

Effect of Sodium fluoride on Hematological parameters in freshwater edible fish, *Rita rita*

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Abstract

In the present investigation, an attempt has been made to determine the acute toxicity of sodium fluoride and its toxicological effects on hematological parameters of freshwater edible fish, *Rita rita* from Bhima river near Solapur. Fishes were exposed to different groups like predetermined LC₀ (30mg/l), LC₁₀ (35mg/l) and LC₅₀ (75mg/l) concentrations of sodium fluoride (during December 2008 and January 2009). Alterations in hematological parameters were studied at the end of 24, 48, 72 and 96 hours of treatment. The findings of this study showed that parameters like R.B.C, W.B.C, Hemoglobin (Hb), Hemocrite (HCT), Mean corpuscular hemocrite (MCH) and Mean corpuscular hemocrite concentration (MCHC) levels were decreased in the sodium fluoride treated fishes. The observed changes indicated that the hematological parameters can be used as bioindicator of sodium fluoride stress in freshwater edible fish, *Rita rita*.

Keywords: sodium fluoride, hematology, *Rita rita*

1. Introduction

Fluoride has a high biological activity with well-established toxic effects on many organisms (Kaul and Susheela, 1974; Groth 1975; Lee, 1983; Shashi, *et al*, 1989) [10, 8, 11, 17]. Fluoride is a widespread pollutant, and is relatively persistent in environment. Fluoride also induces biochemical alterations in human and other mammalian models. Susheela and Jain (1983) [19] have studied the fluoride induced hematological changes in rabbits and mentioned that excessive ingestion of fluoride exert its impact on the overall profile of hematological parameters. Pillai and Mane (1985) [12] have studied the effect of air born fluoride on some hematological parameters of chick and stated that the chicks exposed to the polluted atmosphere retained considerable amount of fluoride in their serum. Jain and Susheela (1987) [9] have studied the effect of sodium fluoride on erythrocyte membrane function with respect to metal ion transport in rabbits and stated that fluoride in circulation and in erythrocyte membranes altered calcium and magnesium ions significantly. However, very little is known about the injury caused by fluoride to aquatic organism (Wright, 1977; Chitra and Ramana Rao, 1980) [23, 4]. Rivers, streams and ponds in India are severely polluted by domestic and industrial waste contains many toxic chemicals that adversely affect aquatic organisms. Several human activities result in substantial fluoride input to aquatic environment. (Dobbs, 1974) [5].

Aquatic organisms take up fluoride directly from water and tend to accumulate in their exoskeleton (Scientific facts on fluoride, 2005). Fish is an important food chain organism and many fishes like other vertebrate haven the ability to accumulate elevated fluoride levels (Fisher and Prival, 1973) [7]. In the present study, an attempt was made to study the toxic effect of fluoride on hematological aspects of freshwater edible fish, *Rita rita*.

2. Material and Methods

Freshwater fishes, *Rita rita* obtained from the Bhima river

near Takli, Solapur District. Healthy fishes were acclimated to laboratory conditions for ten days in well aerated water. Water was replaced every 12 hours in order to maintain healthy environment. Fishes with an average length (8-10 cm) and weight (15-20 gm) were selected for experimental study. External feeding was stopped before 24 hours to experimentation. The physicochemical parameters of water were analyzed by using APHA *et al.*, (1998) [11] methods. LC₅₀ concentration for 24, 48, 72, 96 hours was calculated by probit analysis method (Finney, 1978) [6]. After establishment of lethal concentrations, the fishes were exposed to observed LC₀ group (30mg sodium fluoride/l), LC₁₀ group (35 mg sodium fluoride/l) and LC₅₀ group (75 mg/l) (during December 2008 to January 2009). Ten healthy fishes exposed to each concentration of different groups to sodium fluoride. A concurrent control without toxicant was also run simultaneously.

