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Research Article

Hematological Response of *Clarias batrachus* to Sodium Fluoride Induced Toxicity

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Abstract

This study aimed to assess sodium fluoride's toxic effects on the behaviour and hematology of *Clarias batrachus*. Sodium fluoride is a major pollutant in aquatic environment and exposure can lead to bioaccumulation in fish. In most aquatic habitats, fish are at the top of the food chain and are most vulnerable to the toxic effects of high levels of sodium fluoride. Juvenile *C. batrachus* (Length: 13.6±0.78 cm; Weight: 20±2g) were exposed to sodium fluoride at different concentrations low dose (34.975 mg/L) and high dose (87.43mg/L) for ten days. The behavioural studies showed significant changes in the toxicant group and opercular activity, feeding, aggressiveness, surfacing, heart beat rate and oxygen saturation was found to increase as compared to the control group. The haematological constants such as Red Blood Cells (RBC's), White Blood Cells (WBC's), Haemoglobin percentage (Hb%), Packed Cell Volume (PCV), Clotting Time (CT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were observed. All the parameters except WBC count and CT were found to substantially decrease in experimental groups. The results of this study demonstrates that exposure to Sodium fluoride is toxic to *C. batrachus* and induces alterations in the behavioural and hematological parameters.

Keywords: *Clarias batrachus*; Hematology; Low and High Dose; Sodium Fluoride and Toxicity.

Introduction

Fluoride is an utmost element that is present in nature. Still, elemental fluorine does not occur naturally because it is a reactive non-metal and an electronegative element, so it always integrates, excluding oxygen and noble gases. The fluorine exists in the fluoride compounds which make up minerals of rock and soils. Fluorides make up 0.032% of the earth's crust and are the thirteenth top most element that is present in nature [1]. Naturally, fluoride is present in water and air, but other sources are food. It is present in low concentrations in meat, fruits, and vegetables but a higher concentration of fluoride is present in tea. Fluoride is present in variable concentrations in the water bodies depending on the geographical contribution [2,3]. Living beings depend on the ecosystem for their well-being, growth and development.

Any change in the aquatic ecosystem affects the life of aquatic organisms. As water is the main component on the earth if it meets the toxic changes, it is going to affect not only the aquatic animals but also human beings. The freshwater contaminated by the toxicants becomes a significant concern, as it affects aquaculture, the primary source of protein. Fluoride exceeding the standard level leads to harmful effects and influences the community health and the aquatic environment. In India fish is the primary food source in West Bengal, Andhra Pradesh, Bihar, and many other states. Fishes are sensitive to climatic change, pH, temperature, dissolved oxygen, free carbon dioxide, alkalinity and exposure to toxicity. *Clarias batrachus* is freshwater fish known as Indian walking catfish and is known as magur mas in Assam. The fish exhibits an outstanding level of tolerance and hence good choice for culture.

It is one of the vital fish used as a food source, but is facing a threat due to the contaminant present in the water. The sodium fluoride is a hepatotoxicant and induces severe health problem in *C. batrachus* also altering the behavior of the fish thus an important parameter to study the toxic effect. The toxicant induces opercular movement, surface activity, heart beat rate, oxygen saturation level, mucus secretion, body position, food sensitivity and the changes in the coloration of the body. The immune system of the fish is affected greatly by fluoride toxicity [4,5].

The development in fish is impacted by fluoride, which can alter a fish's length, mass, and growth [6]. The higher fluoride concentration causes decline in the proteins and lipid content leading to slower growth in fish and the weight declines. Thus, the proteins and lipids act as bioindicators of fluoride toxicity [7]. Fluoride toxicity even affects the chromatophores in the fish that specifies the color of the skin and eyes. The exposure to NaF changes chromatophore dimensions, configurations, and dispersal [8]. Due to the high concentration of fluoride, genotoxicity, cytotoxicity and mutagenicity was observed it even hinders cell proliferation and causes cell death [9]. The toxic effect of sodium fluoride severely impacts the hematological parameter of *C. batrachus*. Therefore, it is precious in the assessment of the physiological condition of the fish and are essential indicators of changes in external environment of animals [10].

