Changes in Growth Hormone and Cortisol Profile due to Lead Induced Toxicity in *Labeo rohita*

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Abstract

The present study was conducted to investigate the effect of lead (Pb) on the growth hormone (GH) and plasma cortisol level of common carp, *Labeo rohita*. One hundred *Labeo rohita* were divided in two equal groups; control and treated. Treated fishes were exposed to 1.4mg/l of lead acetate (1.9% lead concentration i,e., LC_{50}) at the interval of 72 hours for a period of five weeks. Blood was collected from the caudal vein of fish from both treated and control groups, after week interval. Mean body weight of treated group decreased significantly as compared to the control group (P<0.001) on weekly basis. GH decreased significantly (P<0.001) in the treated group from first week to the last week of the treatment as compared to the control group. In treated groups, the plasma cortisol level increased gradually from week 1 to week 5. Significant negative correlation between GH concentration and cortisol level (Pearson *r*=-0.94) was observed. It is concluded that Pb acts as endocrine disrupter in fish bodies by altering the pattern of synthesis and metabolism of cortisol as well as GH. Elevation in basal cortisol level markedly influences the growth rate by decreasing the GH ultimately decreasing body weight of animal.

Keywords: Growth hormone, cortisol, lead exposure, carp, endocrine disrupter.

Introduction

Heavy metals are persistent contaminants in the aquatic environment causing serious illness in fish, animals and human ultimately (Shahid, Alkahem Al-Balawi, Al-Misned, Al-Quraishy, & Ahmad, 2014). Among the toxic heavy metals in the water bodies, Lead (Pb) is more abundant than cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn) and mercury (Hg) and is an emerging worldwide concern because it exerts damaging effects on human health. It acts as a cumulative poison and is listed by Environmental Protection Agency as one of the 129 priority pollutants (Kumar, Prasad, Patra, Rajan, & Swarup, 2009). The main natural source of Pb emission is windblown dusts, forest fires, volcanic emission and sea salt sprays (Shahid *et al*., 2014).

Recently, aquaculture industry has gained more importance to cater the quality assurance in fish production, basically a good source of fish production for the nutrition insecure people living in Asia and Sub-Saharan Africa. Furthermore, it aims to fulfill all dietary requirements of human being because fish is a rich source of proteins and other essential nutrients (Beveridge *et al.,* 2013). Moreover, aquaculture industry plays an important role in rural development by alleviating poverty (Stevenson & Irz, 2009). Among the aquatic fauna, fish is more susceptible to water pollutants than other aquatic species (Firidin, 2016) and is an appropriate biological indicator of water pollution (Sudagar & Hajibeglou, 2010). Most water born Pb has anthropogenic origin e.g. mining, smelting, coal burning; cement manufacturing, use of gasoline, batteries and paint, etc. (Ramesh, Saravanan, & Kavitha, 2009). Pb causes serious damage to different organ systems including central nervous system, reproductive, hepatic, renal and hematopoietic etc. by generating reactive oxygen species (ROS) (Gagan, Deepash, & Archana, 2012). Moreover, it has capability to accumulate in different tissues of exposed fish further causing hepatic and renal damage along with growth retardation and induces stress which resulted into impaired cortisol level and metabolic enzymes (Anbu, 2014). Recently, it has been investigated that Pb decreased oxygen consumption in *Labeo rohita* in 28 days (Amin, Ramachandra, Priyadarshini, Nayak, & Sree, 2017). In children, Pb causes behavioral and cognitive impairments (Bellinger, 2008). Pb acts as a teratogenic factor leading to spontaneous abortions,

low birth weight and congenital deformities (Bellinger, 2005). At higher concentrations, Pb disrupts the cortisol secretions through direct toxic effect on adrenocortical cells and enhances cortisol secretion in response to ACTH (Souza-Talarico *et al.*, 2017; Vosyliene, Kazlauskiene, & Svecevicius, 2003).

Pb toxicity resulted from increased ROS production. Glutathione oxidation is one of the elements of ROS detoxification and its decreased levels, accompanied by increased levels of glutathione disulfide (GSSH), are frequently observed on exposure to Pb (Aoyama & Nakaki, 2013). Glutathione reductase (GR) reduces glutathione disulfide back to glutathione using NADPH as a proton donor (Franco, Schoneveld, Pappa, & Panayiotidis, 2007). Glutathione and its glutathione reductase seem to be the major targets of Pb, other enzymes involved in ROS detoxification are also affected by Pb exposure (Kasperczyk *et al.*, 2012). Moreover, Pb can also interact with calmodulin dependent pathways (Kirberger, Wong, Jiang, & Yang, 2013).

