Histological Alterations in The Liver Architecture of Freshwater Fish, *Rasbora Daniconius,* Exposed to Sodium Fluoride

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Abstract

The freshwater weed fishes are very sensitive to the fluoride. The fishes are the major component of food web of humans. Fluoride is considered to be a pollutant to aquatic ecosystem, beyond the normal permissible limits. The freshwater fish Rasbora daniconius were exposed to 200mg/l Sodium fluoride for 30days to study its effect on various organs. The significant to highly significant histological alterations were noted in the gills, liver, kidneys and gonads of the studied fish. The histological alterations were noticed in the liver such as hypertrophy of hepatic cells, loss of polygonal shape of liver cell, and loss of cell boundaries. The vacuolar degeneration, focal areas of coagulative necrosis, destruction of hepato-portal blood vessels and hemorrhage between the hepatocytes were observed. The dilation and congestion were noticed in blood sinusoids. All these alterations in liver architecture were indicative of toxic action of fluorides.

Key words: Sodium fluoride, Liver, Histopathology, *Rasbora daniconius*.

Introduction:

The aquatic environment is subjected to different types of pollutants due to human activities such as industrialization, domestic and agricultural waste. These wastes affect severely on aquatic organisms. For freshwater fishes water-borne fluoride is toxic at low levels but this is highly dependent on exposure time, temperature and water hardness. (Camargo JA. 2003). The first major natural source of inorganic fluoride is the weathering of fluoride minerals. Volcanoes are the second major natural source through the release of gases with hydrogen fluoride (HF) into the atmosphere. (Canadian Environmental Protection Act, 1994). Most important inorganic fluoride minerals in the earth's crust are fluorapatite (Ca5(PO4)3F), fluorite (CaF2) and crayolite (Na3ALF6).

It is desirable in very limited quantities for healthy growth of bones and teeth preventing dental cavities, but in excess causes a disease known as fluorosis. The problems associated with an excess of fluoride exposure is that it amplifies the biochemical stress in the body by generating imbalance between reactive oxygen species and antioxidants there by inducing oxidative stress. At high concentration, it causes adverse changes in the soft tissues like kidney, liver, and brain leading to adverse consequences in neurological, behavioral and physiological functions.

The problems of environmental pollution and its deleterious effects on aquatic organisms received focused interest during the last decades. Human activities results in substantial fluoride input to the

aquatic environment. Exposure of living organisms to abnormal levels of fluoride may result in an alteration of the organism's biochemistry, histology and electrolyte compositions. Although fluoride leads to significant environmental concentrations only in the vicinity of the sources, low level of fluoride pollution appears to be more wide spread.

Growth (1975) stated that freshwater organisms are evolved in an environment, almost fluoride free, thus they are not well equipped to tolerate. Carpenter (1969) stated that slight increase in fluoride in fresh water becomes toxic to the organisms. However, the survey of literature indicates that much of the work is carried out on human beings and less attention has been paid on the effect of fluoride on freshwater fishes and hence an attempt has been made to know any histological alterations in various tissues of the fish, *Rasbora daniconius* exposed to sublethal dose of sodium fluoride.

Material and Methods:

The fresh water fish, *Rasbora daniconius* were collected from Wadali reservoir, Amravati. They were disinfected in 1% $KMNO_4$ solution to avoid infection and then maintained in glass aquaria (183x38x38cm) for two weeks. After acclimation healthy and active fishes of uniform size (11-12cm and 22-29gm) were stored out and kept in separate aquaria for Experimental work. Chlorine free aged tap water was used in both control as well as Experimental aquaria. Fishes were kept away from mechanical disturbances. Fish were fed with Tykio feed, and the aquaria water was renewed on alternate days and supplemented with a fresh dose of NaF.

The physio-chemical characteristics of water were analysed by APHA (1998)

Physio-chemical characteristics of water.

S.N.	Parameter	Result
1	pH	8.2 + 0.2
2	Temperature	25 + 2 ºC
3	Dissolved Oxygen	6.6mg/lit
4	Total Hardness	276mg/lit
5	Alkalinity	312mg/lit
6	Fluoride	0.6mg/lit

The sodium fluoride (NaF) was used as toxicant. That was obtained from Qualigens Fine Chemicals, Mumbai. The Experimental fish, *Rasbora daniconius* was treated with 200 mg/l of sodium fluoride for 10 and 30 days. After 30 days of exposure of the fish were dissected and tissues like bone, gills, liver, kidney and muscles were removed to estimate various biochemical and electrolytes and to study various histological alterations.

The dissected pieces of tissues like gills, liver, kidneys and gonads were immediately isolated and fixed in Bouin's fluid for 24-48 hrs. After fixation, the tissues were washed to get rid of excess fixative and then dehydrated through ascending grades of ethyl alcohol (30% 1hr, 50% 1hr, 70% 1 hr, 80% 1 hr, 90% 1 hr and 100% changed twice during 1 hr). The specimens were cleared in xylene for 15-20 min and infiltrated with and embedded in paraffin wax. The paraffin wax block was sectioned at the thickness 4-6 *µm.* Sections were mounted on clear glass slides and were stained with Harris' haematoxylin and eosin. They were examined under the microscope and microphotographs were taken at X400 for histopathological study.

