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Effect of different sublethal concentrations of Manganese on the levels of cortisol in *Garra gotyla gotyla*

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Abstract- The objective of this study was to evaluate the possible effects of manganese (Mn) exposure on variations of cortisol levels in *Garra gotyla gotyla*. LC_{50} value of manganese after 96 hours was found to be 3.2mg/l for *Garra gotyla gotyla.* Three replicates of 10 fishes were subjected to three sub-lethal concentrations of manganese i.e. 0.64mg/l, 1.28mg/l and 1.92mg/l (20%, 40% and 60% respectively of LC_{50}value of manganese) for 1, 5 & 9 weeks. Blood samples were isolated from the fish following the exposure to measure the levels of cortisol and compared to the control group. We observed significant increase($p<0.01$) in the levels of cortisol of three groups of fish after 1st week of exposure to three doses of manganese as well as significant decrease in the cortisol of three groups exposed for 5th & 9thweek. The rate of decrease in group III (exposed to1.92 mg/l of Mn) was higher than that of group I & II (exposed to 0.64 mg/l of Mn and 1.28 mg/l of Mn).

Index Terms- Manganese exposure, Cortisol, *Garra gotyla gotyla*

I. Introduction

The hormone cortisol in fish has been identified as a metabolic hormone (Vijayan et al., 1994) having multifaceted action. It is also considered as an important stress hormone produced in fish (Mommsen et al., 1999). Cortisol is the predominant corticosteroid in most of the teleost group (Handerson & Garland,1980).The secretion of cortisol is dependent on factors such as temperature (Strange, 1980; Davio et al., 1984; Barton & Schreck, 1980 & Barton & Zitzow, 1995), time of day (Davis et al., 1984 & Barton et al., 1986), wave length of light (Volpato & Barreto, 2001), background of color tanks (Gilham & Baker, 1985), nutritional state (Barton et al., 1988), presence of disease (Barton et al., 1986), season (Pickering & Pottingger, 1984), reproduction (Shankar & Kulkarni, 2007). Besides these factors heavy metals are also known to affect cortisol levels in fish (Richard et al., 1998).

The catabolic action of cortisol is responsible for the mobilization of energy reserves (gluconeogenesis) and lipolysis (Leach & Taylor, 1982; Sheridan, 1986). Cortisol has also been shown to play important roles in intermediary metabolism, growth, ionic and osmotic regulation, reproduction, immune functions (Wendelaar, 1997 & Mommsen et al., 1999). Undoubtedly, the growing evidence supporting the importance of cortisol in maintaining homeostasis, together with the ease of measuring cortisol and the magnitude of cortisol response to stress, make it convenient measure and the predominantly reported stress indicator.

The objective of present study is to evaluate the effect of different sub lethal concentrations of manganese on the levels of cortisol in *Garra gotyla gotyla*.

II. Material and Methods

➢ Animals and experimental conditions

Live specimens of *Garra gotyla gotyla* were collected with the help of cast net and were brought to the laboratory avoiding stress and injury as much as possible. Prior to onset of metal treatment, the fish were acclimatized to the laboratory conditions for approximately a fortnight. During this period fishes were fed with natural diet which included portion of aquatic plants, vegetables, debris and mud as the fishes were herbivores in nature. The 96 hrs LC_{50}value of manganese was determined as 3.2mg/l. Manganese stock solution were made from $MnSO_4$, added subsequently to dechlorinated tap water in the plastic tubs (capacity of 50L) to obtain test concentrations 0.64mg/l, 1.28mg/l and 1.92mg/l (20%, 40% and 60% respectively of LC_{50}value of manganese). The examined fishes were divided in to three groups exposed to three sub lethal concentrations of Mn 0.64mg/l, 1.28mg/l and 1.92mg/l (20%, 40% and 60% respectively of LC_{50} value of manganese). Control groups were the fishes maintained in normal, aerated tap water. The water in each plastic tub was replenished daily to keep the metal concentration unchanged. The experiment had three replicates and in each replicate 40L of water was added and 10 number of *Garra gotyla gotyla* was taken in each experimental tub.

➢ Measurement of the levels of cortisol:

Blood was taken from the heart of the fish with non heparinized syringe, collected in plastic Eppendrof tubes. After centrifugation, blood plasma was removed and the samples were then analyzed for measuring the levels of cortisol by Radioimmunoassay following the methodology adopted by Tort et al., 1998.

➢ Statistical analysis of data :

The data was statistically evaluated by analysis of varience and paired sample t test using SPSS 17 version.