Hematological parameters indicate the variation in an organism effectively. Therefore, the central role of the hematological study is to identify any change due to environmental factors, toxicants, or anything that affect living beings. Fishes are poikilothermic aquatic animals that detect bacteria, parasitic activity, water toxicity, oxygen rate, and pH. Hematology is the leading standard for fish well-being. The study of toxicity on hematological parameters will help detect the quality of the ecosystem and thus will help in improving the health status of fish, leading to increased aquaculture production. In fish toxicology, hematological analysis is helpful in detecting toxicity. The blood indices are quick, sensitive biomarkers of a variety of environmental effects.

Materials and Methods

Collection of experimental animals

Healthy living specimens of freshwater catfish, *C. batrachus* (Length: 13.6 ± 0.78 cm; Weight: 20 ± 2 g), were brought to the laboratory. The samples of the fish *were* collected from Dey Fisheries, Singhati, Ram Sagar, Bankura, West Bengal. They were brought to Banasthali Vidyapith in well-aerated plastic containers.

Maintenance of animal

The fish were disinfected with 2% $KMNO_4$ to avoid any bacterial and fungal infection and acclimatized for 15days with proper aeration. During the acclimation period, fish were supplemented with fish food Tokyo (5% body weight). After acclimatization, healthy and active fish were selected for experimental purposes in different tubs, and water was changed every alternate day.

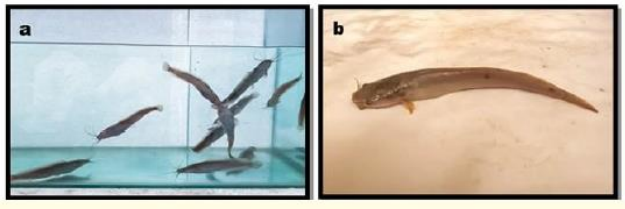

Figure 1: *Clarias batrachus.*

Experimental setup

For the acute study, well-aerated plastic tanks were divided into three groups, each having six fish. The water in all the groups was treated with NaF (LC_{50} 349.75 mg/L) and changed every second day. The experimental setup was divided into three groups control, low dose and high dose (Table 1).

Physicochemical properties of water

Physicochemical properties of an aquatic ecosystem play a significant role in its production and in the growth of organisms. Since water is a great solvent, it facilitates the movement of ions and other molecules needed for metabolism. The physicochemical properties of water such as temperature, pH, total hardness, dissolved oxygen, free carbon dioxide and alkalinity were determined by the method of APHA [11].

pH

A calibrated digital pH meter was utilized in the current study to measure the pH values of the water sample. The pH meter was standardized using standard buffer solutions at pH levels of 4.0, 7.0, and 9.2. The water sample was collected in an uncontaminated, sterile beaker. The electrode was thoroughly cleaned, wiped, and rinsed with distilled water and dipped in the water sample upto the mark. The reading was noted in triplicates.

Total Hardness

The total hardness can be determined by titrating with ethylene diamine tetra acetate (EDTA) solution. It is possible to selectively determine the calcium and magnesium EDTA complexes as the stability constants differ. The EDTA and the metal ions in hard water combine and form stable complexes. The complexometric titration method is used to assess the overall hardness, with the formation of a colored complex as end point. To get the pH between 9 &10 Ammonium acetate buffer (1 mL) is added to water sample (50 mL) taken in a conical flask. Then two drops of Eriochrome Black T indicator was added and stirred. The unstable EBT binds to the Ca^{2+} and Mg^{2+} in the sample, and forms a wine-red complex. A 0.01M EDTA solution is then used to titrate the solution. As the EDTA attaches to the divalent cations, the wine-red hue turns blue, marking the end point. The readings were taken in triplicates and the total hardness in mg/L was determined using the formula given below:

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

Dissolved oxygen

The dissolved oxygen was measured using Wrinkler's technique. The manganese hydroxide ($MnSO_4$) was added followed by the addition of 2mL alkaline-iodide azide at the bottom of the DO bottle using pipette. A white brown precipitate is formed. Then con H_2SO_4 (2 mL) was mixed to the precipitate to acidify and inverted multiple times to mix. Take 50mL of sample in a conical flask and titrate against 0.025N sodium thiosulphate until the solution tinged to pale yellow. Then 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. The readings were tabulated, and calculation was done using the following formula:

$$\textbf{D.O. (mg/L)} = \frac{(V2 \times N)\ titrant \times 8 \times 1000}{V1}$$

Where, Equivalent weight=8; N=Normality of the titrant (0.025 N), V1=Volume of water sample (mL), V2=Volume of titrant used (mL)

Free Carbon dioxide

The carbon dioxide (CO_2) that is dissolved in water when titrated against sodium bicarbonate forms carbonic acid. The formation of which is indicated by phenolphthalein indicator. The sample (50 mL) was taken in 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 the water does not turn pink, 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 30S. The readings were noted, and the formula given below was used to calculate the free CO_2

$$\textbf{Free } CO_2 \textbf{ (mg/L)} = \frac{V2x\ N\ of\ titrant x 1000 x 44}{V1}$$

Where V2= Volume of the titrant used; N = Normality of the titrant; V1 is the volume of water sample taken and 44 is the equivalent weight.