Result:

Fig: 1. The Control group of liver of fish, *Rasbora daniconius*

Fig: 2. Sodium Fluoride low dose treated liver after 10 days treatment from the fish, *Rasbora daniconius*.

Fig: 3. Sodium Fluoride high dose treated liver after 30 days treatment from the fish, *Rasbora daniconius*.

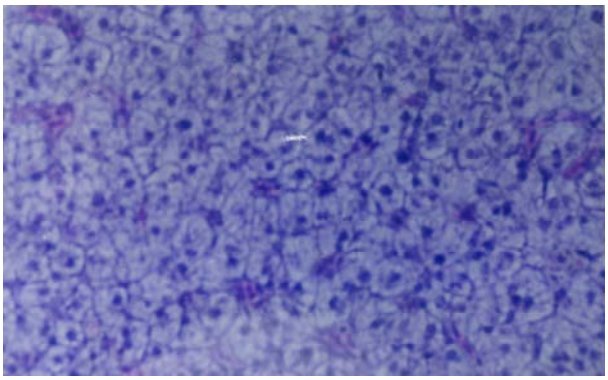

Fig:1. T.S. of liver of freshwater fish, *Rasbora daniconius* showing normal size distinct nuclei and blood vessels.

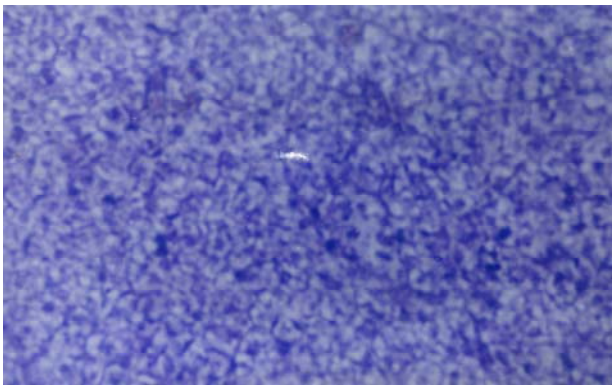

Fig: 2. T.S. of liver of freshwater fish, *Rasbora daniconius* showing degeneration, disruption, and necrosis, loss of cellular matrix, after 10 days (low dose) exposed to Sodium Fluoride.

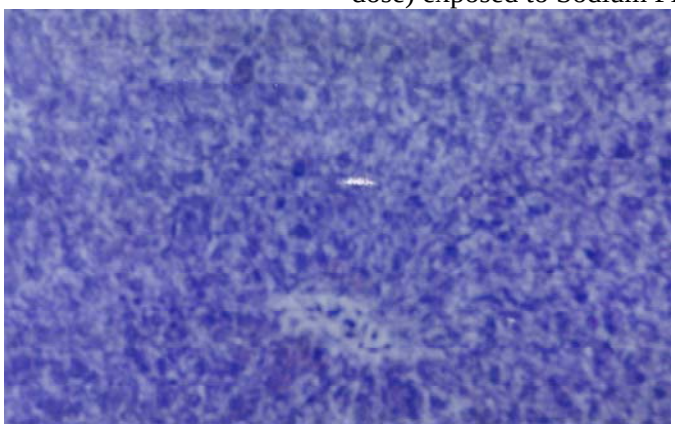

Fig: 3. T.S. of liver of freshwater fish, *Rasbora daniconius* showing necrosis, disorganization, vacuolization, congestion of blood vessels, dilation in sinusoids, nuclear atrophy, and loss in structure of hepatocytes, after 30 days (high dose) exposed to Sodium Fluoride.

Control: Normal liver has a mass of roundish polygonal hepatocytes with centrally located deeply stained nuclei and granular cytoplasm. T.S. liver of freshwater fish, *Rasbora daniconius* showing normal size distinct nuclei and blood vessels.

Experimental group:

Low concentration of Sodium Fluoride (200mg/lit for 10days) exposed group showed degeneration, disruption, and necrosis, loss of cellular matrix, vacuolization and pyknotic nuclei (Fig:2).

High concentration of Sodium Fluoride (200mg/lit for 30days) exposed group showed necrosis, hypertrophy and hyperplasia, congestion in blood vessels, atrophy, pyknotic nuclei, vacuolization and loss in structure of hepatocytes after 30 days exposure (Fig: 3).

