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Sodium Fluoride Toxicity Alter Histopathological Structure in Gill and Liver of Fresh Water Fish, *Cirrhina mrigala* after Long Term Exposure

M. D. KALE

Department of Zoology, Govt. Vidharbha Institute of Science and Humanity, Amravati, Maharashtra, INDIA 444604

Abstract:

The present study was conducted to investigate the histological structures of the gill and liver of fresh water fish *Cirrhina mrigala* after long exposure (30 days) in sodium fluoride (46.75ppm). Several histological alternations were obtained in the selected tissues such as in the gills, the pathological alterations included proliferative, degenerative and necrotic changes in the epithelium of gill filament and secondary lamellae, edema in secondary lamellae, dilation and congestion in blood vessel of gill filaments and mucus cells proliferation. The liver showed vacuolar degeneration in the hepatocytes, focal areas of necrosis and fibrosis, aggregations of inflammatory cells between the hepatocytes, dilation and congestion in blood sinusoids and thrombosis formation in the central veins.

Keywords: Sodium fluoride; *C. mrigala*; histopathology; soft tissue.

INTRODUCTION

The sodium fluoride, through the aquatic organism is entering into the body of terrestrial animals including human and showing many hazardous effects. 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 (rheumatid), mental depression, vascular disturbances, abnormal reproduction and abnormal behavior. 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, we planned to study, at least partly, to investigate the toxicity of sodium fluoride to freshwater fish species such as *Cirrhinus mrigala* these species are commonly consumed as a food in India. The study emphasizes the investigation of toxic effect on important organs of the selected fish species.

Sources of sodium fluorides:

i) Natural Sources: Fluoride, is released into the environment by the natural weathering of rocks, mineral dissolution, emission from volcanic activities and aerosols.

ii) Anthropogenic Sources: The fluoride is also released into the environment via coal combustion, processing of water, various industrial processes of steel manufacturing, primary aluminiation, copper and nickel production and phosphate fertilizer production. The decomposition of pesticides, fluoridation of drinking water supplies also contribute to the release of fluoride from anthropogenic sources. Sodium fluoride (NaF) is used in fluoridation of drinking water, as a preservative in glues, glass, in enamel production, as flux in steel and aluminum production, as an insecticide and as a wood preservative.

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 gill, liver, kidney and intestine 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 hematoxylin and eosin. The observations were made under Olympus Microscope.

Result and Discussion:

Histopathology of Gill:

Control:

1) Primary lamellae projects from posterior edge of gill arch (Fig.1), while secondary lamella originates on superior and inferior surface of primary lamella.

2) Epithelial cell covering of secondary lamella on basement membrane was supported by pillar cells. Arches are supported by mixed bone and cartilage with associated striated abductor and adductor muscles, facilitating movement of gills.

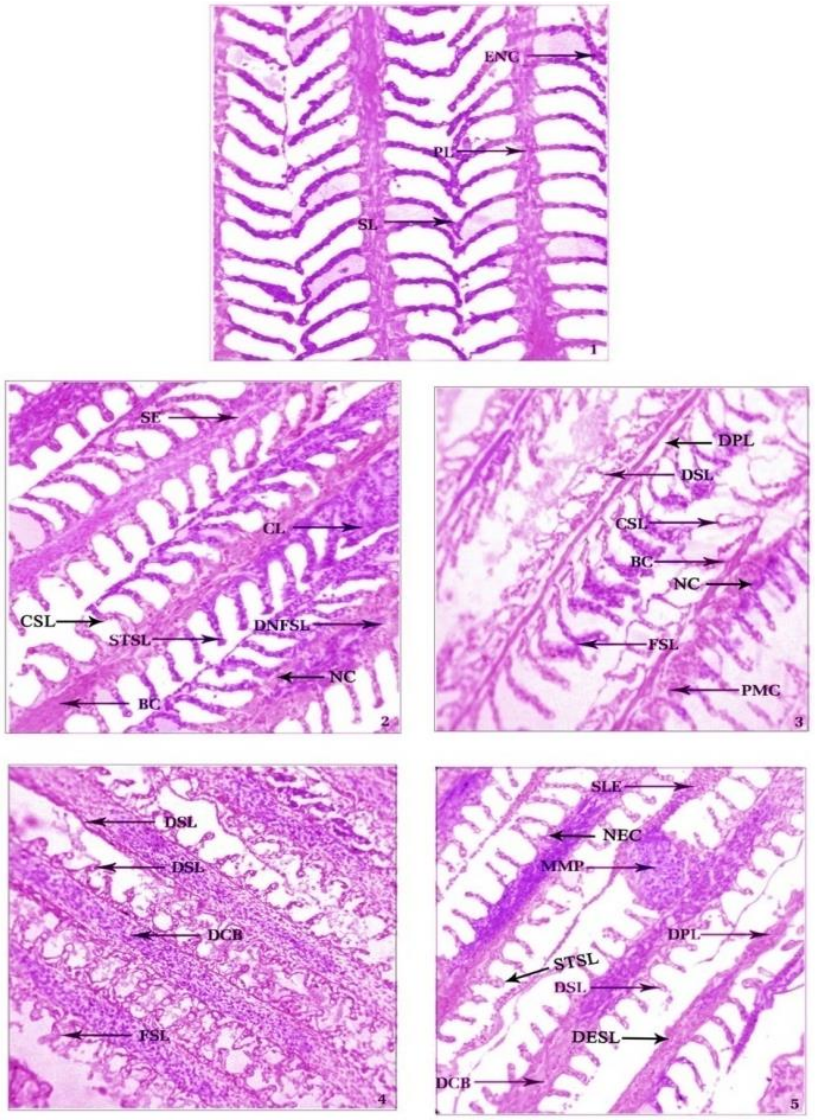
3) The gill arches showed clear boundaries of hypertrophic zone, growth zone and apical zone, which are supported by mucosal epithelium. Normal distribution of macrophages, endothelial cells, mucous cells, and chloride cells were observed (Fig. 1).