Alkalinity

Alkalinity is important for aquatic life because it protects or buffers against rapid pH changes. Alkalinity was determined by taking 50 mL of water sample in a conical flask. Then 2-3 drops of phenolphthalein indicator was added. If the color of the water does not change it means phenolphthalein alkalinity is nil. Moreover, if the pink colour develops titrate against the (0.1N) hydrochloric acid. The first endpoint was noted at pH 8.3 (bicarbonate alkalinity), when the sample becomes colourless. Then for the second point for carbonate alkalinity, 2-3 drops of methyl orange was added colour changes to orange it is then titrated against the 0.1 N hydrochloric acid. The colour changes to pink note the end point.

$$\textbf{Alkalinity (mg/L)} = \frac{\text{V2 of 0.1 N HCl}}{\text{V1}} \times 1000$$

Where V2 is the volume of titrant used and V1 is the volume water sample taken

Behavioural Study

Observations were made regarding the behavioural changes in fish with respect to unanimous body conditions. The activities like movement, swimming patterns, responsiveness, food grasping, change in body coloration and surfacing movements were observed in experimental animals compared with the control group. Behaviour offers a special viewpoint that connects an organism's ecology and physiology with its surroundings.

Heart Beat Rate and Oxygen Saturation

The heartbeat rate and oxygen saturation were measured using the Pulse Oximeter CMS50D. To use this, the tail end of the fish was wiped using a wet tissue and fit onto the equipment without any disturbance. The digital screen shows the pulse and the partial oxygen pressure (pO_2) after a few minutes.

Collection of blood

The blood was collected from the caudal peduncle of the fish with the help of 1mL sterile hypodermic syringe in sterilized vials coated with EDTA. The sample was either used fresh or stored immediately at 4°C until further use.

Analysis of blood

Estimation of the number of RBC (red blood cells)

The blood was taken into the RBC pipette upto 0.5 mark, and then the diluting fluid (Hayem's fluid) was drawn upto 101 mark (dilution is 1:200). The pipette was held horizontally and rotated between finger and thumb with both hands to allow the blood to mix with the diluting fluid. The clear fluid in the capillary section of the pipette was not mixed with blood, it was discarded. Neubauer's counting chamber was used to count the RBCs. The counting chamber was covered with a clean cover slip, and the mixture was quickly lowered to the edge of the coverslip. Capillary action causes diluted blood to flow freely across the counting chamber. After allowing the corpuscles to settle, they were counted using a compound microscope with a 40X objective, the ruled measuring area was focused underneath the microscope, and the numbers of RBCs were recorded in small squares and the number of RBC's/ mm^3 was computed accordingly using the given formula.

$$RBC/mm^3 = \frac{No\ of\ cells\ counted\ x\ dilution}{Area\ of\ 5\ small\ squares\ x\ depth\ of\ squares}$$

Estimation of the number of WBC in blood

WBCs were also counted using the Neubauer counting chamber. Similarly, as mentioned above, the blood was drawn in the WBC pipette up to the 0.5 mark. Immediately after, the diluting fluid (Turk's fluid) was drawn up to the mark11 (dilution is 1:20). By gently rotating the pipette the diluting fluid was mixed with the blood. Again, a drop of fluid was allowed to run beneath the cover slip while maintaining the pipette at an angle of 40 degrees. The ruled area was then focused beneath the microscope, the numbers of WBCs per square millimeter was counted and calculated as per the following formula.

$$WBC/mm^3 = \frac{No\ of\ cells\ counted\ x\ dilution}{Area\ of\ squares\ counted\ x\ depth}$$

Clotting time

Clotting time is the time required for a blood sample of an organism to coagulate under standard conditions. Drop blood on the glass slide and fill the capillary with blood. Break capillary after every 3-5 seconds till blood clots and note the clotting time carefully.

