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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 hematological parameters of *Clarias batrachus*. Sodium fluoride is a major pollutant in aquatic environment and exposure to sodium fluoride in the environment 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 13.4 ± 1.3 cm, and weight 24.3 ± 3.5 g) were exposed to sodium fluoride at different concentrations (34.975 mg/l and 87.43 mg/L)for ten days. The hematological parameters were examined, including RBC, WBC count, PCV, clotting time, hemoglobin%, MCV, MCH, and MCHC. The parameters such as RBCs, Hb%, PCV, MCV, MCH and MCHC PCV substantially decreased whereas, WBC count was found to be increased in experimental groups. The derived hematological indices of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were equally altered compared to control. The results of this study demonstrate that exposure to Sodium fluoride is toxic to *C. batrachus* and induces alterations in the hematological parameters.

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

Introduction

Fluoride is an utmost element that is present in nature. Still, elementally fluorine does not occur naturally because it is a reactive nonmetal and an electronegative element, so it always integrates, excluding oxygen and noble gases; fluorine exists in the fluoride compounds which make up of minerals of rock and soils. Fluorides make up 0.032% of the earth's crust and are the thirteenth topmost element [1] that is present in nature. Naturally, fluoride is present in water and air, but other sources of fluorideare food. Fluoride is present in low concentrations in meat, fruit, and vegetables, but a high concentration of fluoride is present in tea; the industries also produce aquatic animals as a source of food compounds of fluoride. Fluoride is present in variable concentrations in the water bodies depending on the geographical contribution [2,3].

Water is the primary source of life on the earth, but water faces the threat of contaminants; some are naturally present in nature and some are released by industries. Living beings depend on the environment or ecosystem for their well-being, growth, and development; if the climate changes or faces some threat, it also affects the life of an organism, as water is the main component on the earth; if it meets the problem, it is going to affect not only the human beings but also aquatic animals. The freshwater contaminated by the toxicants becomes a significant concern, as it affects aquaculture, the primary source of protein. Fluoride exceeding the required level leads to harmful effects and influences the community's health and the aquatic environment. In India and worldwide, fish is the primary food source in West Bengal, Andhra Pradesh, Bihar, and many other states. Fishes are sensitive to climatic change, pH, temperature, and toxicity. *Clarias batrachus* is an Indian walk-

ing catfish; it is a freshwater fish; it is known for its elegance as magur mas in Assam; it is one of the vital fish which is used as a food source, but it is facing a threat due to the contaminant present in the water, sodium fluoride produced severe health problem in *Clarias batrachus.* The behavioral parameter is an important parameter to understand the behavior of the fishes, depending on environmental changes. The toxicant changes the behavior of the fishes, due to the stress and dramatically affects behavior, such as swimming habits, operculum movement, mucus secretion, body position, food sensitivity, and the coloration of the body. There are many other changes, fluoride also works as an inhibitor. It creates problems in the fish's immune system [4,5].

Fluoride work as a contaminant in the fish and affects the development of the fish such as physical changes, length, mass, and size [6]. Two bioindicators of fluoride toxicity exist lipids and proteins in body tissues. They decrease due to higher concentrations of fluoride, resulting in the reduction of fish growth, and thus the weight of the fish falls [7]. Fluoride toxicity affects the chromatophores of the fish that specifies the color of the skin and eyes. The induction of NaF chromatophores dimension, configuration, and dispersal changes. The toxicity of fluoride affects the reproductive system of the fish. Fluoride toxicity affects the serum and tissue biochemistry, and enzyme and some biomolecules of the fish [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 *Clarias batrachus*. Therefore, it is precious in the assessment of the physiological condition of the fish. Furthermore, hematological responses are essential indicators of changes in the internal as well as external environment of animals [10].

Hematological parameters recall the variation of an organism accurately. Therefore, the central role of the hematological parameter is to identify any change due to environmental factors, chemicals, toxicant, or anything that affect living beings. Moreover, they can remember the slightest difference in an organism because of the changes in hematological parameters due to temperature and climatic change. Fishes are poikilothermic aquatic animals that detect bacteria, parasite activity, water, toxicity, oxygen rate, and pH. Therefore, immunological and hematological usefulness is the leading standard for fish well-being. Thus, 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.

Materials and Methods

Experimental animal

Collection of experimental animals

Healthy living specimens of freshwater catfish, *Clarias batrachus* (Length: 13.6 ± 0.78 cm; Weight: 20 ± 2 gms), 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 0.2% $KMNO_4$ to avoid bacterial and fungal infection. Then acclimatized for 15days in an aquarium filled with tap water, set up of aerator for proper aeration, for feeding the fish were given fish food Tokyo (5% body weight). After acclimation, healthy and active fish were selected for experimental purposes in different tubs, and water was changed every alternate day.

