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Effect of Nitrite Toxicity in Hormones to Freshwater fish, Cirrhinus mrigala

Yesudass Thangam, Mathan Ramesh

Asst. Professor

Department of Zoology

JKKN College of Arts & Science, Komarapalayam, Namakkal (Dt),

Associate Professor, Unit of Toxicology,

Department of Zoology

Bharathiyar University, Coimbatore

Abstract: The present study was to evaluate nitrite toxicity in fish hormone T_4 and T_3 during long term exposure of sublethal concentration of sodium nitrite ($NaNo_2$). The effect of nitrite on thyroid hormones, Thyroxine(T_4) and Triiodothyronine(T_3), were evaluated from 7,14, 21, 28 and 35 days in Indian freshwater fish, Cirrhinus mrigala to a sublethal concentration of nitrite (28.31)ppm for different periods. Exposure of fish to nitrite showed a significant decrease in Thyroxine(T_4) hormone. Plasma T_4 level was found to be decreased at the end of the 7^{th} day showing a percent decrease of 14.38. After 7^{th} day, significant increase in Plasma T_4 observed showing a percent increase of 14.56, 47.02, 52.27 and 64.28 at the end of 14^{th},21^{st}, 28^{th}, 35^{th} days. Where as plasma triiodothyronine(T_3) level was increased throughout the exposure period showing percent increase of 20.00, 25.00, 33.33, 47.05 and 75.00 at the end of 7, 14, 21, 28 and 35 days.

Key words: Cirrhinus mrigala, Sodium nitrite, Thyroid hormones.

Introduction

Nitrite an intermediate compound of nitrification process, does not, predominate among other nitrogenous substances in natural waters, (Kroupova et al., 2008). Aquatic animals in general, adapted to relatively low levels of inorganic nitrogen since natural ecosystems often are not N Saturated and natural concentrations of inorganic nitrogenous compounds usually are not elevated (Wetzel, 2001; camargo et al., 2005a). Therefore high level of nitrite derived from human activities can impair the ability of aquatic animals to survive, grow and reproduce, resulting in direct (acute or chronic toxicity) of nitrogen compound, (Eddy and Williams, 1987; Philips et al., 2002). Contamination of natural water's from anthropogenic sources of nitrogen is a wide spread problem. In the aquatic environment, the most common ionic(reactive) forms of inorganic, nitrogen are ammonium(NH_4), Nitrite (NO_2) and Nitrate (NO_3), (Camargo et al., 2005). These ions present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and ground water runoff, dissolution of nitrogen rich geological deposits.

High levels of nitrite in water is a potential factor triggering stress and cause high mortality in aquatic organisms (Lewis and Morris, 1986; Martinex and souza, 2002; Siikavuopio and Saether, 2006). Nitrite formed due to bacterial nitrification and denitrification processes of ammonia or by the addition of fertilizer to the water (Lewis and morris, 1986). Nitritie from such conditions causes many physiological problems in

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fish (Daene and Woo, 2007). Environmental increase in nitrite impairs the function of several aquatic species. Aquatic animals are more toxic towards nitrite in toxication because nitrite in the ambient water, taken up across gill epithelium and accumulated to very high concentrations in the fish body fluids. Nitrite induced shortage of O_2 results in high stress levels leading to hyperventilation, elevated heartrate, and increased blood pressure in fish (Williams eddy,1986, Jensen 2003). Nitrite found in industrial effluent as a by product of corrosion inhibitors in boiler systems, as a curing agent for meat, and in production of explosives. It also used extensively in agriculture for crop fertilization. Water pollution and industrial waste usually elevate the nitrite concentration due to ammonia oxidation. This nitrification process depends on the bacterial activity and oxygen level. Research on the effect of nitrite in fish has focused mainly on acute and sublethal toxicity of hormones and hence its effects on fish health are discussed. (Lewis and Morris, 1986; Jensen, 2003; Handy and Poxton,1993; Das et al., 2004). During acute and sublethal exposure nitrite concentration leads to several physiological changes like disruption of ion regulatory, cardiovascular, and excretory processes, lead to death of fish (Jensen, 2003).