Hemoglobin %

The hemoglobin was estimated by the acid haematin method. N/10 Hydrochloric acid was taken up to 2 marks in the graduated tube. Blood was sucked into the hemoglobin pipette and then transferred into the graduated tube. It was allowed to remain in the dark for 10 to 20 mins. Distilled water was added drop by drop, until the blood color matched with the standard color. Then the results were read from the scale on the graduated tube and expressed in gram %.

Estimation of Packed cell volume (PCV)

The PCV was estimated through PCV tube. Blood samples were collected in a blood collecting tube coated with an anticoagulant. Blood was taken up to mark 100 in the PCV tube and then centrifuged for 30 min at 3000 rpm. The packed cell volume can be read directly as a percentage.

Estimation of Main corpuscle volume (MCV)

MCV represents the average volume of red blood cells. The MCV is calculated by dividing the Packed Cell Volume by the number of red cells counted and multiplying the result by ten. As a result, MCV is written as cubic micro.

$$\mathbf{MCV} = \frac{Haematocrit\ \%}{RBC in\ millions/mm^3} \times 10$$

Estimation of Mean corpuscular hemoglobin (MCH)

MCH denotes the mean mass of Hb found in each cell. The size of the cell and the concentration of Hb have an effect on MCH. Its values are obtained by dividing the Hb concentration by the number of red blood cells, multiplying the result by ten, and expressing the result in micrograms (µg).

$$\mathrm{MCH} = \frac{\text{Hemoglobin (g/100 ml)}}{\text{RBC in millions/mm}^3} \times 10$$

Estimation of Mean corpuscular hemoglobin concentration (MCHC)

The mean concentrations of Hb in red cells are referred to as MCHC. In contrast to MCH, MCHC is unaffected by cell size. To calculate MCHC, divide Hb by packed cell volume reported in gram percent (g %).

$$\mathrm{MCHC} = \frac{\text{Hemoglobin (g/100ml)}}{\text{Haematocrit}} \times 100$$

Statistical analysis

The observed values as Means ± SE were statistically analyzed with one-way ANOVA using the Graph Pad Prism.

Result and Discussion

Physiochemical analysis of water

In aquatic habitats, the intake of fluoride is affected by physio-chemical parameters like temperature, pH, total hardness, dissolved oxygen, and alkalinity. The Physicochemical properties of the water used during the experimentation was monitored and are given in Table 1. All the parameters were in the optimum range. Water temperature is one of the most essential characteristics of an underwater system, affecting different parameters such as dissolved oxygen. The solubility of oxygen decreases as water temperature increases.

As the water gets warm, and oxygen levels drop, fish become sluggish and in- active. The temperature recorded was 22±3°Cwell within the optimum range. In this study, pH recorded was 7.5 ±0.1 indicating neutral water in aquaria. The high pH harms the fish as it denatures its membranes. The hardness in the study was calculated to be 98.66±5.77 mg/L, within the optimum range. The optimum range leads to better development and growth of fish, and lower value can lead to stress in fish [13]. A higher dissolved oxygen level indicates better water quality. The water used for experimentation had DO of 19.87±2.57 mg/L which is suitable for supporting life as it is the primary requirement for respiration. An appropriate amount of dissolved oxygen is vital for aquatic life [12]. The free carbon dioxide in the water was negligible as the carbon dioxide increases the oxygen level falls and stressful conditions arise for survival.

Parameter	Result
Temperature	22 ± 3 °C
pH	7.5 ± 0.1
Total Hardness	98.66 ± 5.77 mg/L
Dissolved oxygen	19.87 ± 2.57 mg/L
Free CO_2	Negligible
Alkalinity	210.43±1.1mg/L

Table 1: Physicochemical properties of water.

The alkalinity indicates the buffering potential of the water, that is, its ability to resist acidic changes. The value calculated was 210.43±1.1 mg/L which will resist changes in pH and thus maintaining the quality of water [13].

