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Shiv Shankar Yadav

Aquatic Toxicology Research
Laboratory, Department of Zoology,
University of Lucknow, Lucknow
U.P.-226007, India

Rajesh Kumar

Aquatic Toxicology Research
Laboratory, Department of
Zoology, University of Lucknow,
Lucknow U.P.-226007, India

Madhu Tripathi

Aquatic Toxicology Research
Laboratory, Department of
Zoology, University of Lucknow,
Lucknow U.P.-226007, India

Effects of fluoride exposure on some enzymatic and histopathological changes in the liver of *Heteropneustes fossilis* (Bloch).

Shiv Shankar Yadav, Rajesh Kumar, Madhu Tripathi

Abstract

Present study in fresh water fish *Heteropneustes fossilis* (Bloch) were exposed to sub lethal concentrations (35 mg/L and 70 mg/L) of fluoride, for 45 days & 90 days. After fluoride exposure aspartate transaminase (AST) and alanine transaminase (ALT) increased ($P < 0.01$) after 45 & 90 days of low and high dose exposure. There were decreases of protein $P > 0.05$ after 90 days exposure to both low and high dose exposure. After 45 days exposure $p < 0.01$ decreases high concentration of fluoride. The lipid accumulation could have contributed to the increase of liver weight. After 45 & 90 days' exposure of fluoride histopathological observations of liver at low & high dose concentration. As a result foci of necrosis fibrosis, pyknotic nuclei, disruptions and rupture as well as hypertrophy & hyperplasia of hepatocytes were found. The changes included swollen cells, vacuoles filled with cellular debris, focal necrosis, and dilatation of sinusoids.

Keywords: Fluoride, *Heteropneustes fossilis*, AST & ALT, Total protein, Histology of Liver

1. Introduction

Fluoride included as toxicant in the list of UNEP. In human beings chronic fluoride toxicity by the intake of a moderate or high dose of fluoride. These fluoridated water is known to produce fluorosis such as osteosclerosis and symptoms as pain and stiffness in joints ^[1] and fluoride intake through inhalation in industrial area has been reported to be as occupational hazards. For freshwater fish, water-borne fluoride is toxic at low levels but this is highly dependent on exposure time and water hardness ^[2]. 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 ^[3]. Most important inorganic fluoride minerals in the earth's crust are fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$), fluorite (CaF_2) and craxolite (Na_3AlF_6). Fluoride toxicity to fishes increases with increasing fluoride concentration in the aquatic medium, exposure time and water temperature ^[4]. 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 (ROS) and antioxidants there by inducing oxidative stress. At high concentration, it causes adverse changes in the soft tissues like kidney ^[5] liver ^[6] and brain ^[7] leading to adverse consequences in neurological, behavioural and physiological functions. There are evidences indicating metabolic disturbances, enhanced free radical activity ^[8, 9] structural alterations and dysfunctioning ^[10, 11] in soft tissues due to fluoride exposure resulting in oxidative damage ^[12] to bio molecules. Serum enzymes (ALT, AST) are known to be important serum markers to investigate the health of an animal species. Transaminases such as AST and ALT are located in the cytoplasm and mitochondria respectively predominantly in the liver, cardiac cells and striated muscle tissue and playing a vital role in protein metabolism ^[13]. Both AST and ALT have been used to demonstrate tissue damage in fish ^[14, 15]. Moreover the changes in these enzymes have been studied as possible tools for aquatic toxicological research.

Histopathological technique is sensitive, reliable and comparatively inexpensive tool for the assessment of stress-response to toxicant or pollutants. Hence this appears histopathological changes in the liver of fish, *H. fossilis* after exposure to biochemical and other observations reported in organism to sub lethal concentrations of fluoride was included in the present study. Results of present study will be helpful in understanding the mechanism of fluoride toxicity in fishes. This will give a measure to evaluate the role of fluoride contaminated water in aquaculture. *H. fossilis* are widely cultured in tropical and subtropical regions of the world

Correspondence:

Shiv Shankar Yadav

Aquatic Toxicology Research
Laboratory, Department of
Zoology, University of Lucknow,
Lucknow U.P.-226007, India.

and are an important source of protein for human beings. This study emphasized that both enzymatic and nonenzymatic mechanisms may be useful in understanding the degree of fluoride toxicity in fish liver.

