International E-Conference on Life Sciences, Technology and Management
In Association with International Journal of Scientific Research in Science and Technology
Volume 9 | Issue 9 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)

Degenerative Changes in Gills of Poecilia Reticulata Peters on Chronic Exposure to Sodium Fluoride

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ABSTRACT

Background: Fluoride is known as a 'double edged sword' that causes dental, skeletal and non-skeletal fluorosis on excess concentration in body. The effects of toxicant exposure are of particular importance to those concerned with the aquatic environment, since they more closely represent natural situations.

Objective: The damage to the various tissues due to chronic or long-term exposure is more severe than that occurring due to acute or short-term exposure because the organism gets exposed to the deleterious or toxic environment for a considerably long period of time. The study was carried out to assess the applicability of histopathology in aquatic toxicity testing, investigation was carried out on the Guppy, Poecilia reticulata Peters using aqueous solution of Sodium fluoride exposure chronically for a span of 60 days.

Results: Fish were exposed to three sub-lethal concentrations of Sodium fluoride viz. lowest (5.75 ppm), lower intermediate (7.18 ppm) and higher intermediate (9.58 ppm), which were selected on the basis of 96 hrs. LC50 value of 115 ppm. Histopathological deviations in the gills manifestation under intoxication of fluoride were thickening of basement membrane and enlargement of cartilaginous axis cells, degeneration of gill lamellae, swelling of base, proliferation in the inter-lamellar space, pycnosis and necrosis of chloride cells, hyperplasia and fusion of the secondary lamellae characterized by swelling at the tip, which caused failure of the respiratory mechanism resulting in augmentation of mortality of the test fish.

Discussion: The necrosis observed in the cellular architecture of the gill tissue in histopathology slides that revealed the toxic effects of fluoride resulting in physiological imbalance caused by disturbance in primary and secondary gill lamellae with hyperplasia and hypertrophy of gill tissue altering the respiratory potential in the test fish with death due to asphyxiation.

Conclusion: From present investigation it is concluded that fluoride affects dynamic organ like gills in Guppy causing deterioration of gill tissue, the most sensitive indicator disrupting the respiratory potential. Furthermore, the epithelial transport mechanisms which are pretentious in the fish gill model bear a resemblance to those described in the human gut and kidney the sites of action of a variety of environmental contaminants.

KEYWORDS: Fluoride Toxicity, Histopathology, Gill, Poecilia reticulata Peters.

I. INTRODUCTION

A chronic direct effect differs from an acute one in that the toxicant causes a sub-lethal change in the host that may or may not be the eventual cause of death. The effects of toxicant exposure are of particular importance to those concerned with the aquatic environment, since they more closely represent natural situations. The damage to the various tissues due to chronic or long-term exposure is more severe than that occurring due to acute or short-term exposure because the organism gets exposed to the deleterious or toxic environment for a considerably long period of time. In India, about 62 million people are estimated to be afflicted with various stages of skeletal fluorosis from consuming fluoride-contaminated water (Jain et al., 2000). Although uncontaminated freshwater bodies usually have low levels of fluoride, the concentration might increase considerably due to fertilizer run-off, mining activities and industrial emissions. Fluorides also originate in ground water from fluoride rich rocks, volcanic rock and clay mineral, due to industrial effluents discharged directly into them and also by anthropogenic activities. The toxic effects of Sodium fluoride are not just restricted to skeletal or dental fluorosis but even the soft tissues causing soft tissue fluorosis. The test fish, *Poecilia reticulata* Peters is universally known as Guppy fish, which are larvivorous fish thus profusely used as a tool in the biological control of pest. Though not eatable, its momentous involvement in control of mosquitoes' menace cannot be ruled out in space and time. The present study is an effort to reveal influence of fluoride (as NaF) triggering non-skeletal fluorosis in gill tissue of *Poecilia reticulata* Peters.