Behavioural changes in *C. batrachus*

The various behavioural aspects were taken into consideration (Table 2;Figure 2,4,5 and 6) after exposure to high and low doses of sodium fluoride. Fishes are sensitive to their surrounding and their behaviour changes on exposure to the toxicant. The exposed fish were found to be more active as compared to the control and remained confined at the bottom of the tub. The sensitive fish were found in the vertical position in the water column. The swimming movement became erratic, aggressiveness highly increased as compared to the horizontal position and quiet nature of control fish mainly remaining inactive at the bottom of the tub (Figure 3). The increase in swimming behaviour affects the fish appetite, food intake was very rapid in the group exposed to sodium fluoride toxicity as compared to control group [5,14]. The locomotor activity of fish increases for better oxygenation.

Groups	Rate of operculum opening (beats/min)	Surface activity (per 30 min)	Heart beat rate (Per min)	Oxygen saturation (mm/Hg)
Control	38 ± 2.1	3 ± 1.19	45 ± 2.48	96 ± 5.31
Low Dose	52 ± 1.99*	37 ± 1.76*	76 ± 4.20*	75 ± 4.14*
High Dose	54 ± 1.78*	33 ± 2.01*	89 ± 4.92*	71 ± 3.93*
ANOVA at 1%	F value: 118.6 ®	F value: 578.4®	F value: 191.2®	F value: 53.41®

Table2: Behavioural Changes in *C. batrachus* after exposure to different doses of Sodium Fluoride. Values are represented as Mean ± SEM (n=6) in each group. Statistical analysis performed using one way ANOVA (P < 0.001).

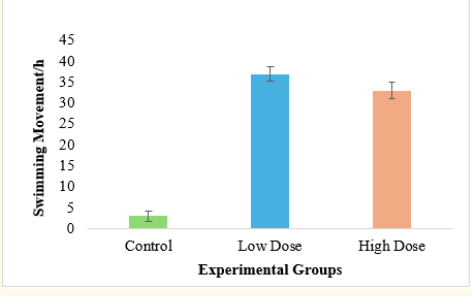

Figure2: Changes in the swimming movements of *C. batrachus* exposed to different doses of sodium fluoride.

Figure3: Behavioral activities in *C. batrachus* exposed to different doses of sodium fluoride: a) Control: Calm nature confined to the bottom of tub b) Exposed: showing increased activity c) Vertical position in the water column of the exposed fish.

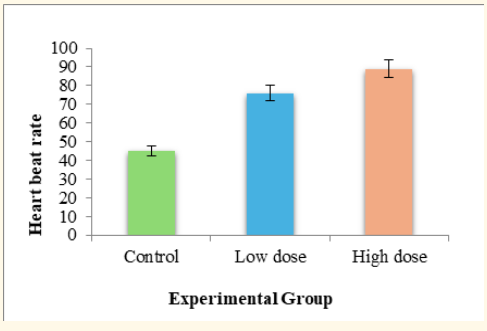

Figure4: Heartbeat rate of *C. batrachus* exposed to different doses of sodium fluoride.

The heartbeat is related to swimming activity, and as the swimming activity increases in the exposed fish, the heartbeat rate also increases to 89 ± 4.92 in the high dose [14]. The decrease in the oxygen saturation to 71 ± 3.93 and increase in the opercular movement to 54 ± 1.78 in the fish exposed to high dose of sodium fluoride may be due to the reduction of RBC's and hemoglobin percentage leading to oxygen deficiency and thus to compensate for the deficiency opercular movement increases [15,16].

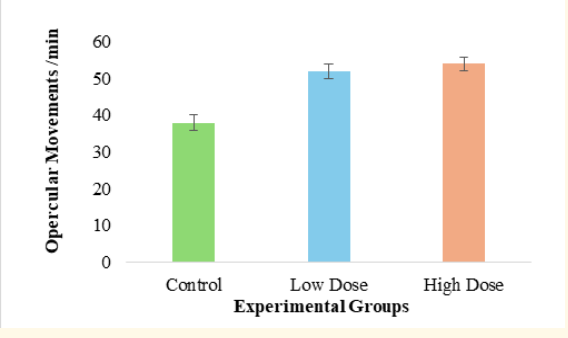

Figure5: Opercular movement of *C. batrachus* exposed to different doses of sodium fluoride.

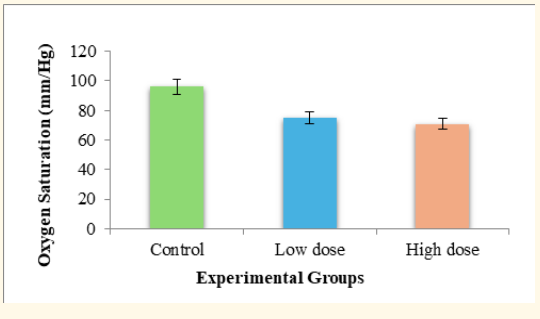

Figure6: Oxygen saturation level in *C. batrachus* exposed to different doses of sodium fluoride.