2. Materials and methods

Healthy specimens of *H. fossilis* (mean weight 28.04 ± 0.20 g and length 17.09 ± 0.20 cm) were purchased from the local market in Lucknow. They were transported to the laboratory in large plastic containers filled with fresh water to minimize stress. Fish were checked for disease as well as injury and rinsed in 0.1% KMnO_4 solution to avoid infection and were acclimated for about 25 days in dechlorinated tap water contained in a large steel tank with proper aeration. For the experiment, healthy fish were sorted out and transferred into aquaria measuring $60 \times 40 \times 45$ cm holding 40 L of water. They were divided into three groups of 12 fish in each group. Group I served as the control (without F added to the water) and group II was exposed to a sublethal concentration of fluoride 35 mg F ion/L (one tenth of 96-hour LC_{50} value). The group III was exposed to a sublethal concentration of fluoride 70 mg F ion/L (one fifth of 96-hour LC_{50} value). The NaF (AR grade) obtained from Qualigens Fine Chemicals Limited, Mumbai, India. The physico-chemical properties of the holding water were determined according to [19] methods. Temperature 27 ± 1.5 °C, pH 7.3 ± 0.2 , dissolved oxygen 7.8 ± 1.5 mg/L, alkalinity as CaCO_3 225–230 mg/L, hardness as CaCO_3 250–290 mg/L, and F 0.1 mg/L. Fish were fed with Tykio feed, and the aquaria water was renewed on alternate days and Supplemented with a fresh dose of NaF.

i. Serum preparation and analysis

At the end of the each experimental duration, six fish were removed from the aquaria and used as replicates. Blood samples were taken from the caudal vein of each fish as described by [16]. This blood was collected in anticoagulant free centrifuge tubes. Serum was obtained by centrifugation of blood at 3500 rpm for 10 min. at 4 °C. Serum samples were then stored at -80 °C until analysis. Biochemical parameters in the serum samples were analysed using Spectrophotometer (Schimadzu).

ii. Enzyme activity

ALT and AST activities were determined spectrophotometrically by Reitman & Frankel [17]. The products of ALT and AST activities, pyruvate and oxaloacetate, were used to oxidise NADH to NAD^+ . The increase rate of the photometrically determined NADH is directly proportional to the rate of formation of pyruvate or oxaloacetate and thus to ALT or AST activity, respectively. Total protein was estimated by the Lowry method [18].

iii. Histopathological examination of tissues

After blood sampling, samples of liver washed with 0.9% saline, were taken for histopathological examinations. The taken samples were immediately fixed in 10% formalin, drained and embedded in paraffin. Sections were made of the paraffin blocks and stained with haematoxylin-eosin.

iv. Statistical calculations

Results were expressed as Mean \pm SEM. Data was subjected to one way analysis of variance (ANOVA). The treatment groups were compared with a control group using Dunnett's test. All the statistics were carried out in Graph Pad In Stat Software Inc., v. 3.06, San Diageo, USA.

3. Results and Discussion

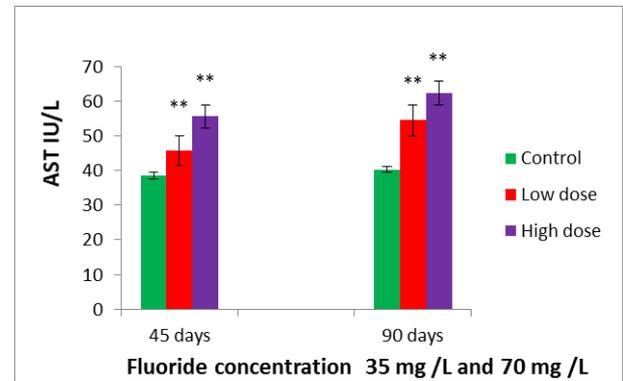


Fig 1: Effects of sublethal fluoride concentrations on serum enzymes aspartate transaminase (AST), after 45 & 90 days exposure. Data are expressed as mean \pm standard error (SE). Comparison with control group are significantly different from each other ($p < 0.01$).