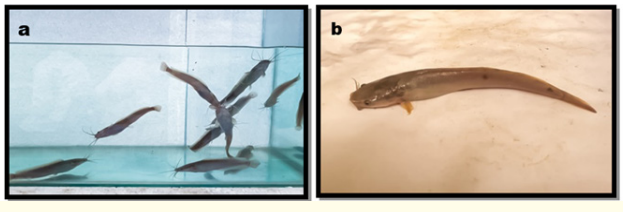

Figure 1: *Clarias batrachus.*

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). The following treatment was given to different groups.

Physicochemical properties of water

Physico-chemical properties of an aquatic ecosystem play a significant role in its production process and, in turn, in the growth of organisms. The physicochemical properties of water were determined by the method of APHA., *et al.* [11], pH, temperature, dissolved oxygen, hardness, and carbon dioxide.

Groups	Acute (10 days)
GROUP I:	Control
GROUP II:	Low dose (34.975 mg/l)
GROUP III:	High dose (87.43 mg/l)

Table 1: Experimental set up.

pH

In the present investigations, the pH values of the water samples were measured using a digital 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 BOD bottle to avoid bubbling. Take 1ml of manganous sulfate solution and alkaline potassium iodine 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. The part of the sample was taken (50 -100 ml) in a conical flask and titrated against 0.025N sodium thiosulphate. Until the solution turns light yellow, add 1ml starch solution in it, and the solution becomes blue. Continue the titration till the solution turns colorless. The quantity of the thiosulphate solution used was calculated.

$$\text{Dissolved oxygen (mg/l)} = \frac{(8 \times 1000 \times N) \times V2}{V1}$$

Where, N = Normality of the titrant (0.025 N); V1 = volume of sample (ml); V2 =importance of titrant used (ml); (1 ml of sodium thiosulphate solution is equivalent to 0.2 mg oxygen).

Hardness

Take 50 ml of sample in a flask, 1 ml ammonium buffer sample was added, 1 ml of sodium disulfide was added, and 2-3 drops Erichrome black T mediator was added, the solution turns wine red, titrate the contents against EDTA solution at the endpoint till the color changes from wine red to blue.

$$\text{Hardness (in mg/L)} = \frac{(V \times N \times 50 \times 1000)}{SV}$$

(Where, V = volume of titrant (mL); N = normality of EDTA; 50 = equivalent weight of $CaCO_3$; SV = sample volume (mL).

Carbon dioxide

50ml of water sample was taken in a conical flask and 2-3 drops of phenolphthalein was added .The sample remains colorless which indicates the presence of free CO2 in water . the sample was titrated with standard alkaline solution (NaOH or Na2 CO3 0.05N) untill pink colour persists for 30 seconds.The reading was noted and free CO2 was calculated by using the below formula**:**

$$\text{Free carbon dioxide (mg/L)} = \frac{\text{ml of titrant used} \times \text{N of NAOH} \times 1000 \times 4}{\text{ml of sample}}$$

(Where, V = volume of titrant (mL); N = normality of EDTA; 50 = equivalent weight of CaCO3; SV = sample volume (mL).

buffer was added, 1 ml of sodium disulfide was added, and 2-3 drops Erichrome black T mediator was added, the solution turns wine red, the contents was titrated against EDTA solution at the endpoint till the color changes from wine red to blue.

Alkalinity

Alkalinity was checked by taking 50 ml of water sample in a conical flask. Add 2-3 drops of phenolphthalein indicator and titrate the sample 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.

$$\text{Total Alkalinity (mg/l)} = \frac{\text{t (total volume of 0.02NHCL)} \times 1000}{\text{ml of sample}}$$

Behavioral study

Observations were made regarding the behavioral changes of fishes with respect to unanimous body conditions and activities like hyperactivity, swimming pattern, responsiveness, food grasping, change in body coloration, and surfacing movements were keenly observed in experimental animals compared with control ones.

Heart beat rate and oxygen saturation

The heartbeat rate and oxygen saturation were recorded using Pulse Oximeter CMS50D. The area is cleaned, making sure it fits easily without being too loose or too tight. Allow several seconds for the pulse oximeter to detect the pulse and calculate the oxygen saturation. Look for the displayed pulse indicator that shows that the machine has seen a pulse. Without a pulse signal, any readings

are meaningless. Once the unit has detected a good beating, the oxygen saturation and pulse rate will be displayed.

