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Hepatotoxic effects of Sodium Fluoride on *Channapunctatus(Blotch.)*

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ABSTRACT

Fishes are major diverse group of vertebrates exposed to environmental contaminants essentially of aquatic origin that accumulate in their body via dietary sources and aquatic ambient sources. Analogous to other animals', fish liver is essentially a producer of bile, aids in fat digestion, red cell turnover, a detoxifier and a potent gut immune defender. By virtue of extensive blood circulation and a potent bioaccumulation, liver is a vital organ for toxicological studies. Fluoride is one such ecotoxicological element that is becoming endemic for hydroflurosisin certain geographical regions of the world including India.Hence hepatotoxicity of fluoride in fish is considered in this study

KEY WORDS: Bio accumulator, detoxifier, fishes, Fluoride, liver,

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INTRODUCTION

Studies reveal that fluoride toxicity has become endemic in regions like Birbhum district of West Bengal because of its higher accumulation in water of those regions i.e. above 1.5 ppm (the permissible limit of aquatic fluoride level). Other regions of India viz. Bihar, Rajasthan, Maharashtra, Orissa, Kanataka etc. are fluoride rampant. Natural sources of fluoride depend on geographical strata including rock fluoride, apatite, and cryolite etc. involving disintegration-dissociation and dissolution reactions in the concerned water table[1]. Anthropogenic sources include effluents aluminium, fertilizer, iron, oil refining, and steel industries[2]. Aquatic biota responds differently to fluorides. The extent of fluoride toxicity in aquatic plants and animals depends on water quality like hardness, alkalinity, pH and temperature[3]. Algae usually exhibit either a weight gain or loss depending on factors influencing aquatic fluoride levels; other aquatic plants are effective in removing fluoride from water. However animals show adverse effects of fluoride as indicated by reduction in body weight, size, poisoning of essential enzyme and inhibition of enzyme activities specific for protein synthetic pathways and glycolysis. Moreover, fluoride accumulation in exoskeleton and hard tissues are reported in both aquatic invertebrates and fishes. Mortality of rainbow trouts increased with increasing fluoride levels and hatching of fresh water fish *Catlacatla* was delayed with aquatic fluoride exposure[4].Shi *et al*., 2009reported that fish exposed to concentrations over 25 mg fluoride/ litre displayed alterations in their respiration and violent erratic movements[5]. This was associated with respiratory enzyme inhibition that was manifested in histopathological sections showing morphological alterations of normal hsistoarchitechture. These changes include the increase in mucous cells in the epithelium of the head region and the gills. Behavioural changes have also been reported in fishes as indicated by movement pattern, response to food, operculum dynamics and delay in trout migration[6]. Fluoride affected fish intestine exhibited flattening and fusion of villi and renal damage has further been reported[7]. Toxicological aspects of fluoride has been monitored wide spread in different species of fishes under diverse water qualities indicated oxidative stress with contradicting results related to the activities of key antioxidant enzyme in organs of fishes, pinned in by fluoride. Yet studies on dose dependent effects of chronic aquatic fluoride exposure on altered activities of key cellular antioxidants mediated hepatic toxicity, and histopathology of liver in fresh water edible fish *Channapunctatus* (Blotch) maintained in Kolkata municipal water has not yet been studied. The perspectives of this study would not only elucidate the status of antioxidant enzymatic defence in hepatic milieu responsible for liver damage but also shall highlight the effects of water quality especially hardness on effective dose of fluoride responsible for such toxicity.

MATERIALS AND METHODS

Fishes (*Channapunctatus*) was procured from local market of Chinsurah, bearing average body weight of 70g and length (16cm) were maintained in laboratory aquarium. A standard prophylactic treatment in 0.05% $KMnO_4$ solution prior to treatment was done to prevent any dermal infection. fish were acclimatized in the laboratory ambient with food *ad libitium* for 15 days before the commencement of the experiments.

Chemicals used

All chemicals used in the present studies were of analytical grade.

Preparation of NaF solution

For 1 ppm 0.00221 g reagent grade sodium fluoride (NaF) was added to 1000ml water; for 3 ppm 0.00661g NaF was added to 1000ml of water ; for 5ppm fluoride preparation 0.011g of reagent grade NaF was added to 1000ml of experimental water.

