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ORIGINAL RESEARCH ARTICLE

Synergistic Impact of $HgCl_2$ and $CdCl_2$ Concentration on Hormonal Changes in the Seabass, *Lates calcarifer*

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ABSTRACT

The sub-lethal effect of mercury chloride and cadmium chloride on biochemical constituents was studies in the fish, *Lates calcarifer* was studied for the period of 35 days. A significant increase in blood prolactin level was noticed. The maximum per cent increase of prolactin was noted at 58.56, 30.10 and 88.65 at the end of 14th, 21st and 14th days. Whereas in all the three experiments minimum percent decrease of 5.38, 2.37 and 6.14 observed at the end of 35th, 7th and 35th days. But in blood cortisol level mixed trend was noticed. The maximum percent increase of 42.08, 3.53 and 42.54 observed at the end of 21st, 35th and 14th day respectively. The alteration in the above assumed parameters were minimum in cadmium treatment. The results are discussed in relation to the significance of the above hormones as non- specific biomarkers against environmental stress.

Key Words: Mercury, cadmium, hormone, *L. calcarifer*, synergistic.

INTRODUCTION

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment having led to various deleterious effects on the aquatic organisms [1]. Heavy metals in aquatic environments are transferred through food chain into humans [2]. Several biochemical and physiological responses can occur when aquatic organisms absorb a toxicant, which may be a compensatory response or a toxicity mechanism [1]. From the heavy metals, mercury (Hg) is the most abundant, and is bio-accumulated by aquatic organisms and biomagnified through the food chain [3]. Mercury causes strong toxicological effects on the cell membrane and many aspects of its toxic action have been attributed to its ability to cross the cell membrane and to disrupt cellular ion transport processes [4]. Cadmium introduced into the environment from natural or human sources is circulated through the system by chemical and physical processes and through biological transport mechanisms of living organisms [5]. The combined action of two factors (two stressors) is also used when estimating the chronic toxicity of low concentrations of toxicants for fish [6].

Hormones have been included, as they are measurable in blood and circulating levels of these hormones can be altered by exposure to xenobiotic chemicals as suggested by [7]. [8] stated that cortisol influences an array of physiological parameters, including carbohydrate and hydro mineral balance, mobilization of amino and fatty acids from cellular stores, gluconeogenesis and plasma protein production. Plasma cortisol has therefore been monitored as a general index of stress. An increase in cortisol concentration was observed in *Oncorhynchus kisutch* under copper treatment by [9] and in tilapia, *Oreochromis mossambicus* under cadmium treatment by [10]. [11] observed a significantly greater serum prolactin levels in rainbow trout, *Oncorhynchus mykiss* held in freshwater than their cohorts held in sea water. [12] in tilapia, *Oreochromis mossambicus* recorded prolactin increase when exposed to cadmium. The aim of this present work is to evaluate the impact of mercury,

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cadmium and mercury plus cadmium toxicity on the stress related hormones like prolactin and cortisol in a fish, *L. calcarifer.*

MATERIALS AND METHODS

Specimens of *L. calcarifer* were obtained from Rajive Gandhi Centre for Aquaculture (RGCA), Thirumullaivasal, Sirkali, Tamil Nadu, India. They were safely transported to the laboratory in well packed polythene bags containing oxygenated water. The experiment was done at CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. Fish were stocked in large cement tank (4x6x3) disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). Fish ranging from 7-8 cm in length and weighing 8-10 g were selected for experimental purpose. The quality of the water was determined according to [13] and were as follows: Dissolved oxygen 5.4 ± 0.02 mg/l; pH 8.6 ± 0.2; Water Temperature 39.0 ± 2.0°C; Salinity 38 ± 0.07 ppt; Total hardness 8.2±2.0 mg/l; Calcium 5.0 ± 0.1 mg/l; Magnesium 3.0 ± 2.0 and Total alkalinity 16.0 ± 06mg/l. Fish were acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. During acclimatization, fish were fed *ad libitum* with dry fish and the fed on flour pellets and ground dried shrimp and fishes 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 8-9 cm and an average weight of 9-10 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. Since the most environmentally relevant metal/H^+ interactions takes place in soft waters, it is worthwhile first to consider the origin and character of such waters [14]. For sublethal toxicity studies a glass aquaria (100 liter capacity) was taken and filled with 90 liter of water. Then 1/10th of value of the LC_{50} 24 h concentration of mercury chloride (3.5) cadmium chloride (4.0) and mercury chloride plus cadmium chloride (3.0 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. At the end of every 7th day treatment live fish from sublethal treatment and control were sacrificed and blood was drawn from the heart regions by cardiac puncture using the cold hypodermic micro syringes pre rinsed with heparin (anticoagulant). The collected blood from the control and experiment were expelled into their respective heparinized plastic vials and placed in the ice cold condition. Analysis of prolactin [15, 16] and cortisol [17] method was followed. All the above values were analyzed statistically.

