Syncytia
OPEN ACCESS

Scaffold

Research

Hepatotoxic alterations due to sodium fluoride-induced toxicity in *Clarius batrachus*

Saya Tyagi[1], Sweety Kumari[1], Nandini Rastogi[1], Varsha Gupta[1], & Rashmi Tripathi [1,*]

Abstract

Although fluoride can be found in nature in a variety of forms and has been widely used in different products. The amount of fluoride higher than the permissible limit is toxic to liver. Fluoride in drinking water in excess (>1.5 mg/l) is unhealthy. Given that fluoride toxicity has been classified as an endemic factor for liver diseases in several nations. It is crucial to pay close attention to the precise toxic effects of fluoride on different aquatic animals. Fluoride is toxic to all the system and causes oxidative stress in various tissues and has been demonstrated to have a number of negative consequences on human and aquatic health. The study was conducted to observe the hepatotoxicity in *C. batrachus* due to fluoride exposure. Changes in behavior could be read as exact indicators of a stressful environment. Fish are an efficient bioindicator of a hazardous environment because they can absorb fluoride from food and water. The toxicity of sodium fluoride (NaF) in fish was evaluated after acute exposure for 10 days. To evaluate the hepatotoxicity caused due to fluoride exposure three groups were set up, control, high dose (87.48 mg/l), and low dose (34.975 mg/l). The behavioral and biochemical parameters in the liver were evaluated. Results show that a high dose of fluoride is more toxic to the liver in contrast to the control group. Characteristic behavioral changes such as hyperactivity, frequent surfacing activity, a vertical swimming pattern, increased rate of opercular movement and heart rate were observed in *C. batrachus*. The liver biomarkers ALT, AST and LPO increased in the low and high dose as compared to the control. The increased liver and oxidative stress biomarkers were a clear indication to liver damage.

Received: 13 January 2023
Accepted: 15 March 2023
Published: 30 June 2023

[1]Department of Biosciences and Biotechnology, Banasthali Vidyapith, Banasthali-304022, Rajasthan, India.

*Correspondence:
tripathi.rashmi@gail.com

Keywords
Acute toxicity;
Behavioral changes;
Biochemical parameters;
Clarius batrachus;
Liver;
Sodium fluoride

Citation
Tyagi, S., Kumari, S., Rastogi, N., Gupta, V., & Tripathi, R. (2023). Hepatotoxic alterations due to sodium fluoride induced toxicity in *Clarius batrachus*. Syncytia, 1(1), 3-15. DOI: 10.52679/syncytia.2023.9cbz7t

Introduction

The primary component of life is water, but water pollution is a global problem that is making water unsafe to drink. The Industrial and domestic waste, as well as some naturally occurring elements, can cause water to become contaminated. Natural sources that can contaminate includes fluoride in groundwater and industrial effluents (Banerjee et al., 1967; Ali et al.,2019; Mondal et al., 2017). The required limit of fluoride in water is 1.0-1.5 ppm is recommended by the World Health Organization. If fluoride content in the body exceeds this limit, it causes fluorosis (Singh et al., 2020). If the level of fluoride is increased more than required in drinking water in our body, some deleterious effectscan take place, and fluorosis of teeth and skeleton is very common (Ali et al., 2019;Kaur et al., 2017; Mukherjee & Singh, 2019). The primary source of fluoride intake in Indians is groundwater, which is a "two-edged sword" because fluoride deficiencyleads to dental caries, and excessive fluoride intake results in fluorosis. Fluoride is both harmful and beneficial. The liver is the primary organ responsible for metabolism and it also aids in detecting compounds that are toxic from entering the body through the environment. Fluoride damages DNA and causes apoptosis in addition to being toxic to the liver (Thivya et al., 2015; Waugh et al., 2016; Campos-Pereira et al., 2017). Fluoride causes damage to the cell and its organelles and excessive intake causes serious health problems.

Figure 1. *Clarius batrachus*

Experimental setup

For the acute study, well-aerated plastic tanks were set up and divided into 3 groups, each having 6 fish. The water in all the groups was treated with NaF and changed every second day. The acute experiment for 10 days was set up. The following treatment was given to different groups:

Experimental groups	Acute (10 days)
Set I	Control
Set II	Low dose (34.975 mg/l)
Set III	High dose (87.43 mg/l)

Physicochemical properties of water

Physico-chemical properties of an aquatic ecosystem play a noteworthy role in productivity and in the growth of aquatic organisms. The physicochemical properties of water were ascertained by the process of Clesceri et al., (1996). The following parameters pH, temperature, dissolved oxygen, total hardness, free CO_2 and alkalinity were studied.

pH

In the present investigations, the pH values of the water samples were determined with the aid of a digital pH meter. The pH meter was calibrated with typical buffer solutions of pH 4.0, 7.0, and 9.2 before taking the readings. The samples of water were taken in a washed and rinsed beaker to measure pH value. Before taking a reading, the electrode was carefully cleaned, rinsed with distilled water, and wiped with tissue paper. The glass electrode was dipped into the water sample and the sample was stirred and the reading when stabilized was recorded after 1 min. The pH value of all water samples was analyzed by direct pH meter.

