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Environmental Changes of Nitrite Toxicity in Blood Indices of Hematocrit (HCT) and Mean Cell Volume (MCV) to Freshwater Fish *Cirrrhinus mrigala*

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Abstract: ***This study represents the data on changes of nitrite in the hematocrit value of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite was presented in the Table 13 and Fig.12. At the end of 7th day, hematocrit value was increased (+4.58%) when compared to that of the control group. In the rest of the study period (14 to 35th days), hematocrit value was declined in nitrite exposed fish showing a percent decrease of 8.79, 8.33, 12.53 and 13.01 at the end of 14th , 21st , 28th and 35th days, respectively. Table 14 and Fig. 13 furnish the data on changes in the MCV value of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days. During the above treatment period, MCV value was found to slightly increased at the end of 7th day showing a percent increase of 2.653. However after 7th day, MCV value was found to be decreased showing a percent decrease of -3.71, -9.35, and -7.28 at the end of 14th, 21st and 28th days. In contrast to the above decrease, a marginal percent increase of 0.38 was noted at the end of 35th day.***

Keywords: Nitrite, Cirrhinus mrigala, HCT, MCV

1. Introduction

Nitrite an important toxicant to fresh water fish present in the environment is relatively low concentration. This anion is an intermediate product in bacterial nitrification processes in ecosystems. Under normal conditions, the ammonia is transformed by bacterial oxidation to nitrite and then to nitrate. However, an imbalance of this oxidation system at high rate of ammonia production can result into disruption of the nitrification process and incremental of environmental nitrite [1]. Thus resulting in a toxicity episode caused by anion, which can be of special importance in habitats receiving nitrogenous materials, in hypoxic environments, and in intensive aquaculture systems with unbalanced water filtering [2,3,4,5]. Studies on fish revealed that nitrite induced a large variety of physiological disturbances many of which contribute to toxicity [6].

Fish peripheral blood analysis is used to assess its physiological state and effect of hazardous substances, to determine the nonspecific resistance. Although fish blood parameters have been increasingly determined in environmental monitoring programs as valuable indicators of physiological changes in the presence of toxicants, the most important barrier to using these findings in environmental studies is the lack of basic information about the blood response to stressors mainly from tropical species[7]. Alteration in the hematological parameters such as hematocrit and mean cell volume in blood corpuscles counts in fish can be stimulated by incidental factors such as capture and sampling as well as chronic factors such as exposure to disease and environmental contaminants pesticides and salts.

The hematocrit (HCT) of blood is the ratio of the volume of erythrocytes to that of the whole blood. Significant increase in HCT values was noted in *Oncorhynchus mykiss* exposed to aluminium[8], and increase in HCT values was reported in different fishes exposed to metals like chromium [9], zinc[10], and cadmium[11], in *Oreochromis niloticus* exposed to copper[12], in *Cyprinus carpio* exposed to cadmium[11], in *Hoplias malabaricus* exposed to methyl mercury[13], in *Clarias gariepinus* exposed to organic selenium [14],and in *Tilapia zilli* exposed to aluminium [15]. Significant decrease in HCT values was also reported in different fish exposed to metals like chromium[9], zinc[10], and cadmium[11]. However many authors have reported a decrease in HCT by cadmium exposure in *Salmo gairdneri*[16],*Tilapia zilli*[17], in *Channa punctatus*[18],copper exposed fish, *Oreochromis niloticus*) and in *Oncorhynchus mykiss*[19]. On the other hand, HCT showed no significant difference in its value in long term methyl mercury exposure [20],in *Oncorhynchus mykiss*. In this study hematocrit values increased and decreased during nitrite exposure in fish *Cirrhinus mrigala*.

[21], reported significant increase in MCV (Mean cell Volume) in lead and nitrate exposed fish *Clarius batrachus*. Similar increase in MCV was reported in *Oncorhynchus mykiss* exposed to copper[19], in *Hoplias malabaricus* exposed to mercury[13], and in *Clarias gariepinus* exposed to zinc[22], increase in MCV in fish exposed to mercury by[23]. Increase in MCV reported in fish *Cirrhinus mrigala* exposed to nitrite by[24]. However significant decrease in MCV was noticed in *Tilapia zilli* exposed to cadmium by[17], in *Clarias gariepinus* exposed to copper[25], and in *Tilapia zilli* exposed to aluminium [15].In this study MCV values increased and decreased during nitrite exposure in fish *Cirrhinus mrigala*.

2. Materials and Methods

Hematocrit

Hematocrit was estimated by microhematocrit (capillary) method as described by Nelson and Morris (1989) using RM 12C micro centrifuge and a microhematocrit reader.

