

Full Length Research Paper

Hormonal responses of the fish, *Cyprinus carpio*, to environmental lead exposure

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The present study reports the acute and sublethal toxicity of lead nitrate on plasma cortisol and prolactin level of a freshwater fish, *Cyprinus carpio*. The median lethal concentration of lead nitrate to fish for 24 h was found to be 4.10 ppm. 1/10th of the LC₅₀ concentration of the lead nitrate (0.41 ppm) was taken for sublethal concentration. During acute and sublethal treatment the plasma cortisol level increased throughout the study period showing a direct relationship with exposure period. Similarly, plasma prolactin level was increased during acute treatment. However during sublethal treatment plasma prolactin level was increased up to 14th day and then declined. The significant increase of plasma cortisol level might have resulted from the release of cortisol from the interrenal tissue as a mechanism of coping up with stress or impaired immune function. The elevated level of plasma prolactin may be a step to re-establish ionic equilibrium due to the disturbances caused by the metal. Whereas the decline in plasma prolactin level indicate the destruction of prolactin cells due to metal toxicity. The alterations of the hormonal levels may be used as a potential biomarker and also can establish the ability of endocrine tissues to respond to their appropriate releasing factors.

Key words: Cortisol, prolactin, lead, *Cyprinus carpio*.

INTRODUCTION

The emission of anthropogenic chemicals has resulted in long-term ecotoxicological effects in different parts of the world. Lead is a naturally occurring metal present in the earth's crust, rock, soil, and water, but most waterborne lead derives from human activities such as mining and smelting, coal burning, cement manufacturing, and use in gasoline, batteries, and paint. Environmental pollutants such as metals pose serious risks to many aquatic organisms by changing genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Among the aquatic habitants, fish is the most susceptible to these elemental contaminants and more vulnerable to metal contamination than any other aquatic habitant

(Alinnor, 2005). The toxic effect of lead is primarily the inactivation of enzymes and proteins via the binding to sulfhydryl groups. It also impairs Ca²⁺ uptake and causing ionoregulatory damage. Recently, attention has been devoted to assess the toxic effect of xenobiotics on endocrine system of stressed animals. In natural and in culture conditions, stress is a common phenomenon; stressors disturb an animal's homeostasis, which in turn can elicit compensatory or adaptive responses. These responses occur in many target organs, especially those under multiple endocrine control (Wendelaar Bonga, 1997). The hypothalmo-pituitary-interrenal (HPI) axis of fish is activated during acute exposure to stressors (Hontela, 2005). Endocrine responses through their integrative and early warning capacity may offer as potential indicators that may be useful in the detection and assessment of sublethal toxic stress in fish exposed to polluted environments (Hontela et al., 1993) and these res-

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ponses are centered on the activation of the hypothalamic-pituitary-interrenal axis. Along this axis, releasing factors are given off from the hypothalamus during stress stimulating the secretion of pituitary hormones such as prolactin (PRL) and adrenocorticotropin (ACTH) (Fu et al., 1990). In turn, ACTH stimulates the synthesis and release of cortisol, the major corticosteroid from the interrenal cells in fish (Fagerlund, 1970). Changes in the concentrations of hormones, particularly those regulating vital functions such as osmoregulation, energy metabolism, reproduction or growth may have potential as early warning indicators of toxic stress in fish. Measurement of circulating levels of hormones can provide additional information on the sublethal effects of many chemicals.

The aim of this present work is to evaluate the impact of acute and sublethal exposure to lead toxicity on the stress related hormones like cortisol and prolactin in a freshwater teleost fish, *Cyprinus carpio*.

MATERIALS AND METHODS

Fish

Specimens of *Cyprinus carpio* var. *communis* were obtained from Tamil Nadu Fisheries Development Corporation, Aliyar, Tamil Nadu, India. They were safely transported to the laboratory in well packed polythene bags containing oxygenated water. Fish were stocked in large cement tank (6'X4'X3') disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). Fish were acclimatized to laboratory conditions for about 20 days before the commencement of the experiment.