Thyroid hormones (THs) plays an important role in the growth, differentiation, development and metabolism of vertebrates (Morgado et al., 2007). In fish (THs) are implicated in reproduction and appear to be important in the regulation of development (Higgs et al., 1982; Brown,1997). High concentration of these hormones are present in fish eggs in increased levels are reported during metamorphosis and larval transition (Power et al., 2001). Larsson et al., (1985) pointed out that almost all (THs) circulating in the plasma are bound to transporter proteins and only free hormones enter cells to elicit response (Ishihara et al., 2003). Natural variation in thyroid status of fish has been demonstrated in response to development state/age (Suchiang,2001), Water temperature (Johnston and Eales 1995) and nutritional status (Eales and Brown,1993) in many cases, the situations shows an increase in thyroid activity.

According to Deane and Woo (2007) nitrite exposure in a teleost fish shows a decrease in T_4 by 43% and 68% in fish that were exposed to 25 and 50 mg/l nitrite, Where T_3 remain unchanged. Decrease in T_3 and T_4 in fresh water fish when exposed to nitrite (Jensen 2003), a decrease in T_4 in silver bream exposed to nitrite (Brown et al., 2004; Vander ven et al., 2006. Aluminium exposure showed increase in hormone in trout (Brown and sadler, 1989). Cadmium known to increase the hormone level in trout (Olsson et al., 1995). Heavy metals like mercury, copper and cadmium showed an increase in hormone levels in Trout (Marc et al., 1995). Atlantic salmon (Salmon solar) induced to acid and limed river waters the T_4 hormone is increased and T_3 triiodothyronine is gradually decreased (Brown et al., 1993) Chemical pollutants have been reported to detrimentally affect thyroidal hormone status in number of fish species (Brown et al., 2004; Vander Ven et al; 2006), but the effect of nitrite on this group of hormones in fish, has yet to be reported. Considering the huge importance of thyroid hormones in fish physiology (reproduction, growth and metamorphosis, regulation), the present study aimed to evaluate the effects of nitrite on T_4 and T_3 levels of an Indian Major carps, Cirrhinus mrigala.

Materials and Methods

The hormonal (T_4 and T_3) of nitrite was estimated by using Enzymes linked immunoabsorbent assay (ELISA of hormones) using kits. The Changes in physico-chemical characteristics, such as temperature, pH, dissolved oxygen, alkalinity , hardness, salinity, calcium and magnesium of experimental water were recorded throughout the experimental period. Fresh water fish Cirrhinus mrigala, weighing 5.0-6.0 gm and measuring 7-8 cm were collected form Tamilnadu Fisheries Development corporation, Aliyar fish form, Aliyar, Tamilnadu, India. Fish of same age and size which hatched form the same lot of eggs

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were collected, the age of the fish being 2 to 3 months old. They were safely brought to the laboratory in well packed polythene bags containing aerated water and stocked in a large cement tanks (36' x18'x19'). Fish were acclimatized for about 20 days before the commencement of the experiment. During acclimatization period, fish were fed with ad libitum, with rice bran and ground nut oil cake in the form of dough once in daily. Water replaced every 24h after feeding in order to maintain a healthy environment for the fish. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic waste. The feeding was withheld for 24h before the commencement of the experiment and to keep the specimens in the same metabolic state. The fish were introduced into glass aquarium (26'x18'x18.5') cm which was washed thoroughly and maintained in the laboratory. Separate circular plastic tubs of 50 litres of water capacity were taken and different concentrations of nitrite were added. 10 healthy fishes were introduced into each tub. A control tub (no toxicant) with 50 litres of water and 10 fishes were also maintained. Three replicates were maintained for each concentration groups. The mortality/ survival of fish in control and nitrite treated tubs was recorded after 24h and the concentration at which 50% mortality of fish occurred was taken as the median lethal concentration (Lc50) for 24h which was 28.31 ppm. A similar experimental set up was also maintained to determine the median lethal concentration of sodium nitrite to fish Cirrhinus mrigala for 96h. The test water was renewed at the end of 24h and freshly prepared solution was added to maintain the concentration of sodium nitrite at a constant level. The median lethal concentration (Lc50) of sodium nitrite for 96h was found to be 19.952 ppm. The median lethal concentration of nitrite was calculated by Probit analysis method (Finney,1978). The sublethal toxicity was conducted at $1/10^{th}$ of Lc50 of 24h value (2.832) ppm.

The data observed in the experiment were statistically analysed for the calculation of standard error of mean (SEM). One way ANOVA and Duncan multiple range test for individual group with comparison was administered for testing the hypothesis. The data shown the average two replicates $\pm$ SE and statistical significance was tested at $P < 0.05$ level.

