm). Several histological alternations were obtained in the intestine and Kidney. In intestine damages internal villi of intestine, mucosal layer showed necrosis resulting into degeneration of cells at few places. Sub-mucosa showed hypertrophy and vacuolation of cells. Beginning of sloughing of mucosal layer is evident from the formation of a gap between mucosal and sub-mucosal layers. In chronic the villi tends to become flattened, and there is sloughing of the mucosal lining. The disorganization of sub-mucosa and formation of vacuolated areas. In kidneys severe degenerative and necrosis changes in the renal tubule with focal area of necrosis, haemorrhage between renal tubule and edema in Bowman's capsule with atrophy in the glomeruli. The necrosis of the renal tubule affects the metabolic activities and promotes metabolic abnormality have been observed. Kidney is responsible for excretion of polluting agents brought after detoxification in liver***

Keywords: histological changes, sodium fluoride exposure, intestinal degeneration, renal damage, *Labeo rohita*

1. Introduction

Environmental exposure to fluoride compounds may occur as a result of natural processes (eg, volcanic emission) or contamination of rocks, soil, and water or from different industrial waste. Fluoride compounds (eg, sodium fluoride, sodium fluorosilicate, fluorosilicic acid) are added to human drinking water at a concentrations of ~1 mg/L in an effort to reduce dental caries. This recommendation is not universally accepted. Both acute and chronic toxicoses may result from ingestion of fluorides of aquatic as well as terrestrial animals. Maximum tolerated concentrations of fluoride in animal depending on age, duration of exposure, nutritional and health status of individual animals. Aquatic animals with a relatively more susceptible to fluorosis because through the gills they are directly come in contact with the contaminated water. Because of potential fluoride contamination of a variety of feed and water sources, it is recommended that feed-grade phosphates contain < 1% fluoride. Acute exposure to high fluoride concentrations will cause corrosive damage to tissues. In contrast, chronic exposure, which is seen more frequently, causes delayed or impaired mineralization of bones and teeth. The solubility of fluoride compound to which the animal is exposed correlates with the extent of severity of toxicosis.

Global prevalence of fluorosis is reported to be about 32% in the world. In India, about 17 states and Union Territories are endemic to fluorosis. Several million people are using drinking water and consuming a food source, that possess a potential risk for fluorosis. In 1993, a report regarding fluoride pollution in India was published by Rajiv Gandhi Drinking Water Mission. According to it, fluorosis is a condition resulting from ingestion of large amount of fluoride, chiefly through drinking water. About 20 million people are suffering from fluorosis, while about 42 million people are exposed to the risk of endemic fluorosis. Fluorosis causes difficulty in movement (rheumatoid), mental depression, vascular disturbances, abnormal reproduction and abnormal behaviour. Vital organs such as liver, kidney, reproductive organs and endocrine glands are reported to be adversely affected by high fluoride intake (Chinoy, 1991; ATSDR 2001). Several metabolic activities are also disturbed due to alteration in regulatory enzymes and biomolecules (Chitra et al., 1983; Kumar et al., 2007) after exposure to fluoride. Recently Sarkar et al., (2004) and Tripathi (2007) have presented an elaborate account of severity of fluorosis. Considering the importance of aquatic life, especially fish, fulfilling the need of food for the mankind, a thorough investigation of the toxicity is needed. In keeping these view, planned to study, at least partly, to investigate the toxicity of sodium fluoride to freshwater fish species such as *Labeo rohita* and *Cirrhinus mrigala* these species are commonly consumed as a food in different region in India. The study emphasizes the investigation of toxic effect on important organs of the selected fish species. www.ijcrt.org © 2021 IJCRT | Volume 9, Issue 8 August 2021 | ISSN: 2320-2882 IJCRT2108080 International Journal of Creative Research Thoughts (IJCRT) www.ijcrt.org a664 Sources of sodium fluorides:

Aquatic animals such as fish and invertebrates can take up fluoride directly from the water or via food (Hemens and Warwick, 1972; Nell and Livanos, 1988). Fluoride tends to be accumulated in the exoskeleton of invertebrates and in the bone tissue of fishes. Fluoride toxicity depends upon increasing fluoride concentration in the aquatic medium, exposure time and water temperature (Neuhold and Sigler, 1960; Angelovic et al., 1961; Hemens and Warwick, 1972).