Water analysis

Analysis of experimental water was done by standard water testing kit.

Dose response study of Sodium fluoride in vivo

After acclimatization to laboratory conditions, the fishes were divided into four groups, with 6 in each group:

Group I: Control

Group II: NaF1 (at a dose of 1 ppm)

Groups III: NaF3 (at a dose of 3 ppm)

Group IV: NaF5 (at a dose of 5 ppm)

Control group received the vehicle only. Each day, the body weight of the fishes were measured and recorded.

Preparation of tissue homogenate and measurement of lipid peroxidation (LPO) level

The liver tissues were separately homogenized (10%) in ice-cold 0.9%saline (pH 7.0) with a Potter Elvenjem glass homogenizer for 30 s and lipid peroxides (LPO) in the homogenate were determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust[8]with little modification[9].

Measurement of reduced glutathione content (GSH)

GSH content (as acid soluble sulfhydryl) of the liver was estimated by its reaction with DTNB (Ellman's reagent) following the method of Sedlak and Lindsey[10].

Measurement of the activities of cytosolic (Cu-Zn type) catalase (CAT), glutathione peroxidase(GPx), glutathione S transferase (GST)

Copper-Zinc superoxide dismutase (Cu-ZnSOD or SOD1) activity was measured by hematoxylinautooxidation method of Martin *et al.*,[11].Catalase activityof Blotch hepatic tissue was assayed by the method of Beers and Sizer[12].Glutathione peroxidase activity of the Blotch hepatic tissue was measured according to the method of Paglia and Valentine[13].The activity of Blotch hepatic GST was measured according to the method of Habig et al.,[14].

Measurement of tissue protein content

The protein content of various samples was estimated by the method of Lowry *et.,al.*,[15]using bovine serum albumin (BSA) as the standard.

Histological studies

Immediately following sacrifice of the fishes,liver was surgically extirpated and a small portion of the tissue was fixed in 10% formalin and embedded in paraffin following routine procedure. Tissue sections (5 μM thick) were prepared and stained with hematoxylin-eosin following routine hematoxylin-eosin staining of fish hepatic tissue sections.

Statistical evaluation

Each experiment was repeated at least three times with different Blotch fish. Data are presented as means ± S.E.M. Significance of mean values of different parameters between the treatment groups were analysed using one way analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatments. Pair-wise comparisons were done bycalculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS & DISCUSSION

The water used for the experiment showed the following physicochemical properties (Table1.):

Table No. 1: "Results of physicochemical analysis of experimental water"

Serial no.	Parameter of testing water	Parameter analytical method	Value
1	Temperature	Mercury thermometer	28±2 °C
2	Ph	pH-meter	7.2
3	Colour	General visualization	Colour less
4	Smell	General olfaction	Odourless
5	Total hardness	EDTA Titrimetric Method	1.2 mg /L
6	Calcium hardness	EDTA Titrimetric Method	0.8 mg /L
7	NH_3 hardness	Colorimetric method	Below the limit
8	Chloride hardness	Colorimetric method	Below the limit
9	Phosphate hardness	Colorimetric method	Below the limit
10	Total iron	Colorimetric method	Below the limit
11	Fluoride hardness	Colorimetric method	Below the limit

In the present study, sodium fluoride has been shown to be toxic for hepatic antioxidant enzymes at doses of 3 and 5 ppm. Level of lipid peroxidation found to be significantly increased (* = $p<0.05$; in all figures) withsodium fluoride at doses of 3 and 5 ppm (Fig.1.).