RESULTS

(Table 1 & Fig 1) shows the changes in blood prolactin level of fish exposed to sublethal concentration of mercury, cadmium and mercury plus cadmium concentration for a period of 35th days. During above treatment highest blood prolactin level was observed in all the three treatments showing maximum percent increase of 58.56, 30.10 and 88.65 at the end of 14th, 21st and 14th days, respectively. Whereas the minimum per cent decrease of 5.38, 2.37 and 6.14 observed at the end of 35th, 7th and 35th day respectively.

(**Table 2 & Fig 2**) shows the changes in blood cortisol level of fish. During the treatment period highest blood cortisol level was observed in all the three treatments showing maximum percent increase of 42.08, 3.53 and 42.54 observed at the end of 21st, 35th and 14th days, respectively. Whereas in minimum per cent decrease of 3.31, -41.84 and 48.71 observed at the end of 28 th , 21st and 21st days of treatment, respectively.

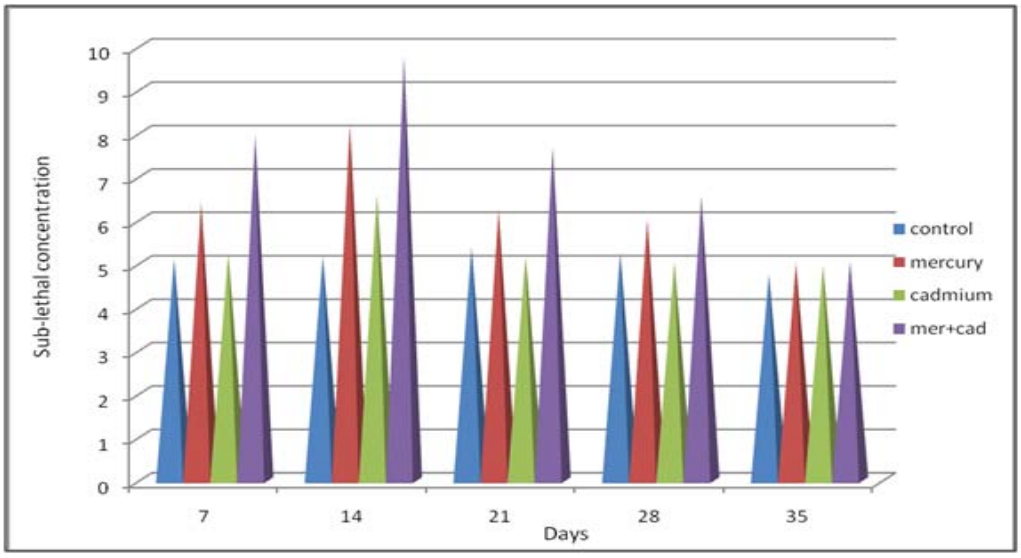

Fig 1: Changes in the plasma prolactin level, exposed to sublethal concentration of mercury, cadmium and mercury plus cadmium in the fish, *L. calcarifer*