Acclimatization

During acclimatization, fish were fed *ad libitum* with rice bran and groundnut oil cake once in a day. Feeding was given at least one hour prior to replacement of water. The feeding was withheld for 24 h before the commencement of the experiment to keep the experimental animals more or less in the same metabolic state. Water was replaced every 24 h after feeding in order to maintain a healthy environment to the fish during both acclimatization and experimental period. After acclimatization, fish with an average length of about 7.5 cm and an average weight of 6.0 g were selected. The fishes were introduced into glass aquarium (75 x 35 x 37 cm) of 150 L capacity which was washed thoroughly. Fish belonging to both the sexes were used. Analytical grade lead nitrate was obtained from Fisher Inorganic and Aromatics Limited, Madras, India.

Water chemistry

In the present study, tap water free from chlorine was used. The physico-chemical features of tap water were estimated following the method of APHA (1998) and are as follows: temperature $24.7 \pm 1.2^\circ\text{C}$; pH 7.2 ± 0.09 ; dissolved oxygen 6.4 ± 0.04 mg/L; salinity 0.4 ± 0.02 ppt; total alkalinity 18.0 ± 10.0 mg/L; total hardness 17.5 ± 0.5 mg/L; calcium 4.3 ± 0.3 mg/L; and magnesium 2.6 ± 0.6 mg/L. In the pre-

sent study, static test method was followed. The LC_{50} value of lead nitrate for 24 h was calculated following the method of Finney (1978) with a confidence limit of 5% level which was 4.10 ppm. The experiment was repeated for four times.

Acute toxicity study

In the present study for acute study 2 plastic tubs each of 15 L capacity were taken and filled with 10 L of water and LC_{50} 24 h concentration of lead nitrate (4.10 mg/L) was added. The experiment was initiated by introducing 10 fish in each tub. A common control was also maintained. At the end of 24 h, there was no mortality of fish in control tub, while 50% mortality occurred in the experimental tubs. The experiment was replicated for 4 times.

Sublethal toxicity study

For sub lethal toxicity studies a glass aquaria (100 liter capacity) was taken and filled with 90 liter of water. Then $1/10^{\text{th}}$ of value of the LC_{50} 24 h concentration of lead nitrate (0.41 ppm) was added to the tank. Subsequently, 90 healthy fishes were introduced in to the tank. Four similar replicates were maintained. Experiments were conducted for a period of 35 days with 7 days sampling frequency. A glass tank of toxicant free water was maintained as control.

Sampling

At the end of 24 h from acute treatment and every 7th day live fish from sub lethal treatment and control were sacrificed and blood was drawn from the heart regions by cardiac puncture using the cold hypodermic micro syringes prerinsed with heparin (anticoagulant). The collected blood from the control and experiment were expelled into their respective heparinised plastic vials and placed in the ice cold condition. Then the pooled blood sample was centrifuged for 15 min at 10,000 rpm and plasma was withdrawn and transferred into clean vials for hormone analysis.

Hormonal assay

Plasma cortisol was estimated quantitatively by direct solid phase enzyme immunoassay method of Tietz (1986) using EIA gen cortisol kit by Automated Microplate Reader (Bio-Tek Instruments, Inc. USA) and plasma prolactin was estimated quantitatively by enzyme immunoassay by following the method of Engvall (1980) and Uotila et al. (1981) using Medix Biotech prolactin enzyme immunoassay test kit by Automated Microplate Reader (Bio-Tek Instruments, Inc. USA). The method followed for the plasma prolactin is a heterologous assay and cross reactivity of the antiserum to native carp growth hormone and somatolactin is not known. This can be referred to as 'immunoreactive prolactin' (ir-PRL). Statistical correlation between control and experimental values were made by students't' test.