Study of hematological parameter

Hematology is the study of blood, blood-forming organs, causes, prognosis, treatment of diseases related to blood and. Hematological parameters are essential in determining the health and physiological status of fish. They reflect the changes in the organism correctly and play important in the detection of diseases and the metabolism of fish living in different ecological environments.

Collection of blood

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

Analysis of blood

Estimation of the number of RBC (red blood cells)

A drop of blood was placed on a clean glass slide, and the blood smear was made with the help of a spreader slide set at an angle of 45° and then stained by Leishman's stain for 5-7 minutes. The excess dye was drained, washed with tap water, air-dried, and then observed under a microscope. Neubauer's counting chamber was used to count the RBCs. The blood was taken into the RBC pipette up to the 0.5 points, and then the diluting fluid (Hayem's fluid) was drawn up to 101 (thus, the dilution is 1:200). The pipette was held vertically and rotated between finger and thumb with both hands to allow the blood to mix with the diluting solution. Because the clear fluid in the capillary section of the pipette was not mixed with blood, it was discarded. The counting chamber was then 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 fine small squares of the RBC column under higher magnification, and the number of RBC's/ mm^3 was computed accordingly.

Calculation:

$$\text{RBC's count} = \frac{\text{No. of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{areas counted}}$$

Estimation of the number of WBC in blood

WBCs were also counted using the Neubauer crystalline counting chamber. Similarly, as mentioned above, the blood was drawn in the WBC pipette upto the 0.5 mark. Immediately after, the diluting fluid (Turk's fluid) was drawn upto the mark11 (thus, the dilution is 1:20). By gently shaking the solution, it was entirely mixed. It was left to settle for 2 to 3 minutes. The controlled area was covered once the counting chamber and cover glass were cleaned. Again, a stem of the solution was evacuated, and a drop of fluid was allowed to run beneath the cover slip while maintaining the pipette at an angle of 40 for 2 to 3 minutes until the WBC settled. The ruled measurement area was then focused beneath the microscope, and the numbers of WBCs were recorded in fine little squares of the WBC column under greater magnification, and the number of WBCs per square millimeter was calculated appropriately.

Calculation:

$$\text{WBC count} = \frac{\text{No. of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{areas counted}}$$

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 30 seconds. Till blood clots and note the clotting time carefully.

Hemoglobin %

Estimation of hemoglobin concentration (Hb) The hemoglobin concentration was estimated by the acid haematin method. N/10 Hydrochloric acid was taken upto ten marks in the graduated tube blood was sucked into the hemoglobin pipe and then transferred into the graduated tube containing N/10 hydrochloric acid. The pipette was rinsed 2 to 3 times with dilute hydrochloric acid. It was allowed to remain in the dark for 10 to 20 mins after mixing N/10 HCl was added drop by drop, mixing each dilution until the blood color matched with the standard color. Then the results were read from the scale on the graduated tube, and Hb concentration was expressed in gram %.

Estimation of Packed cell volume (PCV)

The PCV was estimated through Wintrobe's tube. Blood samples were obtained in a container containing an anticoagulant. Blood was taken upto mark 100 in the Wintrobe's tube and then centrifuged for 30 min at 3000 rpm. The initial blood column in the tube measured 100mm. 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.

Calculation

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

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. MCH is obtained by dividing the Hb concentration by the number of red blood cells, multiplying the result by ten, and expressing the result in micrograms (g).

Calculation:

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

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 %).

Calculation

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

Statistical analysis

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

Result and Discussion

Physio-chemical 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 physio-chemical parameters of the water for the experiment were analyzed. The Physicochemical properties of the water used during the experimentation were monitored and are given in Table 2.All the parameters were in 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 value is found to be excellent and suitable for the existence of aquatic animals, as higher pH leads to the loss of nutrients. Water temperature is one of the most essential characteristics of an underwater system, affecting different parameters such as dissolved oxygen levels. The solubility of oxygen decreases as water temperature increases. 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. A higher dissolved oxygen level indicates better water quality. The water used for experimentation has a value of 16.93 ± 2.57 mg/L and 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]. 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 fish, and lower value can lead to stress in fish, as reported by Kumawat., *et al.* [13]. 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 value calculated was 333.33mg/l which will resist changes in pH and thus maintain the quality of water [13].

Parameter	Result
Temperature	25 ± 3 °C
pH	7.5 ± 0.1
Total Hardness	98.66 ± 5.77 mg/L
Dissolved oxygen	19.87 ± 2.57 mg/L
Alkalinity	423.90 mg/l

Table 2: Physicochemical properties of water.