Sodium fluoride is the most common inorganic fluoride used in aquatic toxicity studies (Damkaer and Dey, 1989; Camargo, 1991). Sodium fluoride interrupts metabolic process such as glycolysis, lipid and protein synthesis in fishes (Camargo, 2003). Inorganic fluoride toxicity is negatively correlated to water hardness and positively correlated to temperature (Pimentel and Bulkley 1983). Fluorine interferes with various metabolic activities and alters the levels of protein, lipids, glycogen, and cholesterol of fish (Kumar et al., 2007). The present studies was under taken to evaluate the toxic effect on sodium fluoride on histopathological changes in kidney of fresh water carp *Labeo rohita* and *Cirrhina mrigala*.

Kidney is responsible for excretion of polluting agents brought after detoxification in liver. Such metabolites reaching the kidney must be causing extra stress. Mathur (1969) and Holden (1972) are of similar view that, if the pollutants are in low concentration, the changes are less acute while, at higher concentrations the changes are drastic. Lantz *et al*., (1987) reported that fluoride toxicity may lead to osteosclerosis and end stage with renal failure. These may finally lead to the failure of kidney and death.

2. Material and Methods

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 intestine and kidney were dissected out 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 double staining method hematoxylin and eosin. The observations were made under Olympus Microscope.

3. Result and Discussion

Intestine: The normal histological structure and changes induced by sodium fluoride in intestine of *Labeo rohita* at acute and chronic concentrations are shown in Plate No: 1 (fig. 1 to 5.)

Control: The control group showed normal histological structure of intestine. Inner most layer of mucosa comprised of absorptive epithelial and mucous secreting goblet cells. The mucosal layer was thrown into folds to increase the surface area, the villi. The sub-mucosa was vascular and extended into the folds of villi. The muscularis was thin layer composed of circular and longitudinal muscle and serosa was outer most layer uniformly thick (Fig.1).

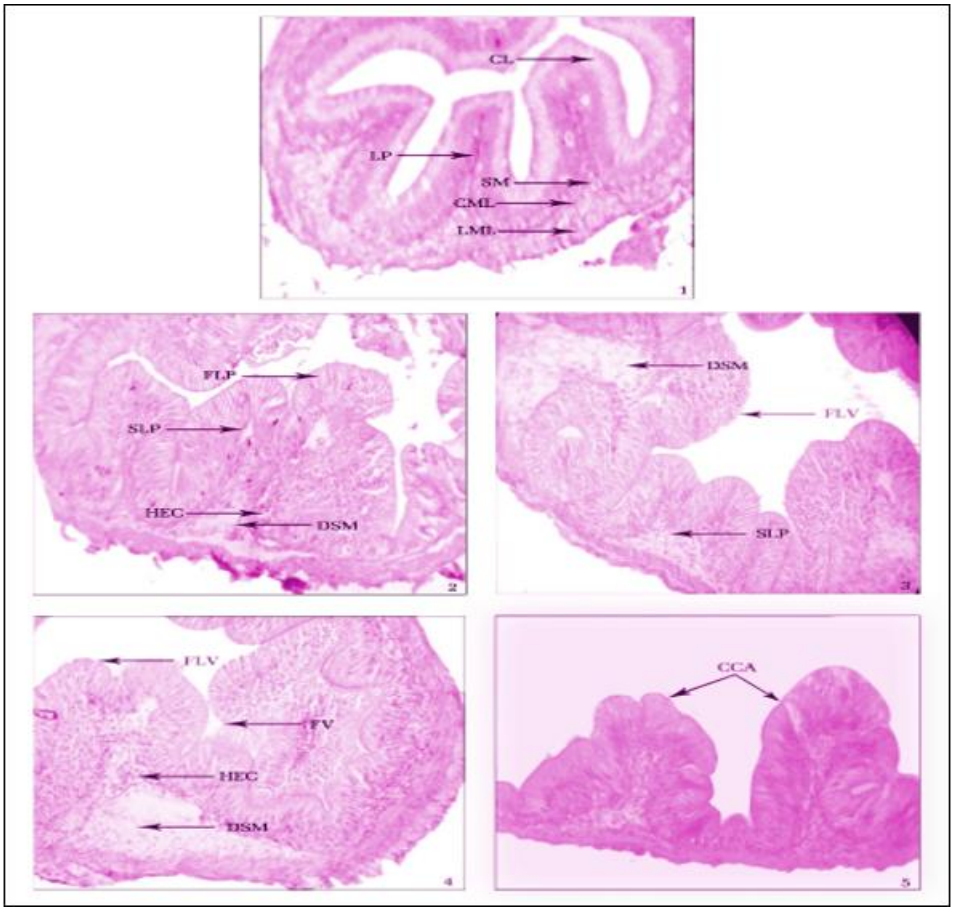

Plate No. 1

Fig. 1: Section passing through intestine of fish L rohita from control group (40 X).
Fig. 2: Effect of 935 ppm Sodium Fluoride on intestine of after 96 h expo L rohita sure (40 X).
Fig. 3: Effect of 960 ppm Sodium Fluoride on intestine of L rohita after 96 h exposure (40 X).
Fig. 4: Effect of 48.00 ppm Sodium Fluoride on intestine of after 30 days e L rohita xposure (40 X).
Fig. 5: Effect of 96.00 ppm Sodium Fluoride on intestine of L rohita after 30 days exposure (40 X).

