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Subtle Toxic Manifestation of Fluoride on Haematological Parameters of Freshwater Carp Catla catla (Hemilton)

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Abstract Fluoride has already been identified as a potential toxic contaminant in the environment causing ailments affecting different systems of body [1]. Toxic manifestation of this element in human beings and other experimental animals is widely documented but very few studies have been conducted on its toxicosis on fish fauna. In this study toxic manifestation of the environmental stress owing to the sub-acute to sub-chronic exposure of fluoride on haematological parameters were evaluated on the chosen fish species Catla catla. The exposure duration was 30, 60, 90 and 120 days. The exposure of excess fluoride concentration in water for long-term duration caused adverse alteration on various parameters of haematopoietic system of the fish. Decrease in haemoglobin, RBC count, MCV, MCH, MCHC and WBC was recorded, and its magnitude was time dependent. These toxic effects are the matter of concern for aquaculture and indicative of the physiological abnormality caused by this environmental contaminant.

Keywords: *fluoride, Catla catla, haematological toxicity, WBC, sub-chronic study*

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1. Introduction

Fluorine is the 17th, most abundant element in the earth's crust, and the weathering of alkalic and silicic igneous and sedimentary rocks, primarily shales, contributes much of the fluoride to natural waters [2]. Hence, exposure to this ion through environmental contamination is imminent. Fluoride ion is described as a double-edged sword and its intake below or above the recommended values have consequences [3]. Acute and Chronic fluoride intoxication exhibits clinical presentations like skeletal Fluorosis, dental Fluorosis and non-skeletal manifestations affecting almost all major physiological systems of body [1]. In many epidemiological and clinical studies conducted on different animal models, effects of fluoride exposure on haematological parameters have been reported [4-11]. Detrimental effects of fluoride on haematopoietic cells have been worked out earlier, and it is also associated with the impediment in the transport of Na^+/ Cl^- ions [8,12,13]. Chemo-kinetically fluoride usually enters in blood circulation by absorption through gastro-intestinal tract [14,15,16]. Fluoride intoxication has been reported to cause premature erythrocyte deaths due to membrane degeneration that turns them into echinocytes and condition eventually leads to anaemia in human subjects [17]. According to Machalinska et al. [18] fluoride is able to disturb haematopoiesis to varying degrees over a wide spectrum. Fish as organism, not only provide the nutrition and cater the demand of food rather they are also the indicator of environmental contamination. As the blood is an important component for studying the effects of toxicants and highly susceptible to environmental fluctuations, study of the subacute and sub-chronic effect of fluoride on haematological parameters gives an insight into the physiological stress caused by this toxicant on an organism, [19].

On fish fauna also, the haematological toxicity of fluoride have been investigated by some workers. But, no study on any Indian major carp has been conducted. On *Channa punctatus* haematological toxicity owing to fluoride exposure has been explored by workers like Saxena et al. [20] and Guru et. al [21]. In these studies, either the exposure concentration was unrealistically high or study duration were not enough to ascertain subtle sub-chronic toxicity. Some cross-references related to work on fluoride toxicity on blood parameters of Clarias and Labeo were noted but those references were either incorrect or missing in literature search. Carps are the main edible fish in India, and susceptible to environmental stress. Major part of the state Rajasthan in India falls in the endemic zone of fluoride excess in ground water [22] and *Catla catla* is one of the major Indian carp on which no such study has been reported. Hence, this study was undertaken to evaluate the

haematological toxicity potential of fluoride on this animal.

2. Materials and Methods

Fish Catla catla, (Hamilton) [23] were collected from the hatchery and breeding unit of the College of Fisheries, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India. Healthy fishes of approximately 25-35 cm, weighing 450-650 gm were kept for acclimatization in the experimental tank for a week. Five groups of fish were segregated randomly. One group of 10 fish each were kept in normal water with fluoride concentration less than 0.01 ppm for the entire duration, while other four groups were kept in high concentration of fluoride for duration of 30, 60, 90 and 120 days respectively. Other physico-chemical parameters of water were in normal defined range. Water temperature was maintained at 27 ± 2.0^{O}C. Fish were fed normal fish feed available commercially. In experimental or treatment tank the concentration of fluoride ion was 9.0 ppm. This dose was determined on the basis of the LC_{50} value of fluoride for this species. After completion of each exposure duration fish were taken out and sacrificed for collection of blood. Blood samples were collected from the heart in EDTA coated vials and proceeded for estimation of its different parameters immediately. Haematological parameters of investigation were determined by the method of Sood [24]. The parameters worked out in this study were Haemoglobin (Hb), Red Blood Cell/ Corpuscles (RBC), Haematocrit/ Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin concentration (MCHC) and White Blood Cell/ Corpuscles (WBC). MCV, MCH and MCHC are indicative of different kinds of anaemic conditions and calculated from values of RBC, Hb and PCV. The values obtained were processed statistically for t-test calculation to determine any significant variation between the treated and control groups.