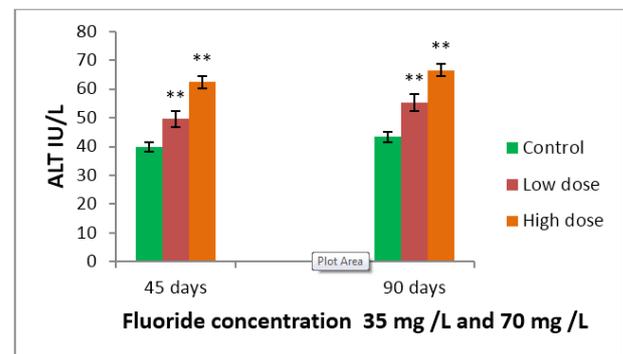


Fig 2: Effects of different sublethal Fluoride concentrations on serum enzyme alanine transaminase (ALT) after 45 & 90 days exposure. Data are expressed as mean \pm standard error (SE). Comparison with control group are significantly different from each other ($p < 0.01$).

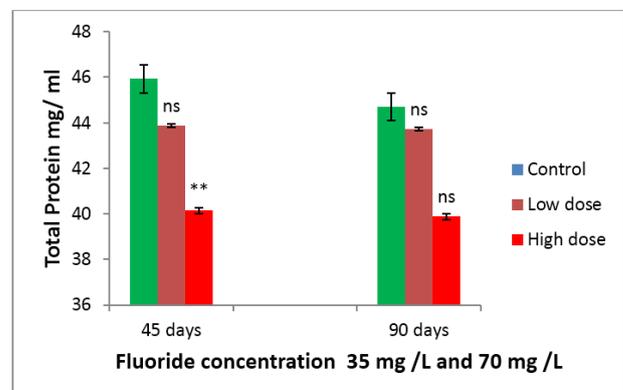


Fig 3: Effects of different sublethal fluoride concentrations of total protein, after 45 & 90 day's exposure. Data are expressed as mean \pm standard error (SE). Comparison with control group are significant and non-significantly from each other ($p < 0.01$) and ($p > 0.05$)

Histology of Liver

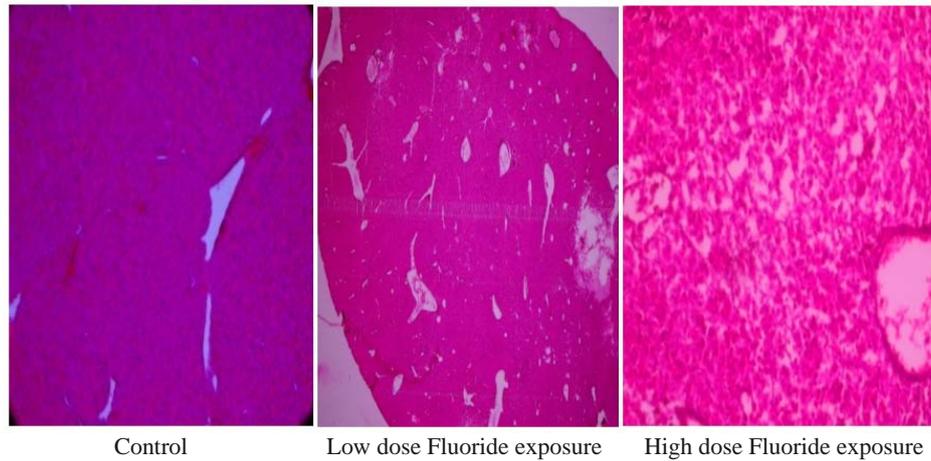


Fig 4: Low dose and high dose fluoride exposure (45 day's) with respect to control histology of liver *H. fossilis*. The Control group showed normal size distinct nuclei. While histological section of Liver at low concentration exposed group show degeneration, disruption, and necrosis, loss of cellular matrix, vacuolization and pyknotic nuclei. At higher concentration exposed group showed necrosis, hypertrophy and hyperplasia, congestion in blood vessels, atrophy, pyknotic nuclei, vacuolization and loss in structure of hepatocytes.