Chronic:

Exposure to $1/20^{th}$ (48 ppm) of LC_{50} concentration of sodium fluoride to *Cirrhinus mrigala* was resulted in chronic effects on gill. The observed changes were destruction of primary and secondary lamellae and clubbed appearance in primary lamellae. A few secondary lamellae can be differentiated at basal part of gill arch, showed destruction as compared to control gill. Blood pools were observed in nearby areas of arch and in arteries. Secondary lamellae appeared foggy with undifferentiated cell mass around the lamella. Few necrotic cells were also observed, which were dominated in the primary gill lamella. The pillar cells architecture was altered by increase in chloride cell number and their subsequent bulging to the surface (Fig. 4).

Chronic exposure of $1/10^{th}$ (96 ppm) of LC_{50} concentration of sodium fluoride to *Cirrhinus mrigala* resulted in complete destruction of primary and secondary lamellae in gill. Blood pools were observed in nearby areas of arch and arteries. The lamellar hyperplasia is derived from primary lamellae and was found to migrate towards the distal end. Secondary lamellae showed lamellar telangiectasis along with edema and mucoid metaplasia. Necrotic cells were also observed which were dominated in the primary gill lamella. There was hypertrophy and hyperplasia of pillar cells (Fig 5).

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



PL - Primary gill lamella, SL - Secondary gill lamella, CSL - Curved secondary lamellae DSL - Degenerating secondary lamellae, LSL - Loss of secondary lamellae ASL - Atrophy secondary lamellae STSL- Swollen tip lamella
BC - Blood clot, FSL – Fused secondary lamellae NEC- Necrotic epithelium cell MMP- Mucoid Meta Plasia , CLS- Clubbing secondary amellae LSL- Loss of secondary lamellae PMC- Proliferation of mucosal cell, PEC- Proliferation of epithelial cell ENC- Endothelial cell

Liver:

The normal histological structure and changes induced by sodium fluoride in liver of *Cirrhinus mrigala* at chronic concentrations are shown in plate II, fig. 1 to 5.

Control:

The control of liver showed wheel like arrangement of hepatocytes with central vein consisting of several blood vessels. The hepatic cells were arranged, forming a continuous hepatic cord. Each hepatic cell contained granular cytoplasm with a central nucleus. A normal distribution of macrophage cell was observed (Fig.1).

LC_{50} dose (960 ppm) showed increase in the hemorrhage of the vein. The pyknotic condition of nuclei was observed in the liver after 96hr. Hepatocytes showed necrosis in all over the area. Other changes observed include a clear dilation of sinusoids and degeneration of cells (Fig. 3).