Temperature

The thermometer was used to measure the temperature of the sample water.

Dissolved oxygen

Dissolved oxygen in the experiment was measured according to Wrinkler's method. The sample was filled in the DO bottle avoiding the bubbling. Take 1mL of manganous sulfate ($MnSO_4$) solution and alkaline potassium iodine (KI) solution added at the bottom of the bottle, if a white precipitate is formed oxygen is low and high, if a brown precipitate is formed. Then Con H_2SO_4 (2 ml) was mixed to the precipitate to acidify and inverted multiple times to mix. A portion of the sample (50-100 mL) was taken in a conical flask. It was then titrated against sodium thiosulfate at 0.025 N until the solution turned light yellow. The starch indicator (1mL) was then added, solution turned blue. The solution was titrated until it becomes colorless. The blue color's disappearance marked the end point. The iodine release was equivalent to the amount of DO in the sample.

$$Dissolved\ oxygen\ (in\ mg/l) = \frac{(8\ x\ 1000\ x\ N)\ \mathrm{x\ V2}}{V1}$$

Where N = normality of the titrant (0.025 N); V1 = volume of sample (mL); V2 = volume of titrant used (mL).

Hardness of water

The 50 mL of sample was taken in a flask, 1 mL ammonium buffer sample was added, 1 mL of sodium disulfide was added and 2-3 drops Erichrome black T indicator, the solution color transformed to wine red, titrate mixture versus EDTA solution. At the endpoint the color changes from wine red to blue.

$$\textbf{Hardness (mg/l)} = \frac{\boldsymbol{mL\ of\ EDTA\ used}}{\boldsymbol{mL\ of\ sample}} \times \mathbf{1000}$$

Carbon dioxide

Sample (50 mL) was introduced to a conical flask. To this phenolphthalein indicator (2-3 drops) were added. In the absence of any free CO_2, the water turns pink. If this does not appear, standard alkali solution (NaOH or Na_2CO_3-0.05N) is used as a titrant and volumetric titration is performed. The end point is indicated by the visualization of pink color that lasts for at least 30 s. The readings were noted, and the formula below was used to calculate the free CO_2:

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{mL\ of\ titrant\ used \times N\ of\ titrant \times 1000 \times 44}{mL\ of\ sample}$$

Alkalinity

Alkalinity was checked by taking a 50 mL water sample in a conical flask. After the addition of 2-3 drops of phenolphthalein indicator, the sample was titrated against the (0.02N) hydrochloric acid. Note the first endpoint at pH 8.3 (bicarbonate alkalinity) then for the second point for carbonate alkalinity add 2-3 drops of methyl orange indicator.

$$Total\ Alkalinity\ (\text{in mg/ml}) = \frac{t\ (total\ vol.\ of\ 0.02N\ HCl)\ x\ 1000}{Vol.\ of\ sample\ (in\ ml)}$$

Behavioral changes

The observations were made regarding the behavioral changes of fish concerning unanimous body conditions and activities like body positions, swimming pattern, responsiveness, food sensitivity, schooling behaviour, and surfacing movements. These parameters were keenly observed in experimental animals and compared with control ones.

Heartbeat rate and oxygen saturation

The heartbeat rate and oxygen saturation were recorded using Pulse Oximeter CMS50D. The area is cleaned making sure it fits allowing the instrument to find the pulse and compute the saturation level of oxygen. The displayed pulse index shows that the machine has detected a pulse. In the absence of a pulse signal, readings of any type are not to be considered. Once detected, the saturation level of oxygen and rate of pulse will be displayed.

Effect of sodium fluoride toxicity on the liver function biomarker in the serum (ALT and AST)

Glutamate oxaloacetate aminotransferase (SGOT) brings about the reversible interconversions between glutamate and aspartate and their 2-oxo analogs. Glutamate pyruvate aminotransferase (SGPT) brings about the reversible interconversions between glutamate and alanine and their 2-oxo analogs. This method was adopted with few modifications. Substrates are incubated at 37 °C for 60 min (SGOT) and 30 min (SGPT) in a medium with a buffer of pH 7.4. The ketoacids reaction takes place with 2,4-dinitro phenyl hydrazine to produce brown-colored hydrazones, which absorb a wavelength of 510 nm (Reitman & Frankel, 1957). For AST and ALT, substrate (0.5 mL) was incubated at 37 ℃ for 5 min, followed by the addition of serum and then again it was incubated for 30 and 60 min, respectively. In control tubes, 0.5 mL of substrate was taken followed by the addition of 0.1 mL of serum to it. Blank was prepared by combining 0.5 mL of substrate with 0.1 mL of water. Reaction with DNPH took place at room temperature for 20 mins in all the tubes. 5 mL of 0.4N NaOH was added to it and mixed well. The contents were then incubated for 10 min at room temperature and OD was taken at wavelength510 nm.

$$AST = \frac{T - C}{S - B} X \frac{0.4\ X\ 1\ X\ 1000}{60\ X\ 0.1} = \frac{T - C}{S - B} X\ 67$$

$$ALT = \frac{T - C}{S - B}\ X\ \frac{0.4\ X\ 1\ X100}{30\ X\ 0.1} = \frac{T - C}{S - B} X\ 133$$