Results and Discussion

In the present study, exposure of fish to sublethal concentration of nitrite for 7, 14, 21, 28 and 35^{th} days caused significant alterations in hormonal parameters of Indian freshwater fish, Cirrhinus mrigala. The alternation observed in various physico-chemical parameters, such as temperature, pH, dissolved oxygen, hardness, alkalinity, salinity, calcium and magnesium of control and experimental systems are calculated. Table 2, and Fig 2, gives data on changes in Plasma thyroxine (T_4) level of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days. Plasma T_4 level was found to be decreased at the end of 7^{th} day when compared to control groups showing a percent decrease of 14.38. However after 7^{th} day a significant increase in plasma T_4 was observed showing a percent increase of 14.56, 47.02, 52.27 and 64.28 at the end of 14^{th}, 21^{st}, 28^{th} and 35^{th} days respectively. There were significant ($P<0.05$) variation among the treatments ($F_{1,}$ 40 = 9104.03; $P<0.05$), Periods (F_4,40=2580.75; $P<0.05$) and their interactions F_4,40=1680.84; $P<0.05$).

Plasma triiodothyronine (T_3) of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite were presented in Table-3 and Fig-3. The Plasma triiodothyronine (T_3) level was increased as the exposure period extended showing percent increase of 20.00, 25.00, 33.33, 47.05 and 75.00 at the end of 7, 14, 21, 28 and 35 days respectively. There were significant($P<0.05$). Variation among the treatments($F_1$40=64.73; $P<0.05$), Periods ($F_{4,}$40=6.39;$P<0.05$) and their interactions ($F_{4,}$40=3.85;$P<0.05$).

Subchronic exposure to water borne Se increase plasma T_3 and T_4 levels in Juvenile rainbow trout, Oncorhynchus mykiss (Miller et al., 2007), Exposure to Se increased plasma cortisol

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and it has been documented that cortisol influences thyroid hormone metabolism(Brown et al., 1991). Moreover, Se is an intergral part of the deiodinase enzymes involved in thyroid hormone synthesis (Kohrle et al., 2005). When Eels were exposed for a longer period of time (7 days), Olivera et al., (2008) noted an unaltered T_4 in plasma and (T_3) decrease. A variety of mechanisms can result in a change of the thyroid status, such as alteration in the hypothalamus or pituitary status, biosynthesis and secretion step of T_3 and T_4 uptake by peripheral tissues or hormone catabolism and clearance rates (Hontela et al., 1995; Oliveira et al., 2008). (Gomez et al; 1997) suggested that the Plasma concentration of T_3 is not influenced (Short-term) by plasma concentration of T_4. Rather, thyroid stimulating hormone stimulates T_4 production, and T_3 concentration is regulated in peripheral tissues by T_3 producing and non degrading deiodinases (Vander Geyten et al., 2001). Since T_3 and T_4 are identical in all vertebrates, it might be that the peak of Plasma T_4 concentration can cause an initial augmentation in metabolism, inorder to handle the first stress response (Eyckmass et al., 2010). They also reported that the altered T_3 concentrations in plasma can be a product of a deprived transformation of T_4 into T_3.

Many toxic chemical such as perfluoroctane sulfonate (PFOS), Polychlorinated biphenyls (PCBS), and heavy metals, have the potential to affect thyroidal status (Bleau et al., Li et al., 2009). Results from mammalian toxicological studies suggest atleast four classical pathways of EDC action on the thyroid system including inhibition of thyroidal iodine uptake, inhibition of thyroid peroxides, displacement of TH from plasma transport proteins, and induction of hepatic T_4 glucuronidation leading to enhanced T_4 excretion. Exposure to $Hgcl_2$ or MeHg caused an increase in plasma T_4 and T_3 in Juvenile rainbow trout, suggesting that Hg activates the hypothalamus pituitary-thyroid (HPT) axis (Bleau et al., 1996). According to Anderson(1996) and khangarot and Rathore (1999) fish exposed to copper showed an increase in hormone level due to the suppression of chemi-luminescence and antibody production in the fish body.