The erratic swimming and lack of equilibrium may be caused by the nervous system disorder affecting essential function was reported by many researchers. The aggressive behaviour may be an avoidance reaction to the toxicant [24,25].

Hematological parameters

Several hematological changes were noticed during the experimental period (Table 3; Figure 7A-H). On acute exposure (10 days) to different concentrations (low and high dose), significant decreases in RBC count, Hb%, PCV, MCV, MCH, and MCHC were noted, indicating anemia. On the otherhand, there was an increase in the number of WBC and clotting time(Figure 7A-H).

Parameter	Control	Low dose	High dose	F value
RBC's count (10^6/µl)	1.95 ± 0.507	0.76 ± 0.086*	0.63 ± 0.015*	35.99®
WBC's Count (10^3/µl)	7.41 ± 1.16	12.70 ± 1.28*	16.56 ± 1.47*	73.82®
Clotting time(s)	20 ± 0.12	31 ± 0.22*	36 ± 0.24*	100.2®
Hemoglobin%	8.00 ± 0.2	7.06 ± 0.305*	6 ± 0.61*	35.68®
PCV%	32.452 ± 1.70	31.94 ± 1.29*	28.46 ± 1*.03	15.12®
MCV (fL)	100 ± 1.34	88 ± 2.12*	74 ± 1.99*	297.4®
MCH (pg)	41 ± 2.98	35 ± 1.65*	28 ± 3.98*	27.77®
MCHC (g/dL)	24.65 ± 0.67	22.1 ± 0.75*	21.08 ± 0.89*	33.75®

Table3: Effect of different doses of sodium fluoride on the hematological parameters in *C. batrachus.* Values are represented as Mean ± SEM (n = 6) in each group. Statistical analysis performed using one way ANOVA ($P < 0.001$).

Estimation of RBCs

Compared to control group the RBC counts significantly declined in experimental groups in 10 days of fluoride exposure. The RBC count after 10 days of experiment was 1.95 ± 0.507 in control which on exposure of fluoride was decreased to 0.76 ± 0.086 and 0.63 ± 0.015 in low and high doses respectively. The decrease in RBC counts following exposure to sodium fluoride may be caused by the toxin's stimulation of the lipid peroxidative system producing lipid peroxides that haemolyze RBC's [26].

Determination of WBCs

After 10 days of fluoride exposure, the WBC counts showed higher values in toxicant groups than in the control group. The WBCcounts after 10 days of the experiment were 7.41 ± 1.16 in Control, and increased to 12.70 ± 1.28 and 16.56 ± 1.47 in low and high doses after fluoride exposure (NaF per se). This increase in leukocytes indicates that fluoride, may cause an immunological response through lymphocytes, which are more susceptible to fluoride and act as evidenced by their rapid increase in number [27,28]

Clotting time

Clotting time was increasingly reported in experimental groups than control. It ranged from 20±0.12 (control) to 31±0.22 and 36±0.24 in experimental groups (Low and high doses). The increased clotting time might be related to the fact that NaF is an anticoagulant as well as a chelator of calcium ions from the blood, which is required for blood clotting [17,18].

Estimation of hemoglobin

Hemoglobin percentage decreases due to the reduction of the RBC count because RBC cell ruptures. This led to a reduction in the number of cells, this in result also decreased the O_2 carrying capacity. This is also the reason for toxicity, given fishes come to the surface of the water more as compared to the control group. The hemoglobin percentage significantly decreases at higher doses, from 8.00±0.2 to 7.06± 0.305 and 6 ± 0.61 at a lower and higher dose as compared to the control (Table 3). The decrease in hemoglobin leads to anemic conditions and is due to the destruction of erythrocytes, and the suppression of erythropoiesis [19].

Determination of PCV

As compared to control group the PCV were significantly decreased in experimental group in 10 days of fluoride exposure. The PCV after 10 days of experiment was 32.452 ± 1.70 in control, which on exposure of fluoride was decreased to 31.94 ± 1.29 and 28.46 ± 1.03 in low and high dose respectively. This can happen as a result of fewer erythrocytes and haemoglobin. Packed cell volume measurements are crucial for assessing the impact of stress on an animal's health and serve as a gauge of the blood's ability to deliver oxygen [17]. The decline in packed cell volume is also a result of a reduction in the RBC count and Hb content as reported [20].