Effect of sodium fluoride on lipid peroxidation (LPO)

Immediately after the necropsy, tissues were excised, washed with normal saline, blotted, and stored frozen. For different enzymatic assays, the tissues were blended with Remi Motor homogenizer (RQ-122) with the help of a glass tube and Teflon pestle, in distinct media following the protocol of parameters. Total thiobarbituric acid s substances (TBARS) (degradation products of peroxidized lipids) were measured with thiobarbituric acid (TBA) reaction to produce TBARS chromophores that were considered as the index of LPO. 1mL of homogenate was prepared in KCl solution and was incubated at 37℃ for 30min. Protein precipitation took place after the addition of 1 mL of 10% TCA and then it was centrifuged at 2,000 rpm for 15 min. Then1 mL of TBA solution was added to 1 mL of supernatant, which was taken in different tubes. The tubes were maintained in a boiling water bath for 10 min. After the temperature came down, the O.D. was taken at wavelength 535 nm (Okhawa et al., 1979).

$$LPO = \frac{O.D.\ X\ 1000}{156\ X\ amount\ of\ protein\ (in\ mg)}$$

Estimation of protein

Proteins react with Folin's – Ciocalteu reagent to produce a colored complex, the color so developed is because of the reaction of alkaline copper with the protein. Proteins react with alkaline reagents forming a complex compound with Cu^{++} ion. This compound reacts with Folin's reagent as a result of which aromatic amino acids reduce the phosphomolybdic acid and oxidize them. The blue color appears whose intensity is directly proportional to the concentration of proteins. The content of protein was decided by using bovine serum albumin (BSA) as per the standard method of Lowry et al. (1951). The Lowry protein assay is a biochemical test to measure the amount of protein in a sample. The isolation of tissues was followed by the preparation of 2% homogenate in 0.33 M of cold sucrose solution. The centrifugation of homogenates took place for 15 min at 1000 x g. For the precipitation of protein equal volume of 10% TCA was added to the supernatant. It was then kept at room temperature for 30 mins and then again it was centrifuged at 1000 x g for 15 mins. In 1 mL of 0.1 NaOH, sediment was dissolved. After diluting, the appropriate volume of the solution was mixed with 5 mL of alkaline copper sulfate reagent. By shaking vigorously, it was mixed. After 10 min, 0.5 mL Folin–Ciocalteu phenol reagent was added to it and mixed well. The tubes were again kept for 30 min. With theuse of a spectrophotometer, absorbance was recorded at 625 nm. No sample was added in the blank. For the standard bovine serum albumin was used. The presentation of value is in mg/g wet wt. of tissue and the concentrations were represented in percentages.

$$Protein = \frac{O.D.\ of\ Unknown}{O.D.\ of\ Known} X100$$

Histopathological changes

Fixation:

The liver tissue was collected from all the experimental groups and fixed for 24h in alcoholic bouin's fluid. The tissues were then washed in 70% alcohol to remove the fixative completely. After the fixative was removed the tissue was proceeded for dehydration.

Dehydration: The tissues were dehydrated using progressively higher grades of ethyl alcohol (30%, 50%, 70%, 90%, and 100%). The tissues were preserved with the successive alcohol grades during the stepwise dehydration.

Clearing and Embedding: The xylene was used as the clearing agent and the tissue was embedded in paraffin wax (58℃ to 60℃). During the embedding process, the tissue is submerged in melted wax. The mould was put in a petri dish that had water in it. After the wax, the impregnated tissue was placed to the mould in the proper plane of section, air bubbles were removed with the help of warm needle. The block was left to solidify and was trimmed to the correct shape for section cutting.

Mounting and flattening the ribbon: The block was cut into sections with the rotatory microtome (8 µm) and sections were serially arranged and flattened on the slide using Mayer's albumen (egg white and glycerol in 1:1 ratio and sodium salicylate 1ml was added to it and mixed) as a glue.

Staining Process: The slides were deparaffinize in xylene for 5 to 10 min. The slides were then subjected to lower concentrations of ethyl alcohol (100%, 90%, 80%, 70%, 50%, and 30%) and stained with haematoxylin for 2 to5 min, dipped in acid water to remove any excess stain, and finally submerged in water for 5 to 10 min. The slides were then dehydrated by being exposed to an alcohol series in ascending sequence that included up to 70% alcohol. The slides were then cleaned in 70% alcohol after being stained with eosin for 2 brief dips. Ascending concentrations of 90% ethyl alcohol and 100% alcohol were used to induce dehydration for 5 to 10min and cleared in xylene (5 to 10 min). The DPX was put on the sections and covered with the cover slip and left to dry and observed. The light microscope was used to observe the haematoxylin-eosin-stained slides. The selected fields were focussed and microphotographed with the microscope camera unit.

Statistical study

A statistical study was performed on numerical data to estimate the significance of the differences in group means. The mean levels of various parameters of the several experimental groups were assessed using ANOVA, and F values were computed at $p<0.001$. The figures that have been tabulated are displayed as Mean ± Standard Deviation (SD). The p-value that was determined has been included in the tables. For a given comparative mean, a calculated p value of less than 0.001 was deemed sufficient to indicate a significant difference.