It is known that nitrite can disrupt fish growth (Woo and chiu,1994) and therefore part of this process may be related to adverse effects on thyroidal hormone status. Nitrite which has direct toxic effects and indirect effects mediated by the stress hormones, confinement is a stressors that has only indirect, hormone mediated effects on the gill (Camargo et al., 2005). However only a few authors reported toxicity of nitrite on primary stress responses of fishes especially the hormones like triiodothyronine (T_3) and thyroxine (T_4) (Brown, 2005). Using in vitro and in vivo mammalian models it was demonstrated the nitrite exposure inhibited steroid hormone synthesis (Panesar and Chan, 2000). The inhibition of steroid hormone systhesis was proposed to occur via conversion of nitrite to nitric oxide that in turn inhibits rate-limiting enzymes of steriodogenesis (Delpunta et al., 1996). In the present study the significant increase in T_4 and T_3 level during acute and treatment might have resulted from activation of hypothalamus- pituitary –thyroid (HPT) axis by nitrite.

Thus it is concluded that the hormonal parameters are most sensitive parameters in monitoring the toxicity of nitrite especially at sublethal concentrations.

ISSN 2278-7763

Table: 2. Changes in the plasma thyroxine (T4) level of Cirrhinus Mrigata exposed to sublethal Concentration of nitrite for 35days.

Plasma Thyroxine (T_4) (ng/ml)

Exposure period (in days)	Control	Experiment	Percentage change
7	1.67±0.007e	1.46±0.004e	-14.38
14	1.58±0.007d	1.81±0.004d	+14.56
21	1.68±0.004c	2.47±00.04c	+41.02
28	1.76±0.004b	2.86±0.004b	+52.27
35	1.82±0.007a	2.99±0.004a	+64.28

Treatment (T) 9104.03**
Period (p) 2580.75***
TXP 1680.84**

Values are mean ± S.E of five individual observations. (+) denotes percentage increase over control. ** Significant at 5% levels. Ns= not significant. Means in a column bearing same letter are significantly different according to DMRT ($p > 0.05$).

Table: 3. Changes in the plasma triiodothyronine (T_3) level of Cirrhinus mrigala exposed to Sub lethal Concentration of nitrite for 35days.

Plasma Triiodothyronine (T_3) (ng/ml)

Exposure period (in days)	Control	Experiment	Percentage change
7	0.15±0.007a	0.18±0.007c	+20.00
14	0.16±0.001a	0.20±0.007c	+25.00
21	0.18±0.007a	0.24±0.008b	+33.33
28	0.17±0.004a	0.25±0.007ab	+47.05
35	0.16±0.007a	0.28±0.007a	+75.00

Treatment (T) 64.73**
Period (p) 6.39**
TXP 3.85**

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

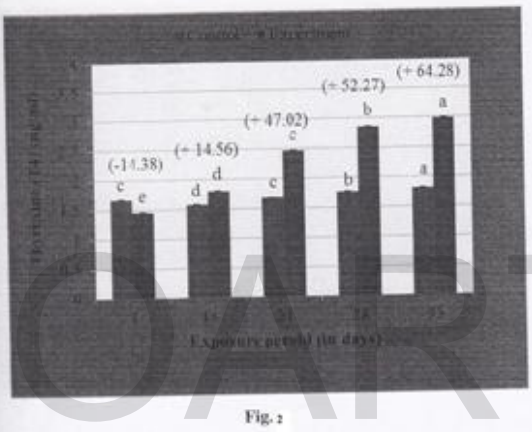

Fig. 2

Fig. 2 Plasma thyroxine (T4) level of *Cirrhinus mrigala* exposed to nitrite at 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.

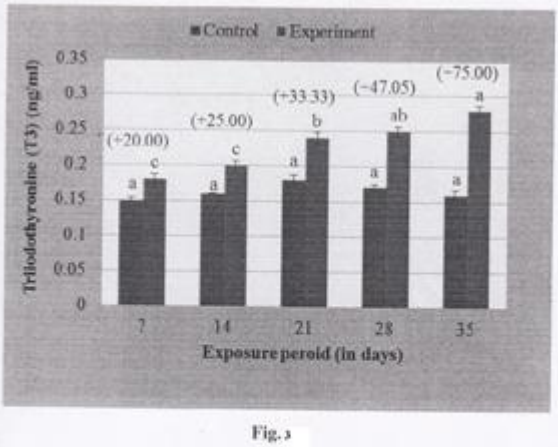

Fig. 3

Fig. 3 . Plasma triiodothyronine (T3) level of *Cirrhinus mrigala* exposed to nitrite at sublethal concentration for 35 days. Error bars indicate the standard error of the mean. Bars bearing same letter(s) are significantly different according to DMRT ($P > 0.05$). The numericals in the parenthesis indicates percent change.

ISSN 2278-7763

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