Determination of MCV

As compared to control group the MCV were significantly decreased in experimental group in 10 days of fluoride exposure. The MCV after 10 days of experiment was 100 ± 1.34 in control which on exposure of fluoride was decreased to 88 ± 2.12 and 74± 1.99 in experimental groups. Depending on the average RBC cell size, MCV is either increased or lowered. A low MCV implies microcytic (small average RBC size) was reported by many researchers [21].

Determination of MCH

After 10 days of fluoride exposure, the MCH was found lower in the experimental group compared to the control group. The MCH in control was 41 ± 2.98; however,after exposure to fluoride, it dropped to 35 ± 1.65 and 28 ± 3.98 in low and high doses respectively. MCH was most suitable as an indicator of iron deficiency anemia [22].

Estimation of MCHC

After 10 days of fluoride exposure, the experimental group's MCHC decreased compared to the control group. The MCHC in control was 24.65 ± 0.67; however, after exposure to fluoride, the MCHC in low dose group was 22.1±0.75 and in high dose it was found to be 21.08 ± 0.89. The average amount of haemoglobin found inside a single red blood cell is calculated as the mean corpuscular haemoglobin concentration (MCHC) [23]. A sensitive indicator of a lower Hb level is a low MCHC.

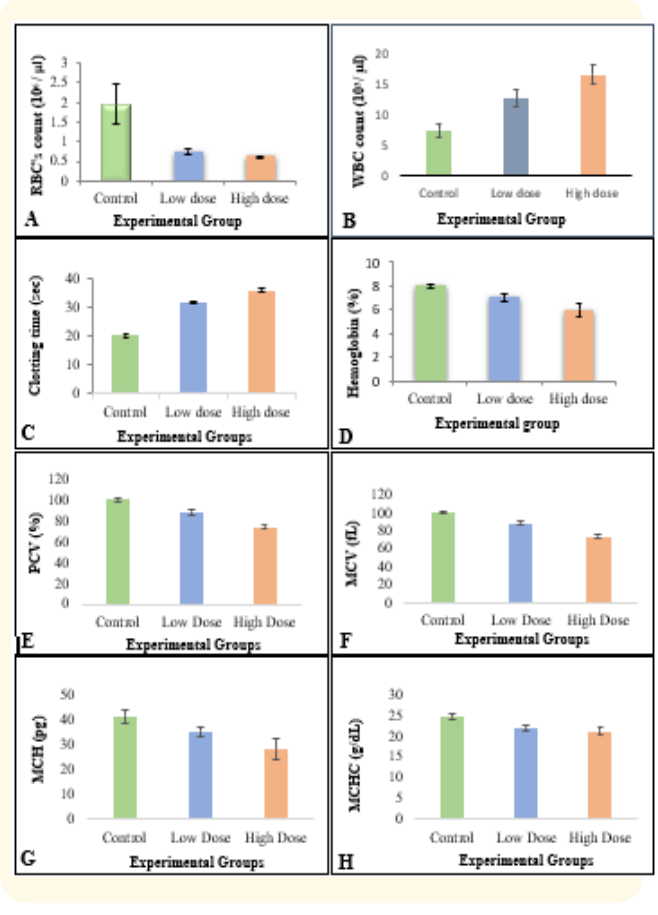

Figure 7 (A-H): Different hematological parameters studied during exposure to sodium fluoride at different doses.

Conclusion

Significant effects on haematological parameters were observed when the fish were exposed to fluoride toxicity during the current study. Blood is a sensitive indicator of the health of almost all body systems. In a variety of fish species hematology is a measure of fish health status to identify physiological changes caused by various stressors such as pollution, illness, hypoxia, and so on. The findings of this study are also consistent with previous research reports. The experimental findings revealed a significant increase in the behavioural activities such as Opercular movement, feeding, aggressiveness, surfacing, heart beat rate and oxygen saturation and the haematological parameters such as RBC, Hb%, PCV, MCV, MCH, and MCHC counts declined. Also, the significant increment in WBC's and clotting time in the experimental groups was noted in comparison to control group.

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Conflicts of Interest

The authors declare no conflict of interest.

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