Results & Discussion

Physicochemical analysis of water

In aquatic habitats, the uptake and intake of fluoride are affected by physio-chemical parameters like temperature, pH, total hardness, dissolved oxygen, and alkalinity. The physio-chemical parameters of the water for the experiment were analyzed and are given in Table 1 and were within the optimum range. High pH levels harm fish by denaturing cellular membranes and variation in pH alters the chemical constituents of water leading to stress. The pH (8 + 0.1) value is found to be suitable for the existence of aquatic animals as higher pH leads to the loss of nutrients. Water temperature is an important characteristic of an aquatic system, affecting different parameters such as dissolved oxygen levels. As there is an increment in water temperature, the oxygen solubility decreases. However warm water does not contain much excess oxygen, which fish need. When it gets too warm and oxygen levels drop, fish become sluggish and inactive. The temperature in the experimental water was recorded to be (23±1°C) within the standard range. A higher dissolved oxygen level indicates better water quality. The water used for experimentation has a DO concentration of 16.93 ± 2.57mg/l and is good for supporting life as it is the primary requirement for respiration (Banerjee et al., 1967; Kumawat et al., 2022). The freshwater has a hardness in the range of 15-375 ppm. The hardness measured was found to be 96.66 ± 5.77 mg/l, within the optimum range. The optimum range leads to better development and growth of fishand lower value can lead to stress in fishes was reported by Kumawat et al. (2022).The free carbon dioxide in the water was negligible, as the carbon dioxide increases the oxygen level falls and stressful conditions arise for survival. The alkalinity indicates the buffering potential of the water that is its ability to resist acidic changes.The alkalinity was 219.31±0.65mg/l, which will resist changes in pH and thus maintain the quality of water (Kumawat et al., 2022).

Table 1. Physicochemical properties of water

Parameter	Result
pH	8 + 0.1
Temperature	23 ± 1 °C
Dissolved oxygen	16.93 + 2.57 mg/l
Total hardness	96.66 + 5.77 mg/l
Free CO_2	-
Alkalinity	219.31±0.65mg/l

Behavioural changes in *C. batrachus*

The various behavioral aspects were taken into consideration (Table 2) after exposure to the high and low doses of sodium fluoride. Fishes are sensitive to their surroundings their behaviour changes on exposure to the toxicant.

Table 2. Behavioral changes in *C. batrachus* after exposure to various concentrations of sodium fluoride. Statistical analysis was performed using one-way ANOVA ($p<0.001$). Means with the same superscript along the same row are highly significant at ($p<0.001$).

Parameters	Control	Low dose	High dose
Body position	Slant	Swimming	Swimming
Habit	Calm	Active	Mostly swimming
Swimming habit	No movement	Erratic movement	Erratic movement
Food sensitivity	Normal	Increases	High
Schooling behavior	Present	Declines	Less prominent
Rate of opercular movement (beats/min)	$38\pm2.10^{\dagger\dagger\dagger}$	$52\pm2.87^{\dagger\dagger\dagger}$	$54\pm2.98^{\dagger\dagger\dagger}$
Swimming activity (per 30 min)	$3\pm0.16^{\dagger\dagger\dagger}$	$37\pm1.82^{\dagger\dagger\dagger}$	$33\pm2.04^{\dagger\dagger\dagger}$
Heartbeat rate	$45\pm2.48^{\dagger\dagger\dagger}$	$76\pm4.20^{\dagger\dagger\dagger}$	$89\pm4.92^{\dagger\dagger\dagger}$

The exposed fish were found to be more active as compared to the control which mainly remained confined to the bottom of the plastic tub (Figure 2). The exposed fish were found in the vertical position in the water column and swimming movement became erratic, aggressiveness highly increased as compared to a horizontal position, and the quiet nature of control fish mainly inactive at the bottom (Figure 3). The rate of opercular movement increased as the fluoride concentration increases. The increase in swimming behavior affects the fish appetite, food intake was very rapid in the group exposed to sodium fluoride toxicity as compared to the control group as reported by (Narwaria & Saksena, 2012; Tripathi & Pandey, 2014; Sabullah et al., 2015).

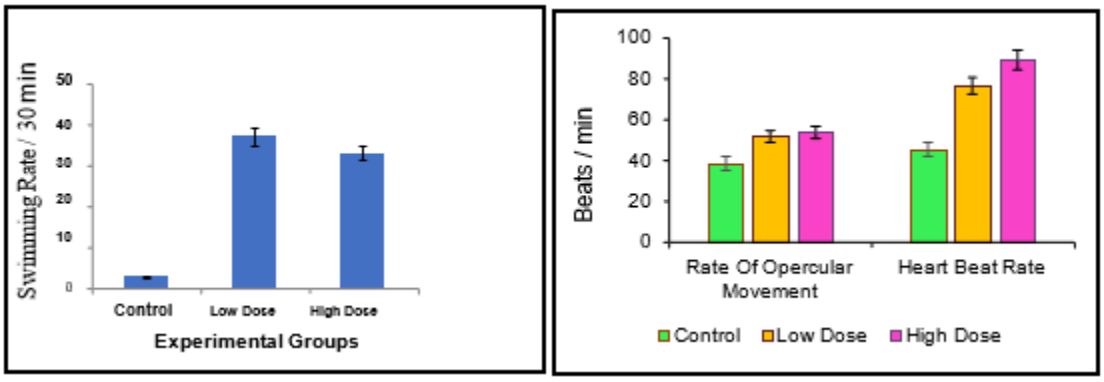

Figure 2. Changes in the swimming movements, rate of opercular movement, and heart beat rate in *C. batrachus* exposed to high and low doses of sodium fluoride.