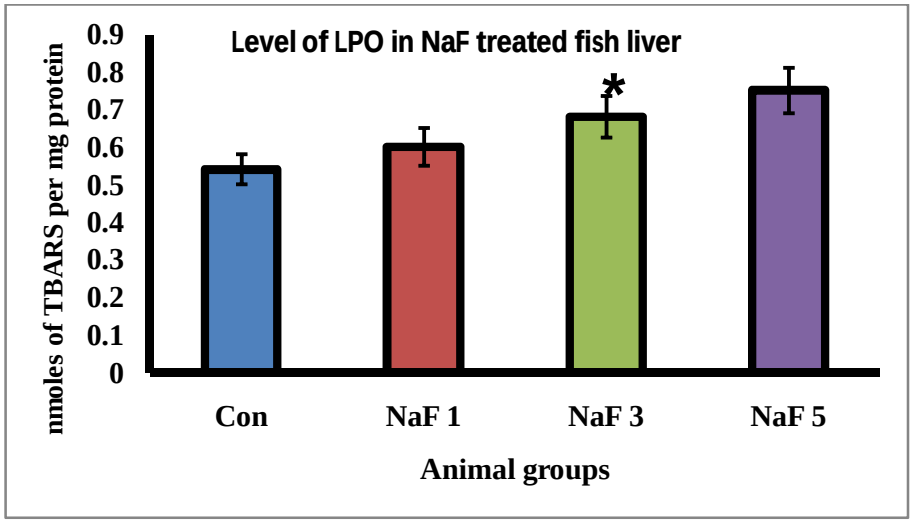

Figure 1. Effect of NaF on the level of lipid peroxidation (LPO) in fish liver

Existing literature proposed that F-concentration above 0.2 mg /l is lethal to Salmon fish preventing its migration[16]. It has been reported that soft water with low ionic strength can adversely affect fish health at doses below 0.5 ppm[4].Thus the selected doses may have toxicological impacts in fresh water fishes. However 1 ppm did not cause much alteration. Fluoride in water gets converted to hydrogen fluoride ion that is easily permeable through membranes leading to generation of

superoxide anions and downstream production of hydrogen-peroxides, hydroxyl radicals, peroxynitries etc. in the cell milieu leading to generation of oxidative stress in the microenvironment[17]. The antioxidant defence enzymes that scavenge such radicals were studied in the present investigation. The primary enzyme in this cascade, superoxide dismutase showed a dose dependent downfall in its activity proving its inability to forage the superoxide anions. Further GSH content of hepatic tissue was reduced significantly following fluoride administration at doses 3 and 5 ppm proofing a shuttering of superoxide dissmutase accompanied GSH mediated transformation of toxic superoxide anions to less harmful hydrogen peroxide and water which might otherwise lead to damage of cellular membranes and nucleic acids[18] (Fig. 2 & 3).

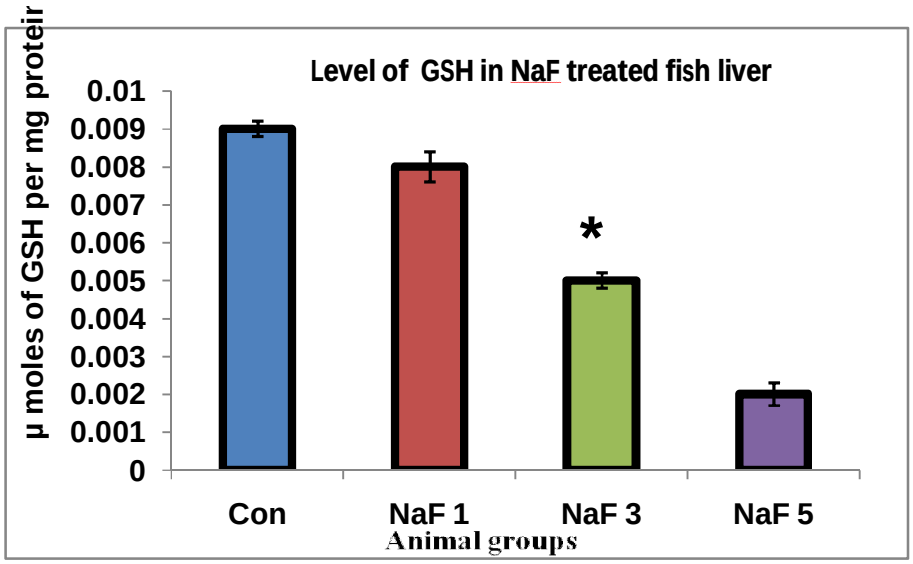

Figure2. Effect of NaF on the level of reduced glutathione (GSH) in fish liver

In addition fluoride anions competitively inhibit SOD by binding to its active site on copper in Cu-SOD [19]. This might be another facet of fluoride mediated inhibition of SOD as found in the present investigation. The later consequences may possibly lead to excess production of superoxide anion and peroxyl anions that inhibit catalase activity[20].The present findings are in line with that of previous studies[21].