S- Serosa; LML- Longitudinal muscle layer; CML- Circular muscle layer; SM- Sub- mucosa; CE- Columnar epithelial cell; DSM- Degenerating submucosa; DLML- Disorganized longitudinal muscle layer; DS- Degenerating serosa; DCML- Degenerating circular muscle layer; HM- Hypertrophy of mucosa; FV- Fusion villi; FLV- Flattened villi; HEC- Hypertrophied epithelial cell; SNC- Severe necrotic changes; CCA- Cracked clay appearance

Acute: The alterations in mucosal and sub-mucosa layer were observed after (910ppm) LC_0 exposure of sodium fluoride in the intestine of *Labeo rohita*. The mucosal layer showed necrosis resulting into degeneration of cells at few places. Sub-mucosa showed hypertrophy and vacuolation of cells. Beginning of sloughing of mucosal layer is evident from the formation of a gap between mucosal and sub-mucosal layers (Fig. 2).

Damage to intestine in (935 ppm) LC_{50} concentration of sodium fluoride was severe as compared to LC_0 exposure. Hyperplasia and hypertrophy of mucosal cell resulting in thickening of mucosa was observed. Lesion was observed in the mucosal layer. The sub-mucosal cell showed degenerative changes and complete disorganization of sub-mucosal cells. Pyknotic nuclei were noticed in many mucosal cells (Fig. 3).

Chronic: Chronic studied revealed a degenerative effect on intestine of *L rohita* at $1/20^{th}$ of LC_{50} concentration (46.75ppm) of sodium fluoride for 30days. The destruction was evident by the observation of the mucosal lining and villi of the intestine. The villi tends to become flattened, and there is sloughing of the mucosal lining. The disorganization of sub-mucosa and formation of vacuolated areas were noticed at few places (Fig. 4).

The exposure at $1/10^{th}$ of LC_{50} concentration (93.50 ppm) of sodium fluoride for 30 days showed intense effects as compared to $1/20^{th}$ (46.75 ppm) exposure. Increase in thickness of mucosa was found. Pyknotic nucleus was seen in the mucosal cell. Necrosis of mucosal cells with breaking at few places leading to vacuolation was evident. Hypertrophy of epithelial cells, swelling of lamina propria and fusion of villi due to excessive hypertrophies are also evident (Fig. 5)