Figure 3. Represents the behavioral activities in *C. batrachus* exposed to different doses of sodium fluoride; A & B) Calm nature confined to the bottom of tub and schooling habit; C) Vertical position in the water column of the exposed fish; D) Schooling habit declining in fish exposed to the toxicant.

Table 3. Toxic effect of different doses of fluoride on ALT and AST after acute exposure (10 days).

Groups	ALT	AST
Control	24.5±1.35	75.5 ± 4.17
NaF Low dose	245 ± 13.54	265 ± 14.64
NaF High Dose	298 ± 16.5	307 ± 16.97
ANOVA at 1%	**F Value: 121.1**[@]	**F value: 390.7** [@]

Values are exhibited as Mean ± SEM (n=6) in each group. A statistical study was performed using one-way ANOVA (P <0.001).

Effect of sodium fluoride on liver function biomarkers in the serum (ALT and AST)

The harmful inclusion of fluoride in the blood increases the levels of AST and ALT (Table 3). The increase in the concentration of aminotransferases (AST and ALT) in the serum was a clear indication of cellular leakage and loss of functional integrity of hepatocyte cell membrane(Anstee et al., 2022; Harrington et al., 2022; Xu et al., 2022).

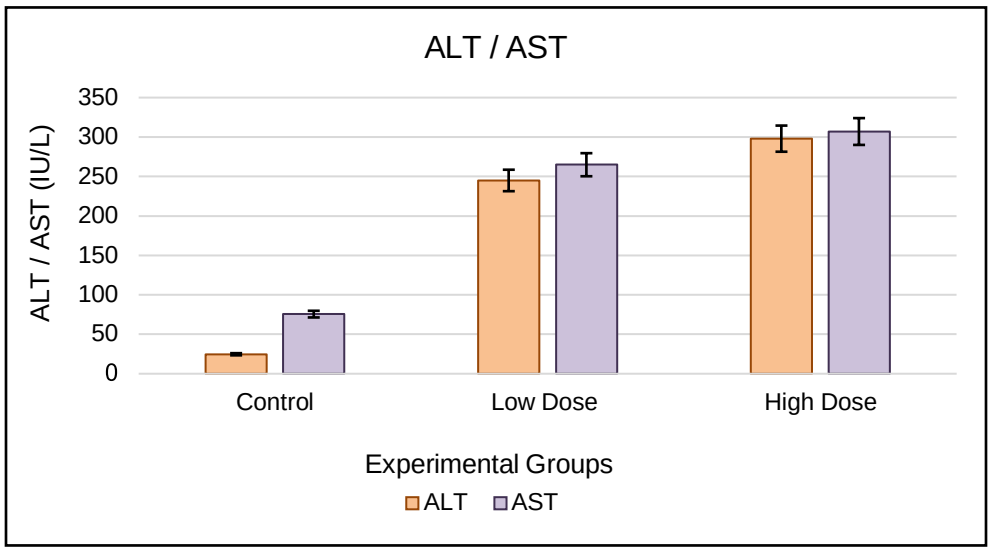

Figure 4. Effects of fluoride concentrations on ALT/AST, after acute exposure (10 days). Data are evaluated as Mean ±Standard error (SE; n=6).

Alanine transaminase (ALT) and aspartate transaminase (AST) is an enzyme present mainly in liver and kidney (Figure 4). Under normal conditions small amount of ALT and AST is present in the serum and is performed to estimate liver damage. After fluoride exposure, ALT and AST level increases in the blood. Fluoride acts as a hepatotoxicant. ALT and AST are helpful in the determination of injury caused by toxicants in different tissues like the liver, muscle, and gills. On fluoride exposure, ALT and AST are progressively increased in high doses as compared to control and low doses. Fluoride causes necrosis in the liver of animals. It is believed that the increment of this enzyme outside the cell fluid and plasma reveals tissue injury. This process is also responsible for altered protein metabolism. Because of this process depletion of protein in different tissues of fluoride-exposed fish takes place which is due to increased hydrolysis of proteins to sustain during metabolic pressure (Madhavan & Elumalai, 2016).

Table 4. Toxic effect of different doses of fluoride on LPO after acute exposure (10 days).

Groups	LPO
Control	1.4 ± 0.07
NaF Low dose	2.14 ± 0.12
NaF High Dose	2.52 ± 0.14
ANOVA at 1%	**F Value: 274.9[@]**

Values are exhibited as Mean ± SEM (n=6) in each group. Statistical study was performed using one-way ANOVA (P <0.001).

