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Erythrocyte Indices of Nitrite Toxicity in Mean Cell Hemoglobin (MCH) and Mean Cell Hemoglobin Concentration (MCHC) to Freshwater Fish *Cirrhinus mrigala*

Yesudass Thangam

Assistant Professor, J. K. K. Nattraja college of Arts and Science, Kumarapalayam, Namakkal (Dt). Tamilnadu, India.

Abstract: ***This study reveals the changes in nitrite toxicity in the MCH value of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days (7, 14, 21, 28, 35 days) was presented in the Table 17 and Fig. 16. At the end of 7th day the MCH value was found to be slightly increased (+0.46) when compared to control groups. In rest of the study period (14th to 28th days), the MCH value was found to be decreased showing a percent decrease of -7.66, -10.04, -4.23 and at the end of 35th day MCH value found to be increased showing a percent increase of 4.09 respectively. The data on changes in the MCHC value of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days was presented in Table 18 and Fig. 17. During the above treatment period, a biphasic trend in MCHC value was observed in nitrite treated fish. At the end of 7th and 21st day, MCHC value was found to be decreased showing a percent decrease of -1.02 and -0.56, respectively. On day, 14th, 28th and 35th day a slight increase in MCHC value was observed when compared to control groups.***

Keywords: Nitrite, Fish Cirrhinus mrigala, MCH, MCHC

1. Introduction

Nitrite is generally more toxic to fresh water organisms (Jensen, 1995, 2003). Consequently, the mechanisms of No_2^- toxicity have been studied more intensively in freshwater fish than in marine animals. In freshwater, nitrite is actively taken up across the gill in competition with chloride (Eddy and Williams, 1987). The principal effect of nitrite loading is a progressive oxidation of hemoglobin to methemoglobin, but several other physiological changes occur (Jensen, 2003). Nitrite induced shortage of O_2 results in high stress levels leading to hyperventilation, elevated heart rate and increased blood pressure in fish (Williams and Eddy, 1987, Jensen, 2003). High levels of nitrite in water is a potential factor triggering stress and may even cause high mortality in aquatic organisms (Lewis and Morris, 1986; Martinez and Souza, 2002; Siikavuopio and Saether, 2006). Fish have been used extensively for monitoring purposes because they concentrate pollutants in their tissues, which are directly absorbed from water and also through their diet, reflecting the level of pollution in the aquatic environment (Cazenave *et al*., 2005). Fish has been proposed as a good animal model for disclosing the physiology that underlies the toxicology of nitrite (Jensen, 2003).

Fish *Cirrhinus mrigala* exposed to nitrite toxicant in the aquatic environment. Mrigal, being a bottom feeder, and is more prone to the stress effect of toxic nitrogenous metabolites as ammonia and nitrite. Number of hematological parameters such as hematocrit (HCT), hemoglobin (Hb), Red blood cells (RBCs), White blood cells (WBCs) and so on are used to assess the functional status of oxygen carrying capacity of the blood streams and have been used as indicator of pollution in aquatic environment (Nussey *et al*., 1995; Maheswaran *et al*., 2008) and also indicate secondary responses of an organism to irritants (Ololade and Ogini; 2009). The calculated blood indices, such as MCH and MCHC have a particular importance in anemia diagnosis in most animals. The analysis of blood indices has proven to be a valuable approach for analyzing the health status and these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Zhou *et al*., 2009). The main toxic action of nitrite on aquatic animals is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimate death (Camargo and Alonso, 2006).

Significant increase in mean cellular hemoglobin (MCH) was noted in lead exposed fish *Clarias batrachus* (Nath and Banerjee, 1995), nickel exposed fish *Silurus glanis* (Sobecka, 2001), and in *Puntius conchonius* exposed to cadmium (Ololade and Ogini, 2009). However, significant decrease in MCH was noted in *Tilapia zilli* exposed to cadmium (Ghazaly, 1992) and in *Clarias gariepinus* exposed to copper (Olaifa *et al*., (2004). Likewise, significant increase in MCHC value was noticed in *Oreochromis mossambicus* exposed to copper (Nussey *et al*., 1995), in *Cyprinus carpio* exposed to cadmium (Drastichova *et al*., 2004) and in *Tilapia zillii* exposed to aluminium (Alwan *et al*., 2009). Whereas, MCHC value was decreased in fish in *Clarius batrachus* exposed to lead (Nath and Banerjee, 1995) and *Clarias gariepinus* treated with copper (Olaifa *et al*., 2004).. Significant decrease in MCH and MCHC was reported in fish exposed to nitrite (Das *et al*., 2004). Teleost *Matrinxa* (*Brycon cephalus*) when exposed to environmental nitrite showed a decrease content of MCH and MCHC (Avilez, et al., 2004). The effects of nitrite toxicity exposure to Indian major carps particularly on Cirrhinus mrigala on MCH and MCHC are very scanty. In the present investigation both increase and decrease of MCH and MCHC were noted during nitrite exposure.