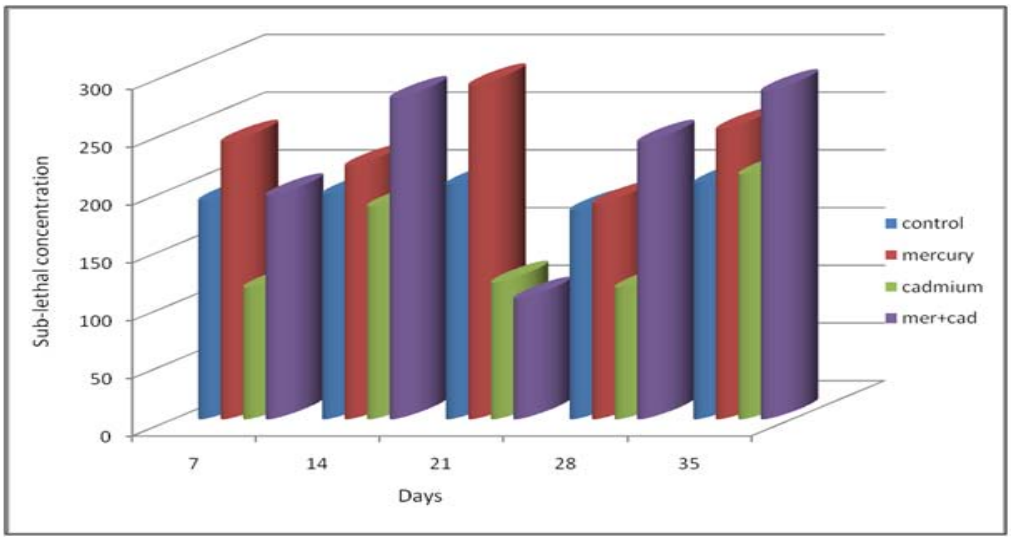

Fig 2: Changes in the plasma cortisol level, exposed to sublethal concentration of mercury, cadmium and mercury plus cadmium in the fish, *L. calcarifer*

Table 1: Changes in the plasma prolactin level exposed to sublethal concentration of mercury, cadmium and mercury plus cadmium in the fish *L. calcarifer*

Exposure period in Days	Plasma Prolactin level in mmol/L						
	Control	Mercury	T-test	Cadmium	T-test	Cadmium Plus Mercury	T-test
7	5.100±0.045	6.402±0.045 (25.52)	0.77	5.221±0.069 (2.37)	1.66*	7.954±0.050 (55.96)	0.49
14	5.167±0.074	8.193±0.089 (58.56)	1.09	6.572±0.102 (27.19)	11.77*	9.748±0.112 (88.65)	0.51
21	5.378±0.046	7.733±0.168 (43.78)	1.71	8.068±0.108 (30.10)	6.94*	7.676±0.124 42.72)	0.63
28	5.250±0.022	6.004±0.179 (14.36)	2.92*	5.728±0.124 (9.10)	9.92*	6.554±0.022 (24.83)	1.68
35	4.750±0.022	5.006±0.032 (5.38)	8.59*	4.954±0.022 (4.29)	10.78*	5.042±0.022 (6.14)	0.20

Treatment 1 (mercury), Treatment 2 (cadmium), Treatment 3 (mercury plus cadmium)
Values are mean ± S.E. of five individual observations; Values in parentheses are per cent change over control
+ Denotes percent increase over control
-Denotes percent decrease over control
T- Test, denotes percent in over control
* Values are significant at 5% level
Degree of freedom 8t = 2.236 (0.05)

Table 2: Changes in the plasma cortisol level, exposed to sublethal concentration of mercury, cadmium and mercury plus cadmium in the fish, *L. calcarifer*

.Exposure period In Days	Plasma Cortisol level in mmol/L						
	Control	Mercury	T-test	Cadmium	T-test	Cadmium Plus Mercury	T-test
7	189.750±1.006	240.625±2.781 (26.81)	0.05	114.250±0.915 (-39.78)	0.01	194.250±1.854 (2.37)	0.46
14	195.375±0.842	219.625±1.174 (12.41)	0.05	184.750±1.280 (-5.43)	0.14	278.500±1.118 (42.54)	0.01
21	203.750±1.854	289.500±2.500 (42.08)	0.03	118.500±2.500 (-41.84)	0.03	104.500±1.118 (-48.71)	0.02
28	181.250±1.157	187.250±1.118 (3.31)	0.26	114.250±0.915 (-36.96)	0.03	240.625±2.781 (32.75)	0.05
35	205.000±2.236	251.500±1.118 (22.68)	0.05	212.250±1.280 (3.53)	0.35	284.500±2.012 (38.78	0.03