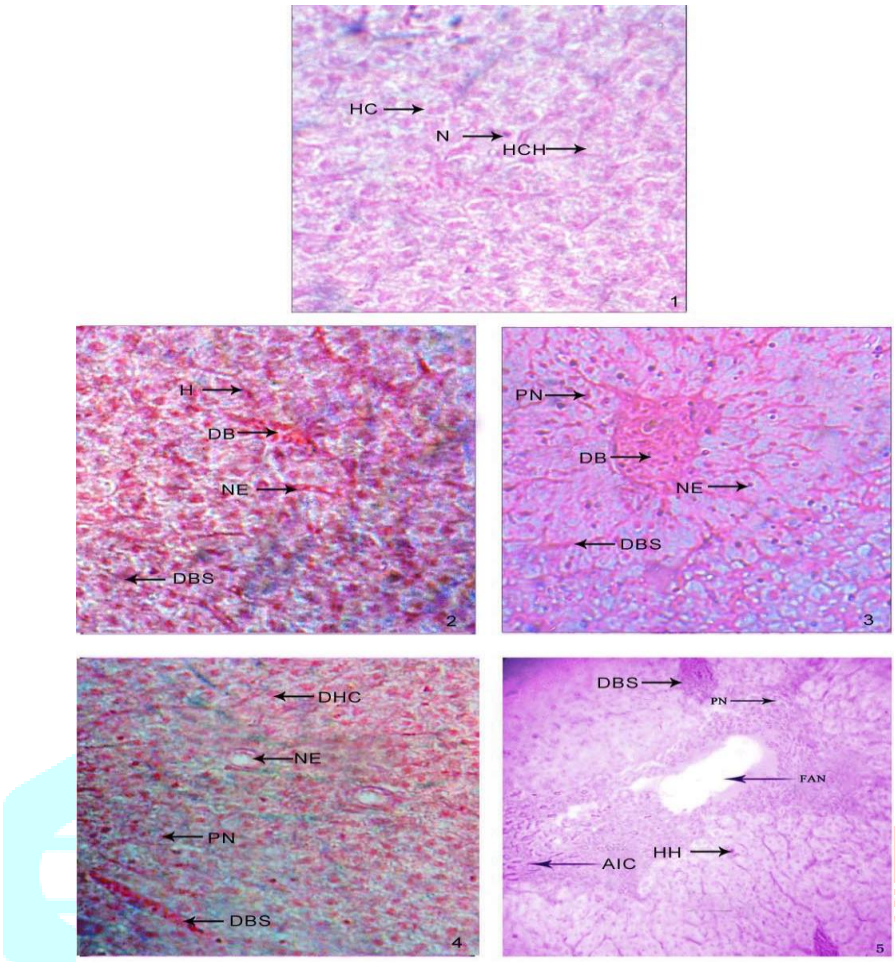


Fig. 1: Section passing through liver of fish *Cirrhinus mrigala* from control group (10 X).
Fig. 4: Effect of 48.00 ppm Sodium Fluoride on liver of *Cirrhinus mrigala* after 30 days exposure (40 X).
Fig. 5: Effect of 96.00 ppm Sodium Fluoride on liver of *Cirrhinus mrigala* after 30 days exposure (40 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

Chronic: Effects of long term exposure at $1/20^{th}$ (48.00 ppm) of LC_{50} concentration of sodium fluoride for 30 days has shown:

A high degree of necrosis in the liver, and sinusoids hemorrhage and congestion state amongst the cells. Hypertrophy of hepatocytes was observed after chronic exposure and congestion resulted in dilation of sinusoids. Degenerated cells showed pyknotic state of nuclei and moderate infiltration of lymphocytes (Fig. 4).

A chronic studies at the exposure of $1/10^{th}$ (96.00 ppm) of LC_{50} concentration of sodium fluoride for 30 days, has resulted in massive necrosis, degenerated cells of gall channels, hemorrhage and congestion state amongst the cells along with a relative hypertrophy on the cells of liver were observed. A clear dilation of sinusoids, frequent degeneration of cells, pyknotic state of nuclei, and infiltration of lymphocytes was observed (Fig. 5).

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

Conclusion:

Degenerated blood sinus Gill and liver, of fishes exposed to acute and chronic concentration of sodium fluoride showed several drastic histopathological changes are shown. The results indicate these drastic degenerative changes will finally leads to malfunctioning of that organ. The effects were more pronounced in fishes treated with higher concentration of sodium fluoride than that of lower concentrations. The changes in the cellular architecture cannot be attributed to a single factor and may be dependent on cumulative effects of many factors induced by presence of sodium fluoride in the body of fishes.

Sodium fluoride can be taken up by aquatic organisms directly from water, or to a lesser extent via food. Uptake of sodium fluorides by fishes will very much depend on the proximity of anthropogenic sources, the

local geology and the physiochemical conditions, which will determine the bioavailability of fluorides. The fishes tend to higher accumulate fluoride in hard tissues than the soft tissues its causes physiological, biochemical and histopathological changes. Absorption of fluoride entering the gastrointestinal tract is affected by a number of factors such as the chemical and physical nature of the ingested fluoride and the characteristic and amount of other component of the ingesta. After absorption fluoride is distributed from the plasma to all the tissues and organ. The rate of distribution may be depends on the blood flow to the tissues. Consequently, steady-state fluoride concentration is achieved more rapid between plasma and well perfuse tissues. Histological changes was observed in the structure of gill and liver such as secondary lamellae appeared foggy with undifferentiated cell mass, necrotic cells, pillar cells architecture was altered by increase in chloride cell number and their subsequent bulging and in the liver clear dilation of sinusoids, frequent degeneration of cells, pyknotic state of nuclei, and infiltration of lymphocytes was observed surface, in long term exposure because may be sodium fluoride inhibit the process of protein synthesis, cell proliferation and inhibit the activity of enzymes

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