2. Materials and Methods

Mean Cell Haemoglobin (MCH)

The MCH is the content (weight) of hemoglobin of the average red cell. It is calculated from the hemoglobin concentration and the red cell count.

$$\text{MCH (pictograms)} = \frac{\text{Hb (g/dl) x 10}}{\text{RBC (millions/cu.mm) x } 10^6}$$

Mean Cell Haemoglobin Concentration (MCHC)

The MCHC is the average concentration of hemoglobin in a given volume of packed red cells. It is calculated from the hemoglobin concentration and the hematocrit.

$$\text{MCHC(g/dl)} = \frac{\text{Hb(g/dl)}}{\text{HCT(\%)}} \text{ X } 100$$

3. Result

The data was presented in the Table 17 and Fig. 16. At the end of 7th day the MCH value was found to be slightly increased (+0.46) when compared to control groups. In rest of the study period (14th to 28th days), the MCH value was found to be decreased showing a percent decrease of -7.66, -10.04, -4.23 and at the end of 35th day MCH value were found to be increased showing a percent increase of 4.09 respectively. There was significant (P<0.05) variation among the treatments ($F_{1, 40}$ = 21208.33; P< 0.05), periods ($F_{4, 40}$ = 5501.69; P< 0.05), and their interactions ($F_{4, 40}$ = 4266.93; P< 0.05). The data on changes in the MCHC value of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days was presented in Table 18 and Fig. 17. During the above treatment period, a biphasic trend in MCHC value was observed in nitrite treated fish. At the end of 7 and 21st day, MCHC value was found to be decreased showing a percent decrease of -1.02 and -0.56, respectively. On 14th, 28th and 35th day a slight increase in MCHC value was observed when compared to control groups. There were significant (P<0.05) variation among the treatments ($F_{1, 40}$ = NS; P< 0.05), periods ($F_{4, 40}$ = NS; P< 0.05), and their interactions ($F_{4, 40}$ = NS; P< 0.05).

Table 17. Changes in the MCH value of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days

Exposure period (in days)	MCH (picograms)		
	Control	Experiment	Percent change
7	238.32 ± 2.782 d	254.36 ± 3.459 d	+0.46
14	244.80 ± 2.885 c	251.50 ± 3.171e	+7.66
21	260.54 ± 8.514 b	298.17 ± 4.016 b	+10.04
28	265.15 ± 3.525 a	339.30 ± 1.316 a	+4.23
35	145.30 ± 3.484 e	292.12 ± 3.225 c	+4.09
Treatment (T)	21208.33**		
Period (P)	5501.69**		
TXP	4266.93**		

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

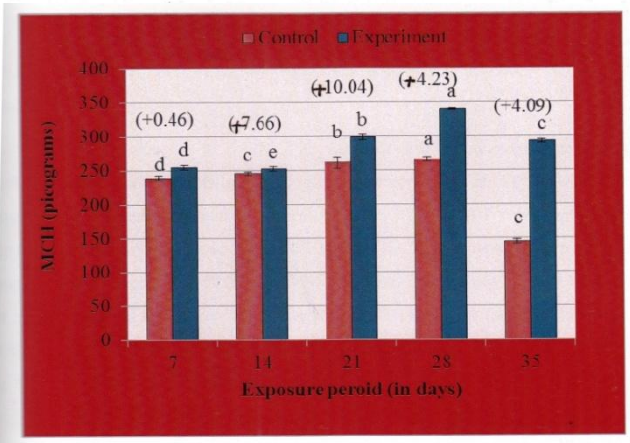

Fig. 16.

Fig. 16. MCH content of *Cirrhinus mrigala* exposed to nitrite sublethal concentration for 35 days. Error bars indicate the standard error of the mean. Bars bearing same letter are significantly different according to DMRT ($P > 0.05$). The numericals in the parenthesis indicates percent change.