Discussion:

Liver is the major metabolic center and any damage to this would subsequently do, so many physiological disturbances leading to subsequent mortality of fish. The main histopathological lesions recorded in the liver of fish from exposure of low and high dose concentration of fluoride. Low concentration exposed group showed degeneration, disruption, and necrosis, loss of cellular matrix, vacuolization and pyknotic nuclei (Fig:2). High concentration exposed group showed necrosis, hypertrophy and hyperplasia, congestion in blood vessels, atrophy, pyknotic nuclei, vacuolization and loss in structure of hepatocytes after 30 days exposure (Fig:3). After 10 days exposure of fluoride low concentration exposed group showed degranulation in hepatocytes, vacuolization, necrosis, congestion of blood vessel, karyolysis. High concentration exposed group showing necrosis, hypertrophy, disorganization, vacuolization, fibrosis, congestion of blood vessels, dilation in sinusoids, nuclear atrophy, disruption and ruptured hepatocytes (Fig:3). Similar results were reported (Blankenship *et al*.,1994) that vacuolization of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in hepatocytes. The hepatocellular dystrophy with pyknotic nuclei in chronic exposure fish indicates cellular death, oxidative stress induced cellular apoptosis of Cr (VI) was reported. (Stentiford *et al*., 2003) Non-neoplastic lesions, such as cellular and nuclear polymorphism have been considered as an initial toxicopathic lesion resulting from exposure to toxic agents. (Gingerich W.H. 1982).

Conclusion:

Liver is the largest gland of the body. It receives pollutants indirectly through blood. It is susceptible to a large number of toxic and metabolic disturbances and serves as an indicator of toxicity of surrounding water. Sodium fluoride produced various deleterious effects such as hypertrophy of hepatic cells (Fig.2), loss of characteristic polygonal shape of liver cells, and loss of cell boundaries (Fig.3). All these alteration in liver architecture are indicative of toxic action of fluoride.

References:

[1]. APHA, AWWA, WEF. (2005). Standard methods for the examination of water and waste water. 21sted. Washington DC: American Public Health Association,

[2]. Blankenship LJ, Manning FC, Orenstin JM, Patierno SR.(1994). Apoptosis is the mode of cell death caused by carcinogenic chromium. Toxicol Appl Pharmacol,; 126:75-83.

[3]. Camargo JA, Tarazona JV. (1991). Short-term toxicity of fluoride ion (F) in soft water to rainbow trout and brown trout. Chemosphere; 22:605-611.

[4]. Camargo JA. (2003). Fluoride toxicity to aquatic organisms: a review. Chemosphere, 50:251-264.

[5]. Canadian Environmental Protection Act, Priority Substances List Supporting Document for Inorganic Fluorides. Prepared by Eco-Health Branch & Environment Canada, Ottawa (Ontario), 1994.

[6]. Carpenter, R. (1969): Factors controlling the marine geochemistry of fluoride.

[7]. Couch, J. A. (1975). Histopathologic effects of pesticides and related chemicals on the livers of fishes. In: The pathology of fishes (W.E. Ribelin and G. Migaki, eds.). Univ. Wisconsin Press, Madison, Wisconsin, pp. 559 - 584.

[8]. Fatma Abd El-Moghney S, Mohamed: (2003). Histopathological studies on some organs of Oreochormis niloticus, Tilapia zilli and Synodontis schall fron El-Salam canal, Egypt., Egypt. J. Aqual. Biol & Fish.. Vol.7 No3: 99-138

[9]. Gingerich WH. (1982). Hepatic toxicology of fishes. In: Aquatic toxicology. (Eds. L.J. Weber). H Raven Press, New York,; 55-105.

[10]. Growth, E. (1969): An evaluation of the potential for ecological damage by chronic low level environmental pollution by fluoride. Fluoride. 8: 224-240.

[11]. Guan ZZ, Wang YN, Xiao KQ, Dai DY, Chen YH, Liu JLP.(1998). Influence of chronic fluorosis on membrane lipids in rat brain. Neurotoxicol Teratol; 20:537-542.

[12]. Karaoz E, Oncu M, Gulle K, Kanter M, Gultekin F Karaoz S. (2004). Effect of chronic fluorosis on lipid peroxidation and histology of kidney tissues in first- and second-generation rats. Biol Trace Elem Res; 102(1-3):199-208.

[13]. Manna P, Sinha M, Sil PC. (2007). A 43 kd protein isolated from the herb Cajanus indicus L attenuates sodium fluoride induced hepatic and renal disorders in vivo. J Biochem Mol Biol 40:382-395

[14]. Patel J.M and A.Bahdur., (2011). Histopathological manifestation of sub lethal toxicity of copper ions in Catla catla American-Eurasian Journal of Toxicological Sciences 3(1):01-05

[15]. Stentiford GD, et al (2003). Histopathological biomarkers in estuarinefish species for the assessment of biological effects ofcontaminants. Marine Environmental Research; 55:137-159.

[16]. Varley, H. (1988): Practical Clinical Biochemistry. 4th Ed. CBS Pub. & Distributors, Delhi. 492-493.

[17]. Yadav S. S., R. Kumar, and Tripathi M. (2014). Effects of fluoride exposure on some enzymatic and histopathological changes in the liver of Heteropneustes fossilis (Bloch). IJFBS; 1 (5): 80-84