The accumulation of heavy metals in aquatic ecosystem also alters the fish growth and development (Mousa & Mousa, 1999). However, much less is known about the effects of heavy metals on growth hormone (GH) in fish. Immunocytochemical and histological studies on fish showed that those living in metal contaminated water had much smaller somatotrophs and weaker GH immune-reactivity compared to those in unpolluted water (Hontela, Rasmussen, & Chevaileir, 2007). The present study is intended to evaluate Pb induced toxicity in *Labeo rohita* with special reference to the growth hormone and cortisol level.

Materials and Methods

Fish Housing Condition

In the current study one hundred and sixty healthy freshwater teleost fish (*Labeo rohita*) with 15-23 g body weight and 8-14cm in length of both sexes were obtained from aqua farms in polythene bags containing oxygenated water. Fish were allowed to acclimatize for 7 days before the commencement of experiment. They were kept in fish laboratory. The animal experiment was performed with the compliance of Research Ethical Review Committee of Lahore College for Woman University.

Chemical Used

- Methylene blue (Westernization International Trading Co. Ltd, 7220-79, Hong Kong, China.
- Heparin (LEO Pharmaceutical Products, Ballerup, Denmark).
- Pb acetate (Ningho Hi-Tech Biochemicals Co; Ltd, China).

Feeding

Fish was given commercially prepared fish feed having a standard ratio of many essential nutrients required for normal fish growth as represented in Table 1 (Royes & Chapman, 2009; Orire & Sadiku, 2011).

Water Chemistry

The water temperature, pH, oxygen concentration, hardness, calcium, magnesium and conductivity were adjusted throughout the period of experiment. pH was recorded by pH meter; HI-8520 and conductivity was recorded by digital meter; HI-9146 (Table 2). Hardness of water was recorded by atomic absorption spectroscopy. Methylene blue was used as a disinfectant in aquaria. The fish were fed with a balanced ration comprised of yeast, wheat flour, soyabean meal, vitamins, nicotinic acid, biotin, folic acid, calcium pantothenic and many minerals.

Experimental Design

Hundred fish were taken for experiment. They were kept into four glass aquaria of 120 liter water capacity provided with tap water and filtered air each. Two aquaria served as control and two as treated. Each aquarium contained 25 fish. Water filters were provided to the aquaria for the removal fish excreta.

Assessment of LC$_{50}$

Before the commencement of experiment sixty fish were taken for the calculation of LC$_{50}$, LC$_{50}$ was

Table 1. Table showing fish feed composition and their relative percent by dry diet

Nutrients in fish feed	Percentage by dry diet
Proteins (10 essential amino acids):	32–45%
Carbohydrates: Major carbohydrates are starch, cellulose, and pectin.	10–30%
Fat: Fatty acids of the linolenic (w-3) and linoleic (w-6) series.	4–28%
Minerals: Calcium, magnesium, phosphorous, copper, iron, manganese, iodine, zinc and selenium.	1.0–2.5%
Vitamins: Fat-soluble (vitamins A, D, E, and K) and water-soluble (vitamins C and the B-complex [thiamin, riboflavin, pyridoxine, pantothenic acid, cyanocobalamin, niacin, biotin, folic acid, choline, and myoinositol).	1.0–2.5%

calculated in three sets [1.4, 15.0 and 30 mg/L of lead acetate (Pb (CH$_3$COO) $_2$)] and 15.0 mg/L was found to be LC$_{50}$ (Table 3). Concentration of 1.4 mg/L containing 1.9% Pb (0.0000266 g) (1/10 of LC$_{50}$) was used for the experiment as the fish size was small. The calculated amount of salt was added to the experimental aquaria at an interval of 72 hour for five weeks to investigate the pathological effects of Pb on the exposed fish.

Blood Sampling

After every 7th day of exposure fish were sacrificed. Blood samples were collected from the caudal vein by using the hypodermic micro syringes pre-rinsed with heparin. Blood was centrifuged at 3200 rpm for 15 min and plasma was stored at -26°C until it was used for the estimation of plasma cortisol and growth hormone.

In the current study biological parameters were measured according to their respective formulae (Halver, 1989).