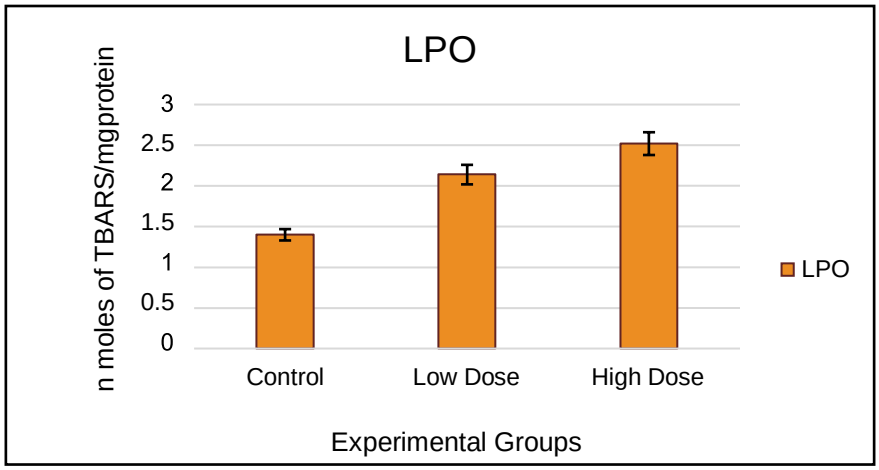

Figure 5. Effect of fluoride on the lipid peroxidation in liver after 10 days of fluoride treatment. Value mean ± standard error mean, n = 6, p***<0.001.

Due to fluoride exposure lipid peroxidation increases considerably in the high-dose group as compared to low dose and control (Figure 5). Malondialdehyde is the end product of lipid peroxidation. It is observed malondialdehyde (MDA) is increased, which is an indication of increased LPO. Fluoride increases lipid peroxidation in cells and creates disturbance in the organization of the cell membranes leading to inhibition of the membrane-bound enzymes as previously reported by (Madhavan & Elumalai, 2016; Huma & Rana, 2022; Shanmugam *et al.,* 2022).

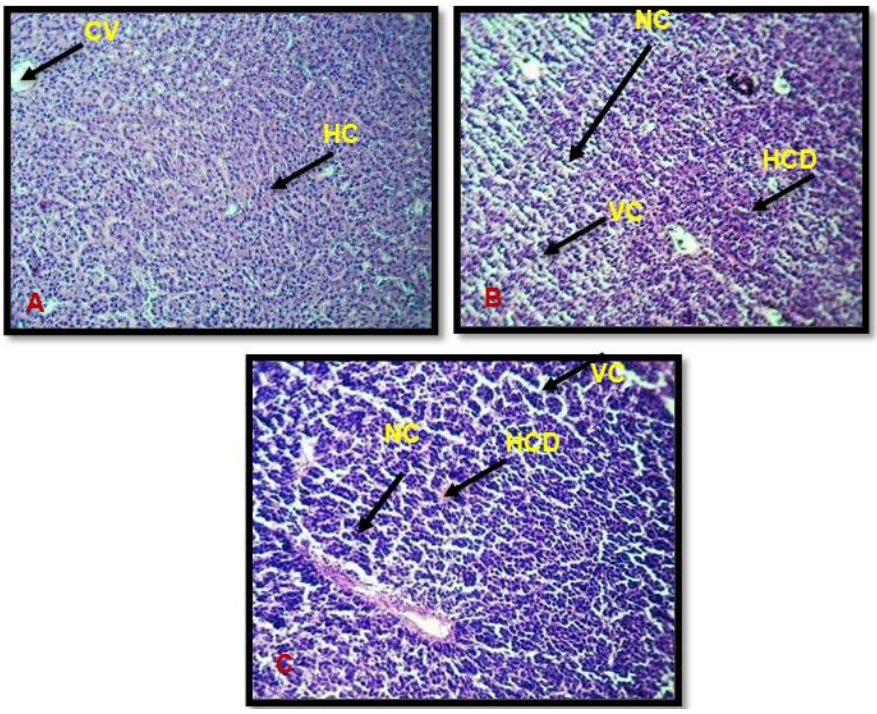

Figure 6. Photomicrographs of T.S of liver of *C. batrachus* showing effects of chronic exposure to sodium fluoride; A) Control group, B) Low dose, C) High dose. Abbreviations: HC: Hepatocytes; CV: Central Vein; NC: Necrosis; HCD: Hepatocellular degeneration; VC: Vacuolization

Histopathological alterations

The liver tissue in the control group is made of veins that are localized in the center that is encircled by strands of hepatocytes revealing normal size distinct central nuclei and eosinophilic cytoplasm (Figure 6A). The fluoride-treated group shows an enlarged vein localized at the center with a huge amount of red blood cells and intense alteration of hepatocytes thus vein at the center gets congested, liver cell degradation, interruption, and localized death of cells increases misplacement of cellular grid, formation of vacuoles and shrinkage and condensing of nuclei (Figure 6B & 6C). Thus, leading to nuclear atrophy, disruption, and ruptured hepatocytes (Kaur et al., 2021; Zhao et al., 2022).