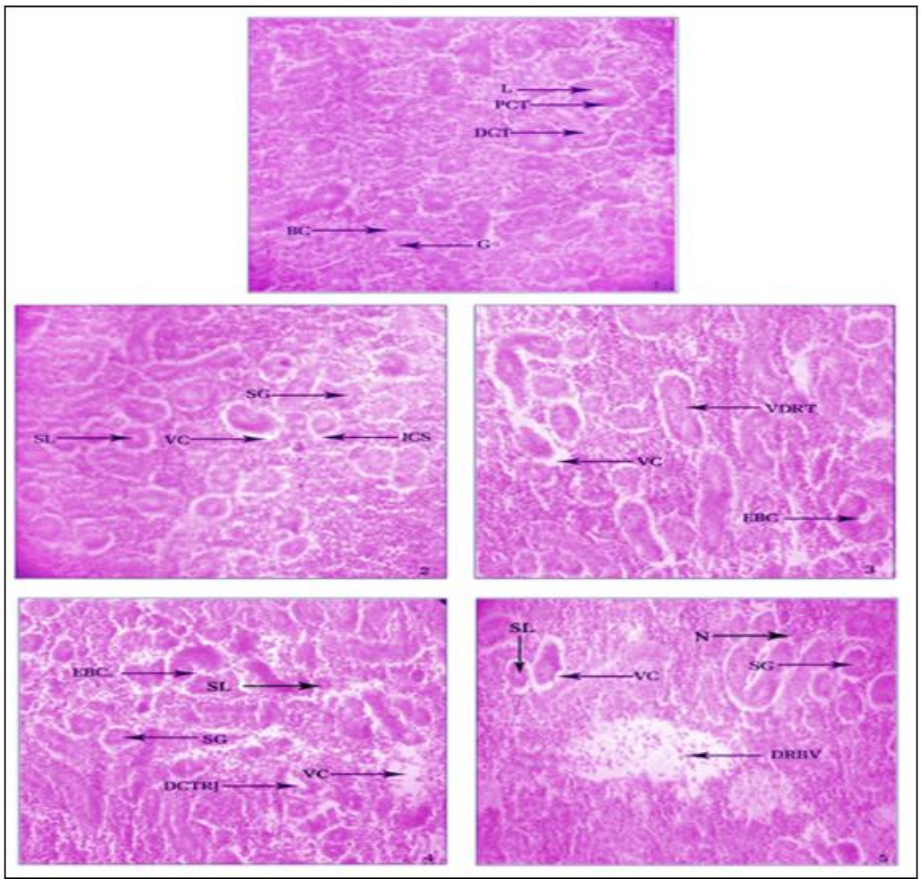

Plate No: 2

Microphotographs of *L. rohita* kidney after acute and chronic exposure to sodium fluoride are presented to (Fig. 1 to 5).

Fig. 1: Section passing through kidney of fish *L. rohita* from control group (40 X).
Fig. 2: Effect of 910 ppm NaF on kidney of *L. rohita* after 96 h exposure (40 X).
Fig. 3: Effect of 935 ppm NaF on kidney of *L. rohita* after 96 h exposure (40 X).
Fig. 4: Effect of 46.75 ppm NaF on kidney of *L. rohita* after 30 days exposure (40 X).
Fig. 5: Effect of 93.50 ppm NaF on kidney of *L. rohita* . 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

The histological changes in the kidney of fish *Labeo rohita* are shown in Plate I and II. The fig. 1 showed normal structure (control) of kidney during experimentation. A control of experimental fish showed a normal architecture of Bowman's capsule enclosing in tuft of blood capillaries and glomerulus. Proximal and distal tubules showed normal distribution with

clear brush border. A distal tubules end in collecting duct has shown well developed epithelium.

Acute: The changes in tubular epithelium could be observed after (910 ppm) LC_0 exposure of sodium fluoride in the kidney of experimental fishes. 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 experimental fishes to $1/20^{th}$ 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 $1/10^{th}$ of LC_{50} concentration (93.50ppm) of sodium fluoride for 30 days was more intense than $1/20^{th}$ 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).

4. Conclusion

In present study, acute (96 h) toxicity experiments were conducted on the fingerling of the freshwater fish *L. rohita* by exposing them to sodium fluoride concentrations. The result indicate that sodium fluoride inhibit the number of activity of fish. The sodium fluoride toxicity on aquatic animals are depends on the number of factors such as size, density of fish per volume of aquarium, water temperature and many other water quality variables. The inorganic fluoride as negatively correlated to water hardness and positively correlated to temperature. In chronic studies, the fish were exposed to sodium fluoride for 30 days period. It is evident from the result that there was no mortality, drastic deleterious effect were recorded on histological aspect of intestine and kidney. The rate of distribution may be depends on absorption and blood flow to the tissues. Consequently, steady-state fluoride concentration is achieved more rapid between membrane layers of intestine and plasma and well perfused tissues of kidney after 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. The present investigation revels that sodium fluoride toxicity induced changes in histopathological architecture in intestine acute and chronic condition wasobserved mucosal layer showed necrosis resulting into degeneration of cells at few places. Sub-mucosa showed hypertrophy and vacuolation of cells. Beginning of sloughing of mucosal layer is evident from the formation of a gap between mucosal and sub-mucosal layers. Pyknotic nucleus was seen in the mucosal cell. Necrosis of mucosal cells with breaking at few places leading to vacuolation was evident. Hypertrophy of epithelial cells, swelling of lamina propria and fusion of villi due to excessive hypertrophies. In kidney in acute and chronic concentrations. Damage to the kidney corresponding with increase in the concentrations of sodium fluoride. The common alterations found in both experimental fish were severe degenerative and necrosis changes in the renal tubule with focal area of necrosis, haemorrhage between renal tubule and edema in Bowman's capsule with atrophy in the glomeruli. The necrosis of the renal tubule affects the metabolic activities and promotes metabolic abnormality have been observed. Kidney is responsible for excretion of polluting agents brought after detoxification in liver. Such metabolites reaching the kidney must be causing extra stress. Sodium Fluoride toxicity may lead to osteosclerosis and end stage with renal failure. These may finally lead to the failure of kidney and death.

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