Inhibition of SOD by NaF may be due to a competitive inhibition of the enzyme by the F– ion. The proposed mechanism for F– inhibition of SOD involves its binding to the active site of Cu on SOD, thus displacing water. The affinity of F^- for the active site has also been shown to vary with the source of SOD[19].

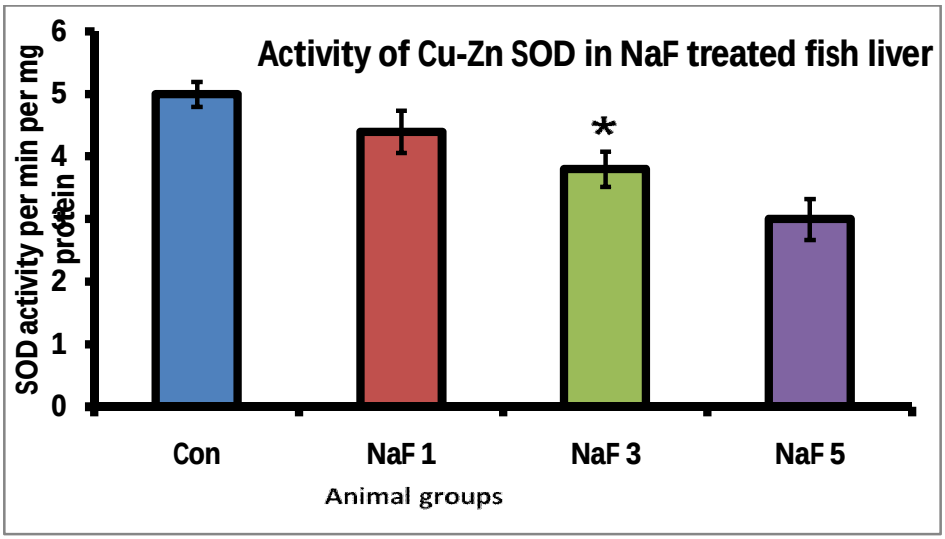

Figure3. Effect of NaF on the activity of superoxide dismutase (SOD) in fish liver

Catalase being a detoxifier of H_2O_2 showed a reduced activity in a dose dependent fashion upon fluoride exposure (Fig.4) which is supportive of previous findings[22]. A fall in the activity of SOD and a consequent rise in supeoxide anion and hydrogen peroxide may contribute to the decline in catalase activity[20]. Further the hydroxyl moiety of iron present in catalase may be replaced by low molecular weight anion possibly fluoride may even slay down the activity of catalase[23].

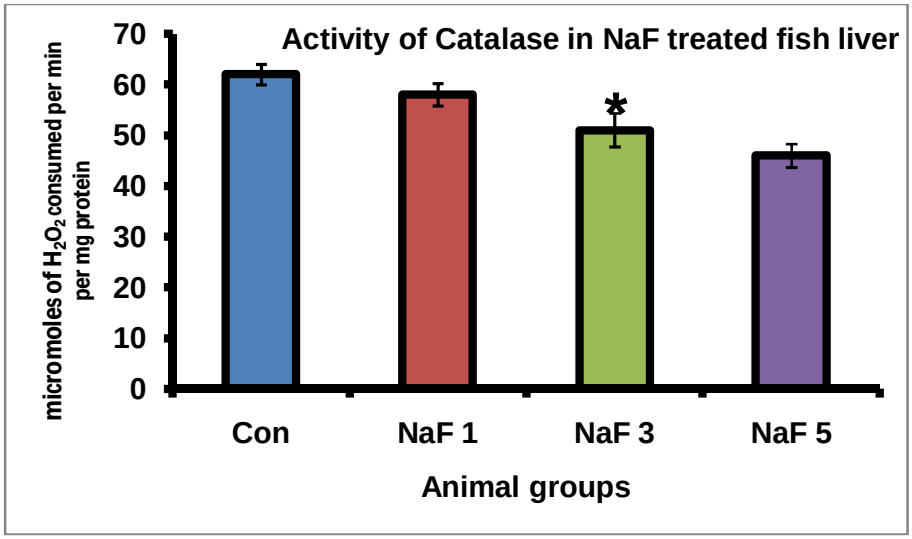

Figure4. Effect of NaF on the activity of ctalase (CAT) in fish liver

Glutathione Peroxidase, the selenium containing enzyme is responsible for decomposition of H_2O_2also showed a fall in its activity (Fig. 5). Selenium is an essential component of this enzyme that catalyzes the conversion of oxidized to reduced glutathione via pentose phosphate pathway.