Behavioural changes in C. batrachus

The various behavioral aspects were taken into consideration (Table 3; Figure 2,4,5 and 6) after exposure to high and low doses of sodium fluoride. Fishes are sensitive to their surrounding their behavior changes on exposure to the toxicant.

The exposed fish were found to be more active as compared to the control that mainly remained confined to the bottom of the plastic tub. The sensitive 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,

Groups	Rate of operculum opening (beats/min)	Surface activity (per 30 min)	Heartbeat	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®

Table 3: Behavioural Changes in *C. batrachus* after exposure to various concentrations of Sodium Fluoride.

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

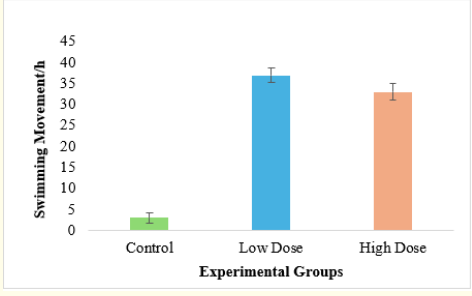

Figure 2: Changes in the swimming movements of *C. batrachus* exposed to different doses of sodium fluoride.

Figure 3: 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.

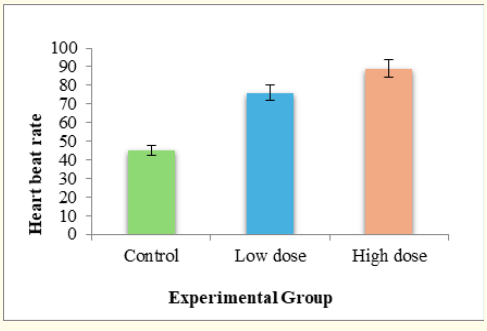

Figure 4: Heartbeat rate of *C. batrachus* exposed to different doses of sodium fluoride.

and the quiet nature of control fish mainly remained inactive at the bottom (Figure 3). 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 reported authors [5,14].

The heartbeat is related to swimming activity, and as the swimming activity increases in the exposed fish, the heartbeat rate also

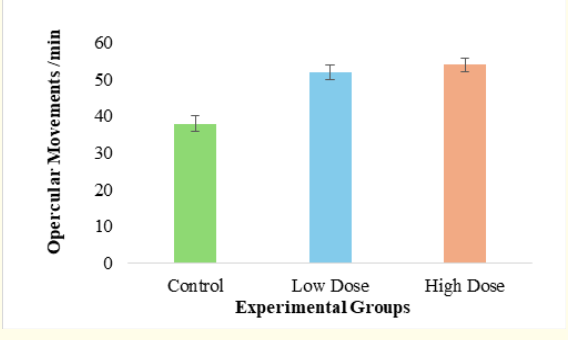

Figure 5: Opercular movement of C. batrachus exposed to different doses of sodium fluoride.

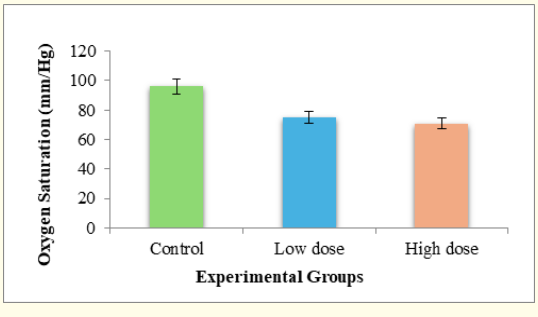

Figure 6: Oxygen saturation level in C. batrachus exposed to different doses of sodium fluoride.

increases, as reported by Sabullah., *et al.* [14]. The decrease in the oxygen saturation in the fish exposed to sodium fluoride may be due to the reduction of RBCs and hemoglobin percentage, and this results in rapid opercular movement to compensate for the deficiency of oxygen reported by authors [15,16].

Hematological parameters

Several hematological changes were noticed during the experimental period (Table 4; Figure 7A-H). On exposure to different concentrations (low and high dose) for different time periods (10 days), significant decreases in RBC count, Hb%, PCV, MCV, MCH, and MCHC were noted, indicating anemia. On the other hand, there was an increase in the number of WBC, 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)	1.4 ± 0.08	1.76 ± 0.09*	2 ± 0.08*	78.55®
Clotting time	20 ± 0.12	31 ± 0.22*	36 ± 0.24*	10017®
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	24.65 ± 0.67	22.1 ± 0.75*	21.08 ± 0.89*	33.75®

Table 4: 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.