For hematological studies, ten fishes each for control, LC₀, LC₁₀ and LC₅₀ were exposed to different concentrations. Fishes from each group were removed and their tails were severed at the caudal peduncle with sterilized surgical blade. Blood was drawn from control and sodium fluoride treated fishes and transferred into small vials containing EDTA as an anticoagulant. The blood samples were analyzed separately from each group after 24, 48, 72 and 96 hours of duration. Blood samples were analyzed by Cransisa Sysmax – KX- 21 cell counter. The experiment was repeated for three times for confirmation. Statistical analysis were done by using standard 't' test to compare values of control and treated fishes (Bailey, 1965) [2].

3. Result

Acute toxicity studies were conducted by exposing fishes, *Rita rita* after treating with sodium fluoride. The regression equation ($Y = a \pm bx$) was established for 24, 48, 72 and 96 hours and LC₅₀ calculated values are given in table No. 1.

Table 1: Acute toxicity of sodium fluoride to freshwater fish, *Rita rita* from Bhima River near Solapur.

S. No.	Duration (hr)	Regression equation $y = a \pm bx$	95 % Fludicial limit (ppm)	Calculated LC ₅₀ (ppm)
1	24	$Y = 2.67 + 0.1523x$	52.6 – 84.13	79.43
2	48	$Y = 2.50 + 0.1238x$	53.08 – 83.17	77.62
3	72	$Y = 2.54 + 0.1321x$	51.28- 75.85	74.13
4	96	$Y = 2.54 + 0.1322x$	50.93 – 76.05	75.8

The results of the present investigation for hematological variations due to acute toxicity of Sodium fluoride toxicity from the freshwater fish, *Rita rita* during different intervals is given in the table No. 2 and 3.

As concentration and time exposure of sodium fluoride increased, fish mortality also increased. There was Linear A relationship between percentage of mortality and Sodium fluoride concentration. Freshwater edible fish, *Rita rita* exposed to sodium fluoride showed significant decrease in red blood cell count and white blood cell count. R.B.C value was significantly ($P < 0.001$) decreased in LC₅₀ treated fishes at 96 hour duration. W.B.C value was significantly ($P < 0.001$) decrease in both LC₁₀ and LC₅₀ group after 72 and 96 hours of exposure. Results of hemoglobin content showed a decreasing trends in LC₀ group after 72 hours of exposure which was significant ($P < 0.05$). Similar results were also observed in

LC₅₀ group at 72 and 96 hours of exposure ($P < 0.001$). Results of hemocrite showed significantly ($P < 0.001$) lowered contents in all groups at 72 and 96 hours of exposure.

The hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration decreased from treated groups when compared with control. Mean corpuscular volume decreased in LC₁₀ and LC₅₀ groups of treatment and the decrease at 96 hour was significant ($P < 0.05$). The mean corpuscular hemoglobin was significantly ($P < 0.001$) decreased from *Rita rita* after 72 hours exposure to sodium fluoride. Mean corpuscular hemoglobin concentration (MCHC) was significantly ($P < 0.01$) decrease after 48 hours of exposure to sodium fluoride.

Table 2: Changes in R.B.C. and W.B.C. count of *Rita rita* after exposure to acute doses of sodium fluoride.

Hematological parameters	Exposure time (h)	Experimental groups			
		Control	LC ₀	LC ₁₀	LC ₅₀
Total R.B.C (10 ⁶ /μl) (Red Blood Corpuscles)	24	1.57 ± 0.003	1.53 ± 0.005** (2.54)	1.56 ± 0.006 (0.63)	1.62 ± 0.003*** (-3.18)
	48	1.51 ± 0.020	1.46 ± 0.016 (3.31)	1.44 ± 0.014* (4.63)	1.42 ± 0.008** (5.95)
	72	1.45 ± 0.035	1.43 ± 0.025 (1.37)	1.34 ± 0.045* (7.58)	1.23 ± 0.046** (15.17)
	96	1.34 ± 0.041	1.13 ± 0.020*** (15.67)	0.91 ± 0.015*** (32.08)	0.85 ± 0.040*** (36.56)
Total W.B.C (10mm ³) (White Blood Corpuscles)	24	3.4 ± 0.100	3.28 ± 0.005 (3.52)	3.35 ± 0.010 (1.47)	3.21 ± 0.115 (5.58)
	48	3.09 ± 0.030	2.94 ± 0.035** (4.85)	2.72 ± 0.026*** (11.97)	2.34 ± 0.041*** (24.27)
	72	2.76 ± 0.047	2.63 ± 0.020** (4.70)	2.46 ± 0.037*** (10.86)	2.16 ± 0.126*** (21.73)
	96	2.74 ± 0.060	2.55 ± 0.045** (6.93)	1.84 ± 0.040*** (32.84)	1.72 ± 0.037*** (37.21)