Treatment 1 (mercury), Treatment 2 (cadmium), Treatment 3(mercury plus cadmium)
Values are mean ± S.E. of five individual observations
Values in parentheses are per cent change over control
+ Denotes percent increase over control
-Denotes percent decrease over control
T- Test, denotes percent in over control
* Values are significant at 5% level
Degree of freedom 8t = 2.236 (0.05)

DISCUSSION

Environmental pollutants such as metals pose serious risks to many aquatic organisms by changing genetic, physiological, biochemical and behavioural parameters. Among the aquatic habitants, fish is the most susceptible to these elemental contaminants and more vulnerable to metal contamination than any other aquatic habitat. Prolactin and cortisol is one of the main osmoregulatory hormones in fish maintaining the plasma electrolyte levels mainly by controlling permeability of the gill epithelium. [12] also have recorded an apparent increase in prolactin levels in tilapia, *O. mossambicus* when exposed to cadmium. The increase in prolactin production is mainly due to a response to a drop in plasma electrolytes [18]. [19] reported that increase in prolactin production after chronic exposure to be mainly a response to a drop in plasma electrolytes. In the freshwater fishes, prolactin has a hypercalcemic action through stimulation of the active Ca^{2+} uptake *via* the gills and alterations of plasma prolactin level in response to stressors have been reported by [20]. [10] opined that increase in prolactin production is mainly due to a response to a drop in plasma electrolytes. An elevated level of plasma prolactin of teleost fish is to almost all forms of environmental stress. In the present study also increased prolactin response observed under sublethal mercury, cadmium and mercury plus cadmium exposure may be due to hypocalcemic action or play a role in counteracting toxicant-induced ionic disturbances supporting the views of the above authors.

According to [21], prolactin reduces the permeability of gill epithelium which is time-dependent and it is mechanism of resistance. 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. [22] in tilapia, *O. mossabicus* under cadmium stress and of [8] in rainbow trout, *O. mykiss* [23] in brown trout, *S. trutta* under acid/aluminum stress. Alterations of plasma prolactin level in response to stressors have been reported by [17]. [18] suggested that severe stress may also cause atrophy of pituitary prolactin cells thereby inhibiting their bio-synthetic activity as observed in book trout, *S. fontinalis* under acute and aluminium exposure. The above decline in plasma prolactin levels in brown trout may be due to a more stressful environment, where mortalities are common. In the present study also decrease prolactin level may be due to in reducing the impact of metal toxicity supporting the observation of the above authors.

Though the increased levels of cortisol is a stress response to favor increased carbohydrate metabolism have been reported in fish under pollution stress. [24], the observed significant reduction in the cortisol levels of endosulfan-exposed *S. mossambicus* appears to be quite different from the earlier reports. In the present study also increase in plasma cortisol concentrations during stress confirmed that confinement (1 or 24 hr) activated the HPI axis and thus disturbed the endocrine equilibrium supporting the views of the above authors. The above decline in blood cortisol levels in brown trout may be due to a more stressful environment, where mortalities are common. [25] suggested that sever stress may also cause atrophy of pituitary cortisol cells thereby inhibiting there bio- synthetic activity as observed in brook trout
S. fontinalis under acute acid and aluminium exposure. In the present study, due to restriction of cortisol cell or sublethal stresses are to mercury, cadmium and mercury plus cadmium supporting the above views.

CONCLUSION

The marine fish, *L. calcarifer* is considered to be one of the chief edible fishes in all regions. In marine 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 Hg and Cd toxicity. The present study, it is concluded that Hg, Cd and Hg plus Cd is highly toxic has a profound influence on the hormonal profiles of fish. These hormones, prolactin and cortisol, could be effectively used as potential biomarker for $HgCl_2$ and $CdCl_2$ toxicity to the

marine fish in the field of environmental bio monitoring.

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