Past few decades many workers have investigated effects of different categories of toxicants and observed their long-term or chronic implication on histopathology of aquatic organisms in general and fish in particular. Mallot (1985) have presented an exceptional statistical review of various gill lesions caused by a number of aquatic pollutants. Muley et al. (1996) treated *Tilapia mossambica* with sodium fluoride and reported damage to the cellular architecture of the tissue. Studies by Palaniappan et al. (2003) on the histopathology of *Cirrhinus mrigala* exposed to sub-lethal concentration of nickel revealed severe damage and changes in the cellular level of gills leading to death of fish. Histopathological changes in *Poecilia reticulata* exposed to sub-lethal concentrations of azodye methyl red as reported by Sharma et al. (2006) includes distortion and disintegration in primary gill lamellae, damage of respiratory epithelium, mucous and blood cells accompanied by thickening of basement membrane, enlargement of cartilaginous axis cells with complete disintegration of secondary gill lamellae. The histopathological alterations reported by them were detached gill lamellae from the filament with broken gill filament and ruptured capillaries. The rate of absorption of oxygen through gills and skin depends on the availability or fluoride concentration. Higher the fluoride concentration lowers the absorption of oxygen which will be later on produced alteration in all biochemical processes occurring in fish (Singh and Tripathi, 2015). Cao et al., (2013) reported accumulation of fluoride to be associated with the inhibition of superoxide dismutase (SOD) activities and a dose-dependent stimulation of malondialdehyde (MDA) levels in the gill tissues of *C. carpio*, suggesting that fluoride promoted oxidative stress in the fish. Microscopic examinations revealed injuries to gill tissues and chloride cells, with the severity of injury increasing with exposure concentration. Histopathological changes induced by fluoride have been adequately documented in

rat by Uprete and Kannan (2005) and fish by Bhatnagar et al., (2007); Shingadia (2011; 2012, 2014); Shingadia and Agharia (2013).

II. MATERIALS AND METHOD

The test fish, *Poecilia reticulata* Peters measuring 3.5±0.1cm & average weight of 0.52±0.002g were acclimated in the laboratory for two weeks and fed with standard pellet food. The physico-chemical parameters like temperature, pH, DO, Free CO_2, Total hardness, Alkalinity and Acidity of aged tap water were analysed using standard methods as given in APHA (2005). Twenty-five acclimated healthy fish were exposed to three sub-lethal concentrations of Sodium fluoride (NaF) viz. lowest (5.75 ppm), lower intermediate (7.18 ppm) and higher intermediate (9.58 ppm). The doses were selected on the basis of 96 hrs. LC_{50} value being 115 ppm. A duplicate set of this experiment was simultaneously run for confirmation of the results. Control set with same number of test fish but without any toxicant was also run simultaneously. The tests were carried out in glass aquaria measuring 60x30x30cm³ dimensions. The amount of water in each tank was 2.0 L/g body weight of the test fish. Entire water from each tank was replaced every alternate day to avoid any accumulation of metabolic wastes and to keep the level of toxicants in the respective tanks constant. At the end of chronic toxicity test period of 60 days, the surviving fish from control & treatment tanks of three sub-lethal concentrations viz. 5.75 ppm, 7.18 ppm and 9.58 ppm were used for the histopathological studies. After sacrificing the fish by decapitation, gills were dissected out and immediately fixed in neutral formalin fixative to prevent autolysis and preserve the shape, structure and chemical constituents of the tissue. After 24 hours of fixation the tissues were dehydrated with alcohol. The tissues were then subjected to the process of infiltration in paraffin wax (M.P. 52-54ºC). Paraffin blocks were prepared and sections were cut on the microtome at 4-5µm thickness. After dehydrated with alcohol the dewaxed tissues were stained with Erhlich's Haematoxylin and Eosin Y stain (Gurr, 1956). Finally, the sections were mounted in Diether Plasticizer Xylene & photomicrographs were taken using digital camera.