Mean Cell Volume (MCV)

The MCV is the average volume of red cells and is calculated from the hematocrit (HCT) and red cell count (RBC).

$$\text{MCV (cubic micro)} = \frac{\text{HCT(\%) X 100}}{\text{RBC (millions/cu.mm x10}^6\text{)}}$$

The Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 46, Tamil Nadu, India, has been registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experiments and the handling of the organisms were carried out as per the guidelines of CPCSEA. (Dr. M. Ramesh, Professor,Bharathiar University, Coimbatore 46, Tamil Nadu, India.

3. Results

The data on changes of nitrite in the hematocrit value of fish *Cirrhinus mrigala* exposed to sublethal concentration of nitrite was presented in the Table 13 and Fig.12. At the end of 7th day, hematocrit value was increased (+4.58%) when compared to that of the control group. In the rest of the study period (14 to 35th days), hematocrit value was declined in nitrite exposed fish showing a percent decrease of 8.79, 8.33, 12.53 and 13.01 at the end of 14th , 21st , 28th and 35th days, respectively. There were significant (P< 0.05) variation among the treatments ($F_{1, 40}$ = 438.34; P< 0.05), periods ($F_{4, 40}$ = 36.34; P< 0.05) and their interactions ($F_{4, 40}$ =71.75; P< 0.05).Table 14 and Fig. 13 furnish the data on changes in the MCV value of fish *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. During the above treatment period, MCV value was found to slightly increased at the end of 7th day showing a percent increase of 2.653. However after 7th day, MCV value was found to be decreased showing a percent decrease of -3.71, -9.35, and -7.28 at the end of 14th, 21st and 28th days, respectively. In contrast to the above decrease, a marginal percent increase of 0.38 was noted at the end of 35th day. There were significant (P<0.05) variation among the treatments ($F_{1, 40}$ = 25.91; P< 0.05), periods ($F_{4, 40}$ = 43.80; P< 0.05), and their interactions ($F_{4, 40}$ = 28.99; P< 0.05).

Table 13: Changes in Hematocrit (HCT) values of Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days

S. No	Exposure period	Hematocrit %		
		Control	experiment	percentage
7	7	39.30±2.990	41.10±1.777	+4.58
14	14	43.20 ±2.119	39.40 ± 2.990	-8.79
21	21	42.00± 1.974	38.50 ± 2.939	-8.33
28	28	41.60 ±1.648	36.30 ± 2.633	-12.53
35	35	41.50 ±1.648	36.10 ± 2.410	-13.01

Values are mean ± of five individual observation. (+) Denotes increase over control. (-) Denotes decrease over control. **Significant at 5% level. Means in a column bearing same letter are significantly different according to DMRT (P> 0.05).

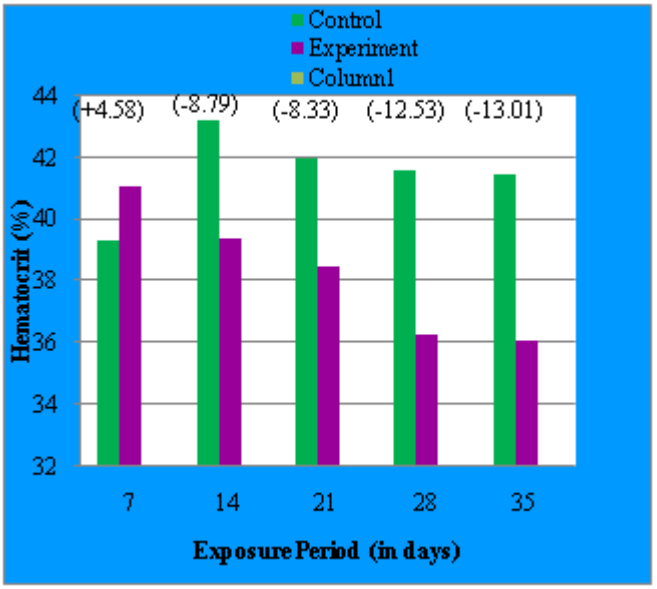

Figure 12

Table 14: Changes in (MCV) values of Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days

S.No	*Exposure period*	*MCV(fl)*		
		Control	*experiment*	*percentage*
7	7	62.55 ± 3.110	64.21 ± 3.094	+2.653
14	14	63.03 ± 4.762	60.69 ± 5.528	-3.712
21	21	64.23 ± 3.392	58.22 ± 3.311	-9.35
28	28	63.14 ± 3.368	58.84 ± 2.527	-7.28
35	35	65.38 ± 2.440	65.63 ± 2.664	+0.382

Values are mean ± of five individual observation. (+) Denotes increase over control. (-) Denotes decrease over control. **Significant at 5% level. Means in a column bearing same letter are significantly different according to DMRT (P> 0.05).