Table 18. Changes in the MCHC value of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days

Exposure period (in days)	MCHC (g/dl)		
	Control	Experiment	Percent change
7	34.38 ± 2.248 a	34.03 ± 2.668 a	-1.02
14	32.75 ± 3.559 a	33.95 ±2.953 b	+3.66
21	34.11 ± 3.315 a	33.92 ± 4.051 a	-0.56
28	33.73 ± 2.939 a	34.95 ±1.286 a	+3.62
35	33.73 ± 2.998 a	34.95 ± 2.203 a	+3.61
Treatment (T)	< 1-NS		
Period (P)	1.48-NS		
TXP	< 1-NS		

Values are mean ± S.E. of five individual observations. (+) Denotes percent increase over control. (-) Denotes percent decrease over control. NS –Non significant. Means in a column bearing same letter are significantly different according to DMRT (P > 0.05).

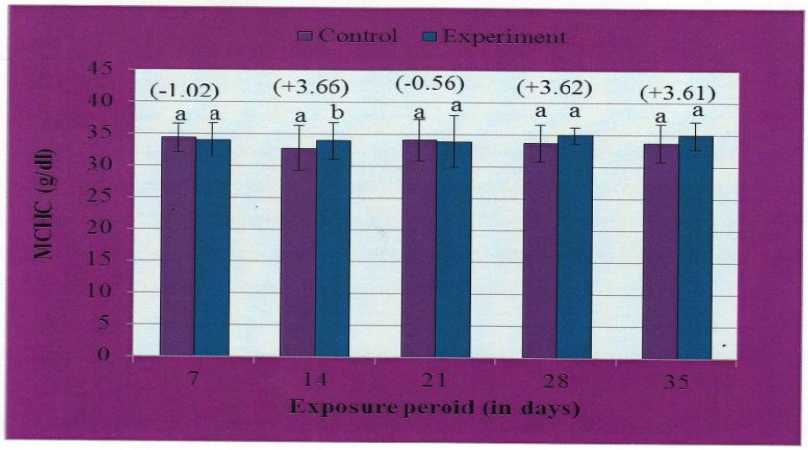

Fig. 17.

Fig. 17. MCHC content of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. Error bars indicate the standard error of the mean. Bars bearing same letter are significantly different according to DMRT ($P > 0.05$). The numericals in the parenthesis indicates percent change.

4. Discussion

Nitrite is an important toxicant to freshwater fish even when it is present in the environment in a relatively low concentration. Nitrites are actively transported into the bloodstream through the gills in freshwater fish by means of chloride cells, which posses a mechanism to actively transport chloride towards the internal medium. Nitrite induces a large variety of physiological disturbances in fish and the toxicity may result from a combination of effects, nevertheless, the most studied is the formation of methemoglobin (metHb) (Tomasso, 1994; Gisbert *et al*., 2004) but several other physiological changes may occur. Nitrite binds competitively to hemoglobin oxidizing it to metHb, a variant causing the blood to appear brown in colour (hence the name "brown blood disease") and vastly reduce the ability to bind and transport oxygen (Jensen, 2003).

In the present investigation the significant decrease and increase in MCH, MCHC in hemoglobin content in *Clarias gariepinus* exposed to effluent may be due to an indication of red blood cell swelling and deleterious oxygen transport and degeneration of the erythrocytes (Adeyemo,2005). Exposure of *Oreochromis mossambicus* to titanium dioxide resulted decrease in MCH, MCHC, may be due to low pH and impaired oxygen uptake and delivery reflecting diffusion limitations at the gill due to mucus accumulation and elevation in haematocrit is associated to an increase in erythropoiesis in response to tissue hypoxia. The high

percentage of immature red blood cells in the circulation might be the reason for MCH decrease in the present investigation. Moreover, increase in the erythrocyte volume during stress condition may be another possible reason. In the present study the decrease in MCH and MCHC during sublethal treatment may be due to swelling of red blood cells and blood dyscrasia.

The significant increase of MCHC value during acute treatment might be resulted from spherocytosis as suggested by Sobecka (2001). However the low concentration of MCHC during sublethal treatment might have resulted from decrease in Hb synthesis due to toxic action (Nussey *et al*., 1995). The increase in MCHC during acute treatment may be due to blood cell lyses (Avilez, *et al*., 2004). The decrease in MCHC value may be due to immature erythrocytes from the erythropoietic tissue and destroyed by the spleen and kidney. The low concentration of MCHC during acute treatment might have resulted from decrease in Hb synthesis due to toxic action of lindane or swelling of erythrocytes by which blood O_2 transport capacity is increased when fish were subjected to less effective gas exchange (Saravanan *et al*., 2011). In the present study increase and decrease in MCHC during acute and sublethal treatment might be due to haemolysis and immature erythrocytes from erythropoietic tissues.

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