III. RESULTS AND DISCUSSIONS

The physico-chemical characteristics like Temperature, pH, DO, Free CO_2, Total hardness, Alkalinity and Acidity of aged tap water used in the bioassay study is as presented in Table 1. The examination of gill tissue exposed to three sub-lethal concentrations of Sodium fluoride at the end of 60 days exposure period revealed that in the lowest concentration (5.75 ppm) of sodium fluoride, the tissues were practically unaffected whereas the damage caused to the tissues of fish treated with lower intermediate (7.18 ppm) and higher intermediate (9.58 ppm) concentrations was of almost similar nature and hence the histopathological changes pertaining to both these concentrations are discussed together.

Histological structure of gills of fish from control tank (Fig. 1):

The gill in fish forms the major site for gaseous exchange. These are situated beneath the operculum and consist of four pairs of arched gill bars. Each bar bears two adjacent rows of slender gill filaments that are flat and

elongated, arising alternately along its length. These are called primary gill lamellae (PGL). On the upper and lower surface of each gill filament there are series of thin parallel folds called secondary gill lamellae (SGL). Blood vessels are extended into each of the secondary gill filaments. These form the sites of gaseous exchange. The gill filaments are supported by a cartilaginous gill ray that acts as a mechanical support and a site of attachment for the adductor muscles. The epithelial wall (EW) of the secondary gill lamellae lies on the basement membrane (BM) and is supported by Pillar cells (PC) and Salt cells (SC). Epithelium of the filament is multilayered and particularly well developed at the tips and also possess numerous mucous cells (MC). The region between the two adjacent secondary gill lamellae is known as inter-lamellar space (ILS).

Histopathological structure of gills of Sodium fluoride treated fish (Fig. 2):

Marked pathological changes in the exposed gill of the treated fish in the apical region as compared with control were observed. There was congestion in the primary gill lamellae (PGL) of gill with accumulation of erythrocytes (RBC). There was proliferation of cells in the secondary gill lamellae (SGL). The epithelial wall (EC) of secondary lamellae appeared ruptured due to necrosis and lifting of respiratory epithelium. Vacuolation (V) was also observed at the base of the secondary lamellae with thickening of basement membrane (BM) and enlargement of cartilaginous axis cells (CAC). Swelling at the tips of the secondary gill lamellae (STSL) was observed. Both the primary and secondary gill lamellae appeared to be ruptured. Gill filaments at the interval showed splitting of gill tissue and rupture of gill filament. Architecture of Pillar cell (PC) was perhaps changed by proliferation in Chloride cell (CC) amount and their consequent bulging to the periphery. The necrosis observed in the cellular architecture of the gill tissue in histopathological observation revealed the toxic effects of fluoride resulting in physiological imbalance triggered by disruption in primary and secondary gill lamellae with hyperplasia and hypertrophy of gill tissue distressing the respiratory function in the test fish.

The above-mentioned results depict the damage caused by sodium fluoride to the histoarchitecture of the vital respiratory tissue of Guppy, *Poecilia reticulata* Peters. Most degenerative changes in cells are initiated by inability to maintain proper ionic balance. Cellular injury causes an intracellular reduction in oxidative phosphorylation with a resultant drop in the level of Adenosine triphosphate and a concomitant shift towards acidosis. The cation pump of the cell, which uses ATP as an energy source, breaks down allowing an influx of sodium, chloride, calcium and water. This causes cellular swelling and damages cell membranes, which leak intracellular ions like Potassium, enzymes and proteins. Most degenerative changes in cells are irreversible to a certain degree before cell death becomes inevitable. Leandro et al. (2004) reported histological anomalies in branchial epithelium with increased mucous secretion and chloride cells and also reported alteration in the content of the granules, suggesting behavioral changes with excessive secretion of mucous to enable Guppy fish to adapt to the toxic environment due to increasing concentration of sodium fluoride. Hitesh Shingadia (2011, 2012, 2014) have earlier reported chronic revelation of sodium fluoride to induce pathohistological alterations in gonads, liver and intestine of *Poecilia reticulata* Peter and & its repercussions on activities of some marker enzymes.