Bracket value indicates percent variations, $P < 0.05 = *$, $P < 0.01 = **$, $P < 0.001 = ***$

Table 3: Changes in various hematological parameters of *Rita rita* after exposure to acute doses of sodium fluoride.

Hematological parameters	Exposure time (h)	Experimental groups			
		Control	LC ₀	LC ₁₀	LC ₅₀
Hemoglobin (g/dl)	24	3.89 ± 0.036	3.54 ± 0.025*** (8.99)	3.34 ± 0.026*** (14.13)	3.43 ± 0.041*** (11.82)
	48	3.63 ± 0.037	3.24 ± 0.005*** (10.74)	2.83 ± 0.043*** (22.03)	2.05 ± 0.046*** (43.51)
	72	3.24 ± 0.040	3.03 ± 0.104* (6.48)	2.12 ± 0.116*** (34.56)	1.73 ± 0.020*** (46.59)
	96	2.52 ± 0.032	1.94 ± 0.020*** (23.01)	1.49 ± 0.010*** (40.87)	1.33 ± 0.036*** (47.21)
Hemocrite (%)	24	11.45 ± 0.225	11.17 ± 0.288 (2.44)	10.33 ± 0.062*** (9.77)	10.01 ± 0.125*** (12.57)
	48	11.23 ± 0.20	10.63 ± 0.020** (5.34)	9.36 ± 0.115*** (16.64)	8.66 ± 0.358*** (22.87)
	72	10.71 ± 0.251	10.05 ± 0.032** (16.15)	8.05 ± 0.015*** (24.81)	7.49 ± 0.658*** (30.04)
	96	8.52 ± 0.025	6.71 ± 0.050*** (21.23)	5.49 ± 0.060*** (35.54)	4.8 ± 0.020*** (43.63)
MCV (fL)	24	72.66 ± 1.608	73.04 ± 1.478 (-0.52)	66.07 ± 0.138** (9.02)	61.70 ± 0.579*** (15.01)
	48	74.38 ± 1.385	72.68 ± 1.311 (2.27)	64.90 ± 0.513*** (12.70)	60.85 ± 2.078*** (18.13)
	72	73.62 ± 3.34	70.17 ± 1.26 (4.65)	60.16 ± 1.93** (18.17)	60.49 ± 2.99** (17.72)
	96	63.48 ± 1.75	59.42 ± 1.35* (6.37)	59.93 ± 0.68* (5.57)	56.55 ± 2.80* (10.88)
MCH (pg)	24	24.67 ± 0.300	23.18 ± 0.174*** (6.03)	21.36 ± 0.326*** (13.40)	21.15 ± 0.286*** (14.25)
	48	24.09 ± 0.643	22.19 ± 0.464** (7.88)	19.61 ± 0.601*** (18.59)	14.42 ± 0.232*** (40.01)
	72	22.27 ± 0.53	21.17 ± 1.08 (4.93)	15.84 ± 0.56*** (28.87)	13.99 ± 0.38*** (37.17)
	96	18.79 ± 0.34	17.23 ± 0.31** (8.29)	16.25 ± 0.21*** (13.51)	15.68 ± 1.16*** (16.54)
MCHC (g/dl)	24	33.95 ± 0.36	31.74 ± 0.65** (6.49)	32.33 ± 0.44** (4.76)	34.28 ± 0.76 (-0.97)
	48	32.38 ± 0.42	30.53 ± 0.10*** (5.69)	30.21 ± 0.70** (6.68)	23.71 ± 0.232*** (26.70)
	72	30.27 ± 0.66	30.16 ± 0.99 (0.36)	26.35 ± 1.42** (12.93)	23.19 ± 1.72** (23.36)
	96	29.60 ± 0.29	28.99 ± 0.23* (2.05)	27.12 ± 0.11*** (8.35)	27.70 ± 0.67** (6.40)