3. Results & Discussion

The fluoride ion as suggested by literature caused alteration in the experimental indices of blood. Up to the duration of 30 days the exposure of the experimental concentration of fluoride did not cause any significant change in any of the parameters taken. But, exposure duration of 60 days and beyond did produce significant lowering in RBC count, Haemoglobin, MVC, MCH, MCHC and haematocrit. The alteration of values in all these haematological indices was found to be time dependent and the significance of variation were more prominent with the elapsed duration of exposure.

In the case of WBC count results were rather different. In 30 day duration slight increase in the value of total leucocyte count (TLC) was noted. But, in the exposure span of 60, 90 and 120 days, significant lowering of was recorded. The variation patterns in different blood indices has been given in the tabulated data (Table 1).

Blood is a very sensitive indicator of the functioning of almost every system of the body. The result of fluoride toxicity recorded during this investigation is similar to the results of fluoride toxicosis study conducted on rabbits by Abbas et al [25]. The lowering the values of RBC, haemoglobin and haematocrit was noted that led to lowering in the values of MCV, MCH, MCHC as well. In contrast to our result, there was no enhancement in the WBC count for short term exposure. The lowering in the erythrocytes and haemoglobin related values may be due to the lysis and lower generation of RBCs in bone marrow as a result of low hemoglobin production [26]. The light microscopic findings in similar studies depicted the presence of macrophages in the spleen, causing more damage to erythrocytes in fluoride treated groups than control groups. This suggests the fluoride induced enhancement in Phagocytosis in spleen localized macrophages, which consequently leads to the development of anemia [27,28]. Moreover, fluoride also retards the normal process of erythropoiesis by interacting with the iron of hemoglobin [25]. Measurement of blood erythrocyte antioxidant enzyme activities shows that Fluoride from drinking water increases levels of superoxide dismutase, glutathione peroxidase and catalase [29]. Therefore, the oxidative stress caused by fluoride ion in the blood may also be the reason for the resulted toxicity. Such reduction in haematological values has been recorded with mammals by other workers also [30]. Reduction in haematocrit value on fluoride poisoning has been recorded by Swarup and Singh [31] and Gujarathi et al [32]. The decreased RBC count in the present study may have been associated with a decreased rate of erythrogenesis due to the negative effect of fluoride on erythropoiesis or to shortened life span of erythrocytes and membrane degeneration [30,33]. It has been reported that fluoride toxicity causes hematopoietic progenitor cells injury in humans [34,35]. The studies of fluoride toxicity on blood parameters conducted on other fish species are also in agreement with the findings of this study [20,21].

Table 1. Changes in haematological parameters of fish Catla catla due to excess concentration of fluoride

Duration → / Parameter ↓	Control	30 Days	60 Days	90 Days	120 days
RBC	1.76 ± 0.046	1.67 ± 0.046	1.55 ± 0.066*	1.43± 0.070**	1.34 ± 0.071***
Haemoglobin	7.8 ± 0.364	7.02 ± 0.406	5.92 ± 0.397**	5.0 ± 0.607**	4.27 ± 0.450***
Haematocrit	26.5 ± 0.711	24.75 ± 0.760	21.95 ± 1.102**	19.8 ± 1.496**	17.77 ± 1.367***
MCV	150.29 ± 1.15	148.15 ± 1.020	141.57 ±1.392*	138.10 ± 3.638**	132.41 ± 3.772***
MCH	44.12 ± 0.899	41.86 ± 1.357	38.06 ± 1.240*	34.51 ± 2.762**	31.55 ± 1.795***
MCHC	29.37 ± 0.692	28.24 ± 0.815	26.88 ± 0.783*	24.83 ± 1.444*	23.81 ± 1.113**
WBC	19.32 ± 0.960	21.03 ± 1.076	16.35 ± 0.980*	14.65 ± 0.927**	12.97 ± 0.924***

Comparisons Vs Control value: *p<0.05; **p<0.01; and ***p<0.001.

In our study there was an increase in the WBC upto 30 days of exposure. Though, insignificant, but the results are akin to the similar study conducted by Oda et al [37]. Decreased WBC in fluoride exposed animal might be the result of its under production from the germinal center of lymphoid organs due to necrosis [36]. Swarup and Singh [31] and Khandare et al [38] also came across the similar results in a study of fluorosis. Initial increase in leucocyte count may be explained through the report of Maheswaran et al. [39] suggesting that the increased in WBC count might be due to the tissue damage, i.e. stimulation of the immune system caused by sodium Fluoride. Overall, it is evident from this study that the long-term exposure to fluoride is detrimental to the haematopoietic system in fish Catla catla.

4. Conclusion

This investigation reaches to the conclusion that the excess exposure of fluoride to fresh water major carp Catla catla leads to deleterious effects on its haematological indices and the results are in agreement with the other similar studies carried out on other species of fish and other animal models. Therefore, freshwater fish fauna are the indicators of the fluoride contamination and may be employed in environmental surveillance of freshwater bodies. As fluoride can exert some pernicious effects on blood, that will affect almost all the systems of the body of a fish, so, from the aquaculture point of view also the ecotoxicology of fluoride is significant.

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