The present investigations with the aforementioned histopathological observations clearly exhibit highly significant changes in the respiratory tissue of test fish under chronic exposure of sodium fluoride. The alteration in the gill tissue might reduce the respiratory area thereby reducing the respiratory and

osmoregulatory potential of the fish. It also indicates a decrease in energy metabolism due to degeneration of respiratory epithelium and the damage of the gill tissues may finally result in tissue hypoxia. Similar observations on fish as test animal have been reported by many researchers that substantiates with the present findings. Bhatnagar et al. (2007) reported fluoride induced pathological irreversible damage to vital tissue like gill of freshwater teleost, *Labeo rohita* characterized by clubbed lamellae, mucoid metaplasia and lamellar hyperplasia that validates with the present findings. Proliferation of mucous cells in the epithelium of the gills and the head region is postulated to be instrumental in the excretion of fluoride from the body and is considered an effective defense mechanism against fluoride intoxication as propounded by John and Williams (2011). Yang et al. (2011) reported that NaF reduced cell viability in a temporal and concentration dependent manner thus promoting osteoblast apoptosis even at lower concentrations (10^{-5}M). Abdo et al. (2011) observed in ultrastructure of kidney of proximal lining cells; some heterochromatic nuclei, numerous cytoplasmic vacuolation of variable sizes and small scattered rounded mitochondria associated with loss of basal infoldings. These results suggest that chronic exposure to elevated concentrations of fluoride might induce toxicity in the test fish. Bajpai and Tripathi (2012) observed that when Catfish, *Hetropneustis fossilis* (Bloch) was exposed to Fluoride, primary and secondary lamellar epithelium became swelled and clubbing on the tip of secondary lamellae of gills, shortening and fusion of secondary lamellae, hyperplasia and hypertrophy in chloride cells of gills that are also observed in the present study. Cao et al. (2014) observed increase in ROS and decrease in antioxidant capacity in the gills of chronically exposed *C. carpio* that eventually caused the apoptosis in the fluoride-exposed gills and cells in the test fish, which according to them played a crucial role in physiological functioning of gill impairment induced by chronic fluorosis.

IV. CONCLUSION

The primary task of toxicological work is providing a scientific basis for the maximum permissible concentration of toxic substances or pollutants to be released in the water bodies and determining the maximum harmless concentration of toxic substances for aquatic animals. Thus, when the concentration exceeds permissible limits irreparable architectural changes in the vital organ like gills damages them making the fish less fit for better survival. The gill epithelium is the site of gas exchange, ionic regulation, acid-base balance and nitrogenous waste excretion in fishes that are controlled by passive and active transport of various solutes across the epithelium. Environmental pollutant like fluoride have been found to affect the morphology of the gill epithelium causing physiological disturbances that underlay the harmfulness of this pollutant. The present study has established toxicity of even otherwise essential element like Fluorine (as NaF) in the freshwater Guppy fish, *Poecilia reticulata* Peters at tissue level that are essential for their body energetics and normal metabolism.

V. ACKNOWLEDGEMENT

Author sincerely thanks the Principal of Mithibai College for constant encouragement & also acknowledges the parent management, Shri Vile Parle Kelvani Mandal for their support. Heart filled gratitude is expressed to madam Veena Sakthivel for her valuable guidance.

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Table 1 Physico-chemical characteristics of test water used in bioassay study

Hydrological Parameters	Range
Temperature (ºC)	29-30
pH	7.2-7.6
Dissolved Oxygen (mg/L)	5.5-6.5
Free CO_2 (mg/L)	Nil
Total Hardness (mg/L as $CaCO_3$)	35-40
Alkalinity (mg/L)	50-55
Acidity (mg/L)	3-4