III. Results and Discussion

Exposure of *Garra gotyla gotyla* to different sub lethal concentrations of manganese (Mn) 0.64mg/l, 1.28mg/l and 1.92mg/l (20%, 40% and 60% respectively of LC_{50}value of manganese) caused a significant variation in plasma cortisol level (Table 1). After first week in the manganese exposed (0.64mg/l,

1.28mg/l and 1.92mg/l sub lethal conc.) fish the cortisol levels were significantly elevated (175.2ng/ml in 20%group., 198.5ng/ml in 40% group and 230.0ng/ml in 60%group) compared to control (109.8ng/ml). On the 5th week, cortisol titer decreased to 156.0ng/ml in 20% group, 170.2 ng/ml in 40% group and 160ng/ml in 60% group. A further sharp decline was evident by 9th week in 60%group (125.0ng/ml) whereas, cortisol titer was 142.5mg/l and 156.5 mg/l respectively in 40% and 60%groups and at this point the cortisol levels were still not restored to normal values and significantly different from those observed in the control groups.

Exposure to manganese appears to elicit a transient stress response in *Garra gotyla gotyla*. Significantly elevated cortisol titers were evident on first week. Our results contrast with earlier reports on the relationship between cadmium and cortisol (Pratap & Wendelaar, 1990). Elevated cortisol was also observed in Sockeye salmon exposed to different concentrations of Cu for 1-24 hrs. (Donaldson & Dye, 1975), in rain bow trout exposed to different concentrations of chromium (Hill & Fromm, 1986), in rainbow trout exposed to different sublethal concentrations of cadmium (James & Wigham, 1986) and in adult *Onchorhynchus* exposed to sub lethal levels of cadmium (Richer et al., 1998).

Changes in cortisol levels under the effect of manganese in *Garra gotyla gotyla* might show that energy needed under the effect of metal was compensated through glucogenic pathway. Increase in cortisol level implies an increase in glucose level in fish blood. Similar viewpoint have been given by Karaytug et al., 2010 while studying the changes in cortisol level under the effect of cadmium in *Clarias gariepinus.*

Data from table clearly indicates that fish subjected to lower concentration of manganese (0.64mg/l, 1.28mg/l) showed a slight cortisol decrease, whereas, those treated with the higher metal value (1.92mg/L) revealed a higher decrease. This might be because the fish could provide an adaptation to the low Mn values, while that for the higher concentration seem to characterize exhaustion. During this phase some physiological changes have been adapted by fish, permitting homeostasis return. The hypothalamus-pituitary interregnal axis (HPI) is a system responsible for the adaptative potential and stimulation of this system leads to intensified or reduced secretion of numerous hormones (Donaldson et al., 1990). Exhaustion leads to down regulation of the system through negative feedback. This disables the fish to react appropriately to other eventual stressors. HPI axis involves atrophy of the interrenal tissue, which is a slow process that caused decreased cortisol levels (Flodmark et al., 2002). Lowered or absent of cortisol response in exhaustion status, observed in fish exposed to toxicants for long period of time (Friedman et al, 1996 & Hontela et al., 1997) and present author is opined of existence of such exhaustion status in fish, *Garra gotyla gotyla* in response to Mn metal and thus a subsequent decline in the level of cortisol.

As the major mineral corticoid in fish cortisol is known to control the branchial sodium uptake in fish, by stimulating of Na^+/K^+ -ATPase activity and by promoting the proliferation of the chloride cells, the location of this enzyme activity (Dharmamba, 1975). Altered Na^+/K^+-ATPase activity of the gills has been reported for mercury intoxicated rainbow trout (Lock et al., 1981) and rainbow trout exposed to zinc (Watson & Beamusch, 1980) and presently though Na^+/K^+-ATPase activity has not been determined in gill cell but thought to be really affected in fish, *Garra gotyla gotyla* exposed to manganese metal and exhibiting elevated levels of cortisol.

IV. Conclusion

To conclude, above results and discussion denotes that the fish *Garra gotyla gotyla* responded to the manganese exposure as represented by the changes in its blood cortisol level and that the response was dose and time dependent.

Table 1: The levels of cortisol (ng/ml) in blood serum of three groups of Garra gotyla gotyla exposed to three sublethal concentrations of manganese i.e. 0.64mg/l, 1.28mg/l and 1.92mg/l (20%, 40% and 60% respectively of LC50value of manganese)

Time Interval	0.64mg/l of Mn (20%)	1.28mg/l of Mn (40%)	1.92mg/l of Mn (60%)
Control	109.8±0.82	109.8±0.82	109.8±0.82
1st week	175.2±0.15	198.5±0.25	230.0±0.13
5th week	156.0±0.35	170.2±0.28	160.0±0.09
9thweek	142.5±0.52	156.5±0.46	125.0±0.22

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