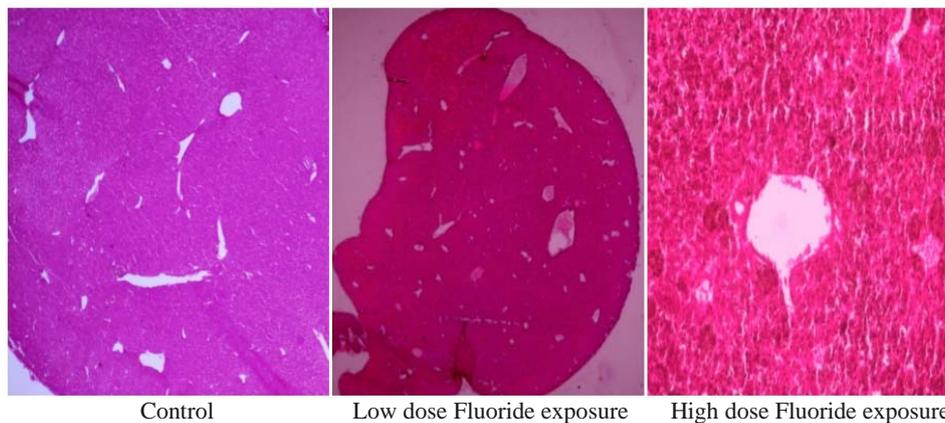


Fig 5: Low and high dose fluoride exposure (90 day's) with respect to control liver histology of *H. fossilis*. The Control group showed normal sized distinct nuclei and blood vessels while at low concentration exposed group showed degranulation in hepatocytes, vacuolization, necrosis, congestion of blood vessel, karyolysis. At higher concentration exposed group showed necrosis, fibrosis, hypertrophy, disorganization, vacuolization, congestion of blood vessels, dilation in sinusoids, nuclear atrophy, disruption and ruptured hepatocytes.

Both aspartate transaminase (AST) and alanine transaminase (ALT) activity was changed significantly by Fluoride exposure in *H. fossilis* after 45 & 90 days as compared to the control (Fig. 1 and 2). It was significantly increasing the level of both concentrations compared with control group ($p < 0.01$). ALT and AST are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills [20]. It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage [21]. According to [22] concluded that blood levels of ALT and AST may increase because of cellular damage in the liver and that high levels of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals. Our present study indicates that increased ALT and AST activity in the serum of *H. fossilis* is caused mainly by leakage of these enzymes from liver cytosol into the bloodstream as a result of liver damage caused by fluoride exposure. The similar result shows that the increased levels in AST and ALT observed in our fluoride-exposed fish could alter protein metabolism [23]. Tissue protein

content has been suggested as an indicator of xenobiotic induced stress in aquatic organisms [24]. The loss of protein in different tissues of toxicant exposed fish is probably due to the excessive proteolysis to overcome the metabolic stress. Similar results supported to the highest reduction of protein in liver of *H. fossilis* exposed to long term chronic toxicity of copper sulphate [25]. It shows the similarity results effect of Cadmium Chloride on the biochemical content in different tissues of the freshwater fish *Ophicephalus striatus* [26]. It was reported a decrease in protein content in *Channa punctata* exposed to sub lethal concentration of fenvalerate [27]. The similar decreasing trend in total proteins was also reported in the liver, brain and gill tissues of *Catla catla* under sub lethal and lethal concentrations of fenvalerate by [28]. Similar result reported the effect of fluoride on total protein content decline in different tissues of *H. fossilis* after exposure to fluoride for 45 and 90 days [29].

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. High concentration exposed group showed necrosis, hypertrophy and hyperplasia, congestion in blood vessels, atrophy, pyknotic nuclei, vacuolization and loss in structure of hepatocytes after 45 days exposure (Fig 4). After 90 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 5). Similar results were reported that vacuolization of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in hepatocytes^[30]. The hepatocellular dystrophy with pyknotic nuclei in chronic exposure fish indicates cellular death, oxidative stress induced cellular apoptosis of Cr (VI) was reported^[31]. Non-neoplastic lesions, such as cellular and nuclear polymorphism have been considered as an initial toxicopathic lesion resulting from exposure to toxic agents^[32].

4. Conclusion

Fluoride treatment induces liver damage as indicated by the elevation of AST, ALT & decline of the protein activity. A fluoride induced histopathological alterations in liver tissues as well as extensive vacuolated pycnosis and necrosis were highly evident at points and likely initiation of fibrosis was also observed.

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