RESULTS AND DISCUSSION

The alteration of the plasma cortisol and prolactin level during acute treatment in lead exposed fish was presented in Table 1. In acute study, both plasma cortisol and prolac-

Table 1. Cortisol and prolactin levels in the plasma of *Cyprinus carpio* var. *communis* exposed to acute lead toxicity.

Parameter	Control	Experiment	Percent change	't' value
Cortisol (ng/ml)	4.995 ± 0.249	6.537 ± 0.503	+30.87	2.747*
Prolactin (ng/ml)	4.267 ± 0.439	6.096 ± 0.222	+42.86	3.718*

Values are mean ± S.E. of five individual observation, '+' denotes percent increase over control, *Values are significant at 5% level, Degrees of freedom at 8 t_{0.05} = 2.30

Table 2. Changes in the plasma cortisol and prolactin levels of *Cyprinus carpio* var. *communis* exposed to varying periods of sublethal lead toxicity.

Exposure period (days)	Plasma cortisol level (ng/ml)		Plasma prolactin level (ng/ml)	
	Control	Experiment	Control	Experiment
7	4.700 ± 0.411	4.900 ± 0.189* (+4.25)	3.489 ± 0.290	3.179 ± 0.276* (-8.88)
14	5.420 ± 0.174	5.620 ± 0.233* (+3.69)	6.331 ± 0.278	5.979 ± 0.313* (-5.55)
21	6.240 ± 0.222	11.420 ± 0.205* (+83.01)	6.458 ± 0.209	6.924 ± 4.025* (+7.21)
28	5.900 ± 0.289	12.260 ± 0.240* (+107.79)	7.248 ± 0.254	7.534 ± 0.304* (+3.94)
35	5.960 ± 0.246	13.180 ± 0.229* (+121.14)	8.584 ± 0.259	8.495 ± 0.298 (+1.03)

Values are mean ± S.E. of five individual observation, Values in parenthesis indicate per cent change over control, *Values are significant at 5% level, Degrees of freedom at 8 t_{0.05} = 2.306.

tin level was increased in the experimental fish showing an increase of 30.87 and 42.86% over that of their controls respectively. Statistical analysis of data by students 't' test indicated that values were significant at 5% level.

In sublethal treatment, plasma cortisol level increased in the experimental fish and was directly proportional to the exposure period showing a maximum increase of 121.14% at the end of the 35th day (Table 2). On the other hand, plasma prolactin level has decreased from -8.88% of 7th day to -5.55 on the 14th day, after that it started increasing slightly i.e. +7.21% of 21st day to +1.03% on the 35th day'.

The endocrine response to pollutants is an integral part of the homeostatic physiological process activated in response to environmental stressors including pollutants. The hypothalamo-pituitary-adrenal (HPA) axis is crucial for the ability of vertebrates to cope with stressors. In fish, the end product of this axis (called HPI axis in fish as they have interrenal cells in their head kidney rather than adrenals) is cortisol, which has both gluco- and mineralocorticoid functions in these animals. But as in other vertebrates, the synthesis and release of cortisol by the interrenal cells in fish is controlled primarily by adrenocor-

ticotropic hormone (ACTH) produced in and released by the pars distalis of the pituitary gland (Wendelaar Bonga, 1997). Cortisol is the most active and abundant corticosteroid in fish blood and its structure has been highly conserved in all of the vertebrate species in which it is found. The primary targets of cortisol action are the gills, intestine, and liver, which reflect the two main adaptive functions of cortisol identified to date: osmoregulation and the maintenance of a balanced energy metabolism. With respect to the latter, cortisol plays an important role in mobilizing fuels such as glucose, lipids, and fatty acids for the maintenance of homeostasis and exerts direct and indirect effects on intermediary metabolism, particularly in response to stress (Van der Boon et al., 1991). Plasma cortisol is an excellent indicator of functional alterations in the HPI axis (Hontela, 2005).