Conclusion

Behavioral changes are based on different parameters and are the first signs of stress on exposure to the toxicants. Fluoride exposure causes liver injury as revealed by the increment of AST and ALT.The increased level of these enzymes also alters protein metabolism and leads to minor cellular damage to the tissue. Oxidative stress in fish is caused by fluoride which results in lipid peroxidation. Imbalance in membrane lipids and synthesis of lipid peroxides, results in lipid peroxidation. The malondialdehyde is produced by disintegration of polyunsaturated fatty acids and it is appropriate in determining the range of lipid peroxidation. It can be considered one of the effective biologically active markers for the presence of membrane LPO. In this study, the liver of the fish treated to different concentrationsof fluoride showed increased MDA levels, which was time and dose-dependent. In the study, the liver of fish revealed the highest MDA content in the high dose as compared with that of the control and low dose.

Declarations

Acknowledgments: The authors would like to take this opportunity to express our gratitude and thanks to the Hon'ble V.C. Prof. InaAditya Shastri, Banasthali Vidyapith, Banasthali, Rajasthan for providing us the research facilities, encouragement, and support. Weacknowledge the Bioinformatics Center, Banasthali Vidyapith, supported by the DBT, DST, FIST, and CURIE program at the Department of Biosciences and Biotechnology for providing the computation and networking support.

Author Contribution: Saya Tyagi performed and contributed to writing the manuscript. Sweety Kumari, Nandani Rastogi, and Varsha Gupta performed the work. Rashmi Tripathi designed analyzed the data, and contributed to the writing and approval of the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received no external funding.

References

Ali, S. K., Fakhri, Y., Golbini, M., Thakur, S. K., Alinejad, A., Parseh, I., Shekhar, S. & Bhattacharya, P. (2019). Concentration of fluoride in groundwater of India: A systematic review, meta-analysis and risk assessment. Groundwater for Sustainable Development, 9, 100224. [DOI]

Anstee, Q. M., Castera, L., & Loomba, R. (2022). Impact of non-invasive biomarkers on hepatology practice: past, present and future. Journal of Hepatology, 76(6), 1362-1378. [DOI] [PMID]

Banerjee, S. K., Kundu, K. K., & Das, M. N. (1967). Autoprotolysis constants of ethylene glycol–water mixtures at different temperatures and related thermodynamic quantities. Journal of the Chemical Society A: Inorganic, Physical, Theoretical, 166-169. [DOI]

Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. Chemico-Biological Interactions, 188(2), 319-333. [DOI] [PMID]

Basha, S. K., & Rao, K. J. (2014). Sodium fluoride induced histopathological changes in liver and kidney of albino mice. Acta Chimica and Pharmaceutica Indica, 4(1), 58-62. [DOI] [PMID]

Brindha, K., Jagadeshan, G., Kalpana, L., & Elango, L. (2016). Fluoride in weathered rock aquifers of southern India: managed aquifer recharge for mitigation. Environment Science and Pollution Research International, 23(9), 8302-8316. [DOI] [PMID]

Campos-Pereira, F. D., Lopes-Aguiar, L., Renosto, F. L., Nogueira, G. A. S., Costa, E. F. D., Pulz, R. B., Silva-Zacarin, E. C. M., Oliveira, C. A., Pigoso, A. A., & Severi-Aguiar, G. D. C. (2017). Genotoxic effect and rat hepatocyte death occurred after oxidative stress induction and antioxidant gene downregulation caused by long term fluoride exposure. Chemico-Biological Interactions, 264, 25-33. [DOI] [PMID]

Chattopadhyay, A., Podder, S., Agarwal, S., & Bhattacharya, S. (2011). Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. Arch Toxicol, 85, 327-335. [DOI] [PMID]

Clesceri, L. S., Eaton, A. D., Greenberg, A. E., & Franson, M. A. H. (1996). Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, DC.

Ghosh, S., Malloum, A., Igwegbe, C. A., Ighalo, J. O., Ahmadi, S., Dehghani, M. H., Othmani, A., Gökkuş, Ö., & Mubarak, N. M. (2022). New generation adsorbents for the removal of fluoride from water and wastewater: A review. Journal of Molecular Liquids, 346, 118257. [DOI]

Harrington, C., Krishnan, S., Mack, C. L., Cravedi, P., Assis, D. N., & Levitsky, J. (2022). Noninvasive biomarkers for the diagnosis and management of autoimmune hepatitis. Hepatology, 76(6), 1862-1879. [DOI] [PMID]

Huma, Y. V., & Rana, S. V. S. (2022). Duration of exposure modulates combined effects of arsenic and fluoride on oxidative stress in the liver of rat. Journal of Environmental Biology, 43(4), 514-519. [DOI]

Kaur, R., Saxena, A., & Batra, M. (2017). A review study on fluoride toxicity in water and fishes: current status, toxicology and remedial measures. International Journal of Environment, Agriculture and Biotechnology, 2(1), 456-466. [DOI]

Kaur, R., Batra, M., & Saxena, A. (2021). Histopathological investigations on the effect of vitamin C on sodium fluoride exposed fresh water amur carp, Cyprinus carpio haematopterus. Fluoride, 54(3), 241-256.