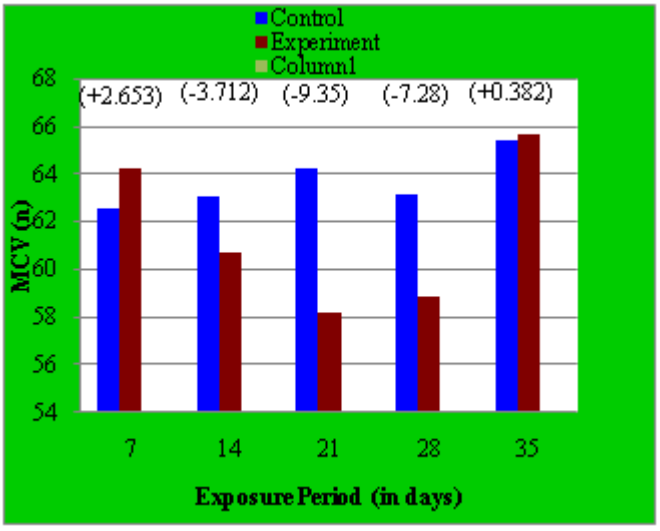

Figure 13

4. Discussion

Nitrite toxicity to fish, as a result of high environmental levels, is due to various processes including the formation of methaemoglobin which are a non-functional haemoglobin form and the cytolysis of hepatocytes and gill chloride cells[26]. Fish can be especially sensitive to nitrite because of its competition with chloride to bind sites in chloride cells[2]. Nitrite can actually be found in high concentrations in plasma of fish exposed to high nitrite levels [27]. The presence of small amount of nitrite in water seems to be highly toxic to fish[4]. Nitrite concentrations in blood plasma may be more than 60 times higher than the concentrations in the surrounding medium[28]. Neverthless, the blood appears to be the primary target of nitrite action. From the blood plasma, nitrite diffuses into red blood cells, where it oxidizes iron in haemoglobin (Hb) to the 3 oxidation state.

Blood of fish may be one of the indicators for diagnosis of fish diseases. Thus hematological investigations are very essential in monitoring the fish health. The most prominent effects to nitrite increases in methemoglobin content and the plasma nitrite concentrations[29]. Methemoglobin formation is supported to result in tissue hypoxia which causes significant stress [26, 10]. This could be caused by increases in red cell counts and the content of hemoglobin to maintain the system delivery[30]. The reduction of methemoglobin can be mediated by the NADH-Methemoglobin reductase system. The enzyme is presumed to be present in several species, including fish [31],because basal levels of methemoglobin were reached whenever, the fish were allowed to recover nitrite in free water.

The increase of MCV observed in individuals of *H. malabaricus* exposed to Hg may be explained by the presence of a larger amount of older or larger red blood cells as described by[32]. In the present study the significant increase in MCV during acute and sublethal treatment indicate swelling of red blood cells due to hypoxia or impaired of water balance, lysis of the red blood cells spend in circulation is reduced. Swelling of RBC's due to hypoxic condition in the toxicant treated organisms may lead to a significant increase in MCV value. The increase in MCV may also result from an increase of immature RBC[33]. In the present investigation the significant decrease of MCV during acute and sublethal treatment might be due to high percentage of immature red blood cells in the circulation. An increased MCV during stress were due to the swelling of red blood cells or the release of large red blood cells into the circulation. The increase and decrease in MCV with all concentrations of NP can be attributed to direct of feedback responses of structural damage to RBC membranes, resulting in hemolysis and impairment in hemoglobin synthesis, and stress related release of RBCs form the spleen.

In the present investigation fish Cirrhinus mrigala exposed to nitrite concentration were it disrupts in the blood of fish. There occurs changes in the hematological parameters especially the changes in hematocrit The increase in hematocrit (HCT) may be due to increase in erythropoises which leads to blood lysis. The passage of nitrite into blood stream causes lysis which means shrinkage of RBC. The decrease might be due to asphyxia leads to an increase in hemoglobin, RBC and other constinuents of the blood. Disrupted hematological patterns appear very quickly and precede changes in fish behavioural and visible lesions. Evaluation of toxic of the salts is facilitated by results of the toxicological variables have been used more to determine the sublethal concentration of pollutants. The hematological profiles have been used as stress inhabitors.

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