Supplementation of F- may interact with the transition metal group present in the enzyme leading to its inhibition[24].

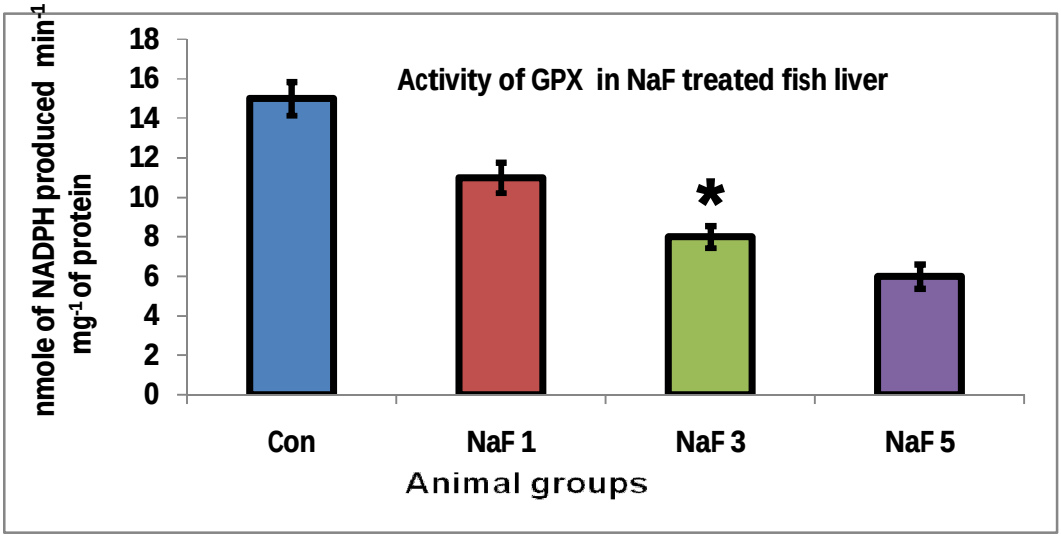

Figure5. Effect of NaF on the activity of Glutathione Peroxidase (GPx) in fish liver

In contrast to previous findings, GST activity was reduced in our study which may sustain the findings of Abdel-Wahab[25]hopefully due to fluoride interference of transition metal induced intonation of the activity of GST (Fig. 6).

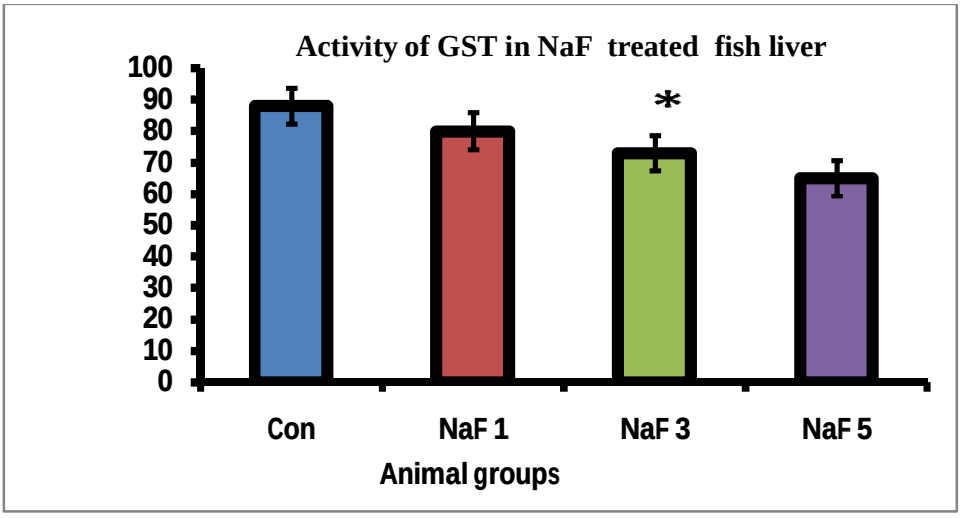

Figure6. Effect of NaF on the activity of Glutathione -S- transferase (GST) in fish liver

It is highly accepted that changes in tissues antioxidant defence may lead to ageing or damage to tissues by mechanism of apoptosis[26].