Kumar, S., Lata, S., Yadav, J., & Yadav, J. P. (2017). Relationship between water, urine and serum fluoride and fluorosis in school children of Jhajjar District, Haryana, India. Applied Water Science, 7, 3377-3384. [DOI]

Kumawat, M., Sharma, P., Pal, N., James, M. M., Verma, V., Tiwari, R. R., Shubham, S., Sarma, D. K., & Kumar, M. (2022). Occurrence and seasonal disparity of emerging endocrine disrupting chemicals in a drinking water supply system and associated health risk. Scientific Reports, 12(1), 9252. [DOI]

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193: 265-275. [PMID]

Machiwal, D., & Jha, M. K. (2015). Identifying sources of groundwater contamination in a hard-rock aquifer system using multivariate statistical analyses and GIS-based geostatistical modelling techniques. Journal of Hydrology: Regional Studies, 4, 80-110. [DOI]

Madhavan, P., & Elumalai, K. (2016). Effects of chromium (VI) on the lipid peroxidation and antioxidant parameters in the gill and kidney tissues of catfish, Clarias batrachus (Linnaeus,1758) (Actinopterygii: Siluriformes). International Journal of Advanced Research in Biological Sciences, 3(4), 249-255.

Mondal, D., Gupta, S., Reddy, D. V., & Dutta, G. (2017). Fluoride enrichment in an alluvial aquifer with its subsequent effect on human health in Birbhum district, West Bengal, India. Chemosphere, 168, 817-824. [DOI] [PMID]

Mukherjee, I., & Singh, U. K. (2018). Groundwater fluoride contamination, probable release, and containment mechanisms: a review on Indian context. Environmental Geochemistry and Health, 40(6), 2259-2301. [DOI] [PMID]

Mukherjee, I., Singh, U. K., & Patra, P. K. (2019). Exploring a multi-exposure-pathway approach to assess human health risk associated with groundwater fluoride exposure in the semi-arid region of east India. Chemosphere, 233, 164-173. [DOI] [PMID]

Narwaria, Y. S., & Saksena, D. N. (2012). Acute toxicity bioassay and behavioural responses induced by sodium fluoride in freshwater fish Puntius sophore (Bloch). Fluoride, 45(1), 7-12.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351-358. [DOI] [PMID]

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28, 56-63. [DOI] [PMID]

Sabullah, M. K., Ahmad, S. A., Shukor, M. Y., Gansau, A. J., Syed, M. A., Sulaiman, M. R., & Shamaan, N. A. (2015). Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. International Food Research Journal, 22(2), 435-454.

Shanmugam, K. R., Shanmugam, B., Siva, M., Ravi, S., & Reddy, K. S. (2022). Effect of Ocimum sanctum in sodium fluoride (NaF) induced fluorosis in rats: A study with respect to antioxidant enzymes and fluorosis markers. Indian Journal of Pharmaceutical Education and Research, 56(1), 175-183. [DOI]

Singh, S., Khare, A., & Chaudhari, S. (2020). Enhanced fluoride removal from drinking water using un-calcined synthetic hydroxyapatite. J Environ Chem Eng, 8(2), 103704. [DOI]

Tripathi, R., & Pandey, A. K. (2014). Alterations in behavior, scaphognathite oscillations and heart beat rate in freshwater prawn, Macrobrachium dayanum (Crustacea: Decapoda) induced by cadmium chloride exposure. Journal of Experimental Zoology India, 17(1), 155-164.

Thivya, C., Chidambaram, S., Rao, M. S., Thilagavathi, R., Prasanna, M. V., & Manikandan, S. (2015). Assessment of fluoride contaminations in groundwater of hard rock aquifers in Madurai district, Tamil Nadu (India). Applied Water Science, 7, 1011-1023. [DOI]

Waugh, D. T., Potter, W., Limeback, H., & Godfrey, M. (2016). Risk assessment of fluoride intake from tea in the Republic of Ireland and its implications for public health and water fluoridation. International Journal of Environmental Research and Public Health, 13(3), 259. [DOI] [PMID]

Xu, Z., Weng, Z., Liang, J., Liu, Q., Zhang, X., Xu, J., Xu, C., & Gu, A. (2022). Association between urinary cadmium concentrations and liver function in adolescents. Environmental Science and Pollution Research International, 29(26), 39768-39776. [DOI] [PMID]

Yadav, S., Bansal, S.K., Yadav, S., & Kumar, S. (2019). Fluoride distribution in underground water of district Mahendergarh, Haryana, India. Applied Water Science, 9, 1-11. [DOI]

Zhao, Y., Wang, J., Zhang, J., Sun, Z., Niu, R., Manthari, R. K., Ommati, M. M., Wang, S., & Wang, J. (2022). Fluoride exposure induces mitochondrial damage and mitophagy via activation of the IL-17A pathway in hepatocytes. Science of the Total Environment, 804, 150184. [DOI] [PMID]

0