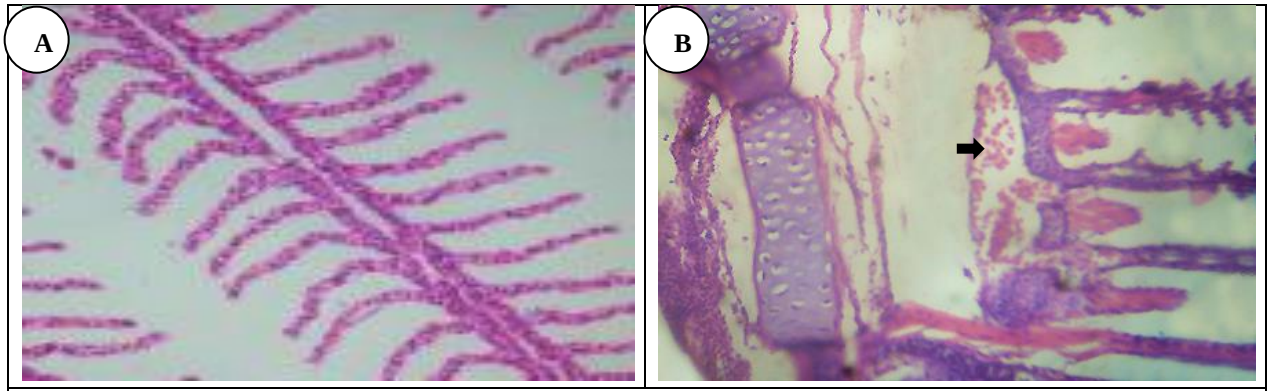

Fig. 1 Showing normal histological structure of Gills of *P. reticulata* Peters from control tank. (A=40x & B=100x)

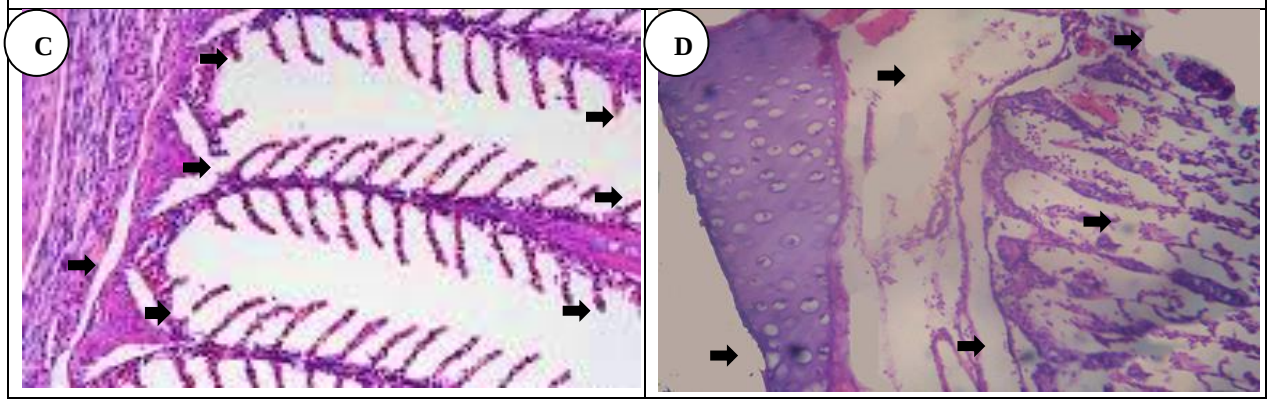

Fig. 2 Showing Sodium fluoride induced histopathological changes in Gills of *P. reticulata* Peters from treated tank. Arrow indicates disintegration of gill lamellae, degeneration of gill tissue, thinning of mucosa & vacuolation. (C=40x & D=100x)

[CL (Clubbed lamellae); EC (Epithelial cell); ENC (Endothelial cell); ILS (Interlamellar space); LF (Lamellar fusion); PC (Pillar cell); PGL (Primary gill lamella); RBC (Red blood cells); SC (Salt cell); SGL (Secondary gill lamellae); STSL (Swollen tip of secondary lamellae)]