In the present investigation the marke dhistopathologicalalterations were observed.Eosin hematoxylin staining of control fishes showed normal his toarchitecture at lower magnification (40X) and at higher magnification normal vacuolated cell with nucleus stained purplish with hematoxylin (Fig.7).

At a dose of 1 ppm fluoride, the eosinophilic tendency of the hepatocytes increased although the cellular orientation was not affected. At a higher magnification enlarged hepatocytes with pyknotic appearance of nucleus was visible and some cellular infiltrations was observed(Fig.7).

Dose 3ppm fluoride showed moth eaten like morphology at lower magnification indicating loss of hepatic cell population. Higher magnification revealed enlarged hepatocytes with pleomorphic nucleus; pyknotic nucleus were also abundant.

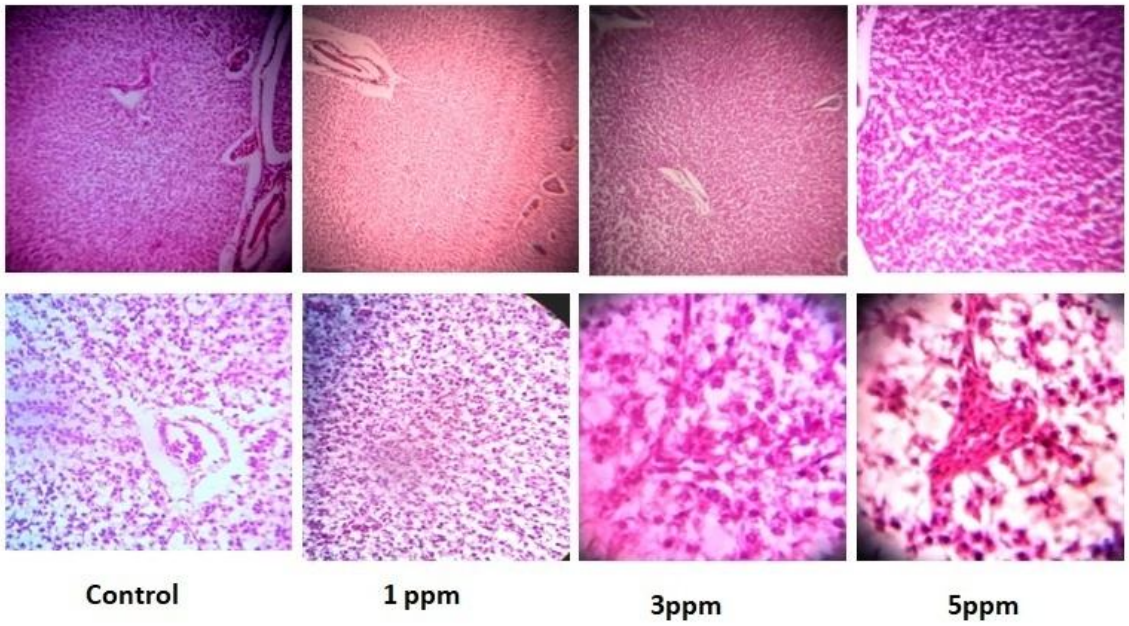

Figure 7. Histological studies of effect of different doses of NaF on liver tissue of *Channapunctatus*at 40X (upper panel) and 400X magnifications(lower panel).

At a dose of 5ppm moth eaten like appearance indicating loss of cells were featured at 40X magnification and at 400X eosinophilic tendency of cells with loss of vacuolar morphology was viewed. Pyknotic nucleus and hepatic duct and sinusoidal congestion were prominent. Some enlarged cells with loss of nucleus were monitored. Cellular infiltration was prominent. Fibrotic tendency of liver of such fishes also might not be overruled.

CONCLUSION

It may be inferred the water used for the study having low hardness is effective in bringing toxicity of fluoride at a relative lower concentration leading to shuttering of the entire antioxidant enzymatic defence and free radical mediated hepatic tissue damage as reflected in the pathological alterations in the hepatic architecture signifying damage of hepatocytes. A future study is required to assess whether apoptosis and inflammatory conditions are involved in this free radical induced hepatic tissue damage.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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