Bracket value indicates percent variations, $P < 0.05 = *$, $P < 0.01 = **$, $P < 0.001 = ***$

4. Discussion

Acute exposure to Sodium fluoride proved to be highly toxic to the freshwater fish, *Rita rita* from Bhima river. It has induced various detrimental effects on the different physiological functions like hematological variations. The total R.B.C, W.B.C, Hemoglobin, Hemocrite, MCV, MCH, MCHC content were found to be significantly decreased after exposing to sodium fluoride from different intervals as well as from different groups in freshwater edible fish, *Rita rita*. The severity of sodium fluoride was found to be more pronounced after 72 and 96 hours of the treatments. Since the hematological indices are very important parameters which will reflect the internal physiological status under toxic stress. This may be true for our present investigation. There is an overall decrease from erythrocyte count during experimentation initial stages, the fish tends to acclimatize for maintaining a steady state however after 48 hours of exposure to the sodium fluoride the *Rita rita* might have not adjusted to the toxic stress resulted in the decline of erythrocyte count.

Hemoglobin content is an important parameter involved in transporting respiratory gases. The aquatic animals generally maintained a normal state of hemoglobin concentration. In the present investigation due to toxic impact of sodium fluoride resulted decline of the hemoglobin content after all exposure duration (Singh and Singh, 1982) while studying the hematological parameter of *Mystus vittatus* due to copper and zinc sulphate impact observed variations in R.B.C content. Few workers have also noticed diminished contents of total R.B.C, total W.B.C count, hemoglobin percentage, PCV, MCH, MCHC and MCV from the freshwater fishes like *Channa punctatus*, *Catla catla*, *Heteropneustes fossilis* after exposing them to cadmium, nickel, and zinc respectively. They have stated that the decrease in major hematological parameters might be due to anemia, erythropenia and leucopoiesis (Vincent and Ambrose, 1996; Sen *et al.*, 1992; Prashant Nanda and Milan Kumar, 1996). This may be true for the present investigation, since the major hematological parameters after exposing to different experimental durations and also from different experimental groups (LC₀, LC₁₀ and LC₅₀) showed diminishing trends. Salarkumari and Ramakrishna Rao (2004) have stated that chronic induction of fluoride through drinking water showed significant decrease in Hb, R.B.C, PCV, MCV, MCH and indicated the existence of hypochromic microcytic anemia.

Bindu and Geeta (2009)^[3] while studying acute toxicity and hemopoietic alterations induced by endosulfan from the freshwater fish, *Anabus testudineus* stated that major hematological parameters showed that major parameters revealed decreasing trends. They have also observed the decrease of hemocrite and stated that stress induction of endosulfan is responsible for alterations. In the present study the MCV, MCH and MCHC and hemocrite contents decreased up to 96 hours to sodium fluoride stress on *Rita rita*. Earlier investigators have suggested that the diminishing levels of MCH value might be due to reduction in the hemoglobin content (Wepner *et al.*, 1992; Verma and Panigrahi, 1998)^[22, 20].

5. Conclusion

From the above results it can be concluded that the sodium fluoride is found to be toxic to the edible fish, *Rita rita* and it has induced variations from various hematological parameters.

It can also be stated that the aquatic fauna has to be protected from contamination through media or by leaching of toxic chemicals into the aquatic animals. However, further investigations are recommended to understand histochemical variations due to toxic stress of sodium fluoride to the freshwater fish, *Rita rita* for the final confirmations.

6. References

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