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 0.7 ± 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, which produces lipid peroxides that haemolyze RBCs.

Determination of WBCs

After 10 days of fluoride exposure, the WBC counts showed higher in experimental groups than in the control group. The WBC counts after 10 days of the experiment were 1.4 ± 0.08 in Control, and increased to 1.76 ± 0.09 and 2 ± 0.08 in high and low doses after fluoride exposure (NaF per se). This increase in leukocytes indicates that fluoride, a foreign agent, may cause an immunological response through lymphocytes, which are more susceptible to fluoride and act as evidenced by their rapid increase in number. Increased WBC count indicates an active response to the toxicant NaF in the water.

Clotting time

Clotting time was increasingly reported in experimental groups than control. It ranged from 20 seconds (control) to 31 seconds and 36 seconds 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 alive 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 of sodium fluoride-treated fish 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 due to the destruction of erythrocytes, and the suppression of erythropoiesis was reported by Kumar., *et al*. [19]. The decline in packed cell volume is also a result of a reduction in the RBC count and Hb content. Similar results were reported by Saxena., *et al.* [20]. Sodium fluoride is anticoagulant and also leads to the Chelation of calcium ions; hence the clotting time increases.

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, fewer haemoglobin molecules per erythrocyte, both, or none. 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].

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 74.54 ± 1.99 in experimental groups. Depending on the average red cell size, MCV is either increased or lowered; a low MCV implies microcytic (small average RBC size) was reported by Dai *et al.* [21].

Determination of MCH

After 10 days of fluoride exposure, the MCH was found lower in the experimental group compared to the control group. After 10 days of the experiment, the MCH in control was41 ± 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. After 10 days of the experiment, the MCHC in control was 24.65 ± 0.67; however, after exposure to fluoride, the MCHC 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.

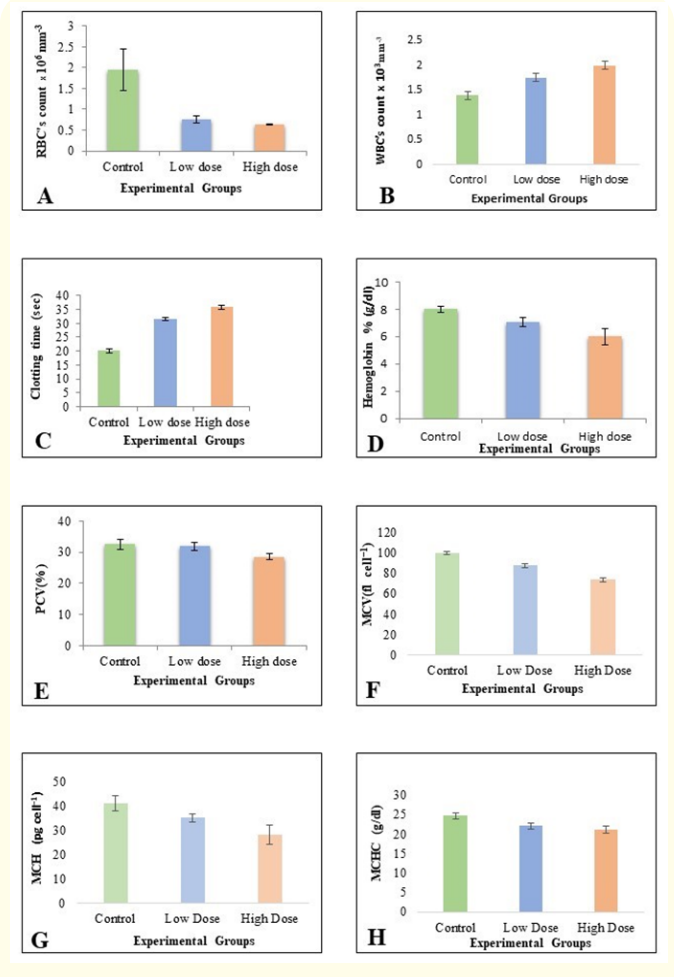

Figure 7: Hematological parameters (A-H) in *C. batrachus* after acute exposure (10 days) in different experimental groups.

Conclusion

Significant effects on haematological parameters were observed when subjects were exposed to fluoride toxicity during the current study. Blood is a sensitive indicator of the health of almost all body systems. A variety of fish species use haematology as 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 on fluoride toxicity on blood parameters in other fish species. The study findings also revealed a significant decrease in RBC, Hb%, PCV, MCV, MCH, and MCHC counts and significant increment in heart beat rate, oscillatory movement and clotting time in the experimental groups when compared to controls.

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