The elevation of plasma cortisol levels in response to various stressors is generally considered of adaptive value, primarily related to the energy-mobilizing properties of cortisol and its role in maintaining ion homeostasis (Sheridan, 1994). Secretion of the steroid hormone cortisol by the interrenal tissue is a characteristic reaction

of teleost fish to almost all forms of environmental stress. Exposure to metals and other toxicants that impair cortisol secretion could then influence social interactions and cortisol-dependent processes (Gagnon et al., 2006). An elevation of plasma cortisol is the most widely used indicator of stress in fish. This may be considered as the reaction of the fish to recognize the presence of a noxious or potential harmful substance in the environment. Scott et al. (2003) reported that plasma cortisol levels in rainbow trout increased when fish were exposed to an alarm substance, a chemical released from skin epithelium, and this increase was inhibited by cadmium. Hontela et al. (2006) observed that copper at high concentrations disrupts cortisol secretion through a direct toxic effect on adrenocortical cells while low concentrations resulting from a 30-day exposure to environmentally relevant Cu concentrations enhances cortisol secretion in response to ACTH *in vitro*. It is assumed that this represents part of a more general adaptive response in which cortisol mobilizes by virtue of its catabolic properties, stored food reserves, thereby enabling the fish to cope with the energy demands (Parxton et al., 1984). Perry and Wood (1985) reported that cortisol may be of a particular importance in *Salmo gairdneri* during aluminium stress in fighting ionic disturbances. Elevated cortisol level is probably related either to create the abnormal chloride and ATPase level or to the process of trying to restore the values to normal, since corticoids have been implicated in electrolyte balance and gill ATPase activity (Johnson, 1973). In the present study, significant increase in plasma cortisol level during acute and sublethal treatments might have resulted from the release of cortisol from the interrenal tissue as a mechanism of coping with stress or abnormal plasma chloride level or the process of trying to restore the values to normal.

Prolactin with cortisol is one of the main osmoregulatory hormones in fish maintaining the plasma electrolyte levels mainly by controlling permeability of the gill epithelium. In the freshwater fishes, prolactin has a hypercalcemic action through stimulation of the active Ca^{2+} uptake via the gills (Filk et al., 1989). Prolactin has also a well known ionoregulatory role in freshwater fishes and has been suggested to play a role in counteracting toxicant-induced ionic disturbances (Bern and Madsen, 1992). Alterations of plasma prolactin level in response to stressors have been reported by Fu and Lock (1990) and Fiess et al. (2007). Reduction in gill permeability by an increased response of prolactin could further explain the maintenance of a stable Na^+ and water content in lead-treated fish, since prolactin has been able to decrease branchial Na^+ efflux and osmotic water influx. Wendelaar Bonga et al. (1987) opined that increase in prolactin production is mainly due to a response to a drop in plasma electrolytes. Elevated levels of plasma prolactin

during the acute and sublethal treatment may be a step to re-establish ionic harmony (osmoregulatory function) or might be due to hypercalcemic action through stimulation of the active Ca^{2+} uptake. In the present study the increased level of prolactin during acute treatment of lead might be due to the lead-induced hypocalcemic action as a result of which prolactin is released to counteract this effect. Waring et al. (1996) observed a decline in plasma prolactin levels in brown trout *Salmo trutta* due to a more stressful environment, where mortalities are common. In the present study the decrease in the level of plasma prolactin from sublethal lead-treated fish resulted from destruction of prolactin cells leading to inhibited prolactin secretion.

Conclusion

In freshwater fish, prolactin and cortisol are thought to be involved in the maintenance of ionic and osmotic balance and since this regulation is apparently disrupted by lead, related changes in prolactin and cortisol might occur. From the present study, it is concluded that lead nitrate is highly toxic and has a profound influence on the hormonal profiles of fish. These hormones, prolactin and cortisol, could be effectively used as potential biomarker for metal toxicity to the freshwater fish in the field of environmental biomonitoring.

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