Specific growth rate (SGR) (Orire, Omotoyinbo, & Sadiku, 2013) was calculated by using the following formula:

i. SGR= Ln MFW (Mean final weight) – Ln MIW (Mean initial weight)*100/Times in days.

ii. Mortality (%) = Total no dead fish/ Total no stocked fish*100

iii. Percent increase or decrease in Body weight was calculated by following formula:

= Final weight – Initial weight / Final weight + Initial weight *x* 100

Kits Used

Active Cortisol ELISA kit (Accu-Monobind), Monobind Inc., 100 Lake Forest, CA 92630, USA and Growth Hormone ELISA kit (Biosource Europe S.A-Rue de 1' Industrie, 8-B-1400 Nivelles-Belgium, 090423/1 KAP1081) was used for the determination of plasma cortisol and growth hormone respectively.

Statistical Analysis

One way ANOVA, Dunnett T3 post hoc were applied for establishing significant difference between different groups and *Pearson's* correlation test was used for finding the correlation between GH, Cortisol and body weight by using SPSS ver 19 and Microsoft excel 2007. P<0.05 was considered as significant and P<0.001 was considered as highly significant.

Results

The average body weight in the control group increased significantly (P<0.001) from first week to fifth week while in the treated group decreased significantly (P<0.001) in the same progression (Table 4) when compared with student's *t*-test and Dunnett T3 post hoc test. Moreover, control group showed 15.62 % increase in mean body weight with specific growth rate (SGR) of 0.391 % (Table 5). On the other hand treated group showed 8.09 % decrease in mean body weight showing SGR value of -0.201 % (Table 5). The results also indicated significantly higher (P<0.001) plasma GH concentration in control group. On the other hand the GH concentrations significantly decreased (P<0.001) in the treated group. The highest mean concentration of plasma GH in the control group was found to be 0.59 ± 0.04 ng/mL in fifth week while the lowest mean concentration was 0.50 ± 0.07 ng/mL during first week. The highest mean concentration of plasma GH in the treated animals was 0.48 ± 0.06 ng/mL in first week whereas the lowest mean concentration was 0.33 ± 0.03 ng/mL in week five (Table 4.)

In control group, there was gradual increase in plasma cortisol level from first to last week of study (2.60 ± 0.09 µg/dL in first and 5.07 ± 0.23 µg/dL in last week). In the treated groups, the increment was gradual but more pronounced as compared to the control group (5.04 ± 0.14 µg/dl and 13.16 ± 0.94 µg/dL in the first and last week respectively) and results were significantly different in control and experimental groups (P<0.001) (Table 4). The data

Table 2. Mean ± SEM of Temperature, pH, conductivity, Ca and Mg throughout the experimental protocol

Groups	Aquarium	Temp °C.	pH	Conductivity µS	Calcium (Ca^{+2}) mg/dL	Magnesium (Mg^{+2}) mg/dL
Control	Aquarium 1	33.07 ± 0.54	7.14 ± 0.20	1190.8 ± 4.76	745.62 ± 14.38	134.23 ± 0.12
	Aquarium 2	31.84 ± 0.34	7.2 ± 0.30	1187.40 ± 4.87	734.81 ± 18.53	133.86 ± 0.17
Treated	Aquarium 1	33.23 ± 0.56	7.09 ± 0.19	1238 ± 9.02	746.52 ± 14.35	134.11 ± 0.09
	Aquarium 2	32.7 ± 0.45	7.14 ± 0.25	1241.4 ± 10.24	733.03 ± 14.40	134.357 ± 0.14

Table 3. Assessment of LC$_{50}$ and % mortality of *Labeo rohita*

Dose (mg/L)	%age of Pb	No. of fish	No. of deaths	Body weight (g)	Body length (cm)	Mortality %
1.4	1.9	20	2	15-19	08-14	10%
15	20.4	20	9	15-17	09-11	45%
30	40.9	20	14	17-19	08-12	70%

Table 4. Mean body weight (g), Mean plasma GH concentration (ng/mL) and cortisol level (µg/dL) in control and Pb acetate treated group during 5 weeks of study

Parameters	Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Body weight	Control	15.80 ± 0.28	17.34 ± 0.55	18.84 ± 0.42	$20.33 \pm 0.35^{*}$	$21.65 \pm 0.31^{*}$
(g)	Treated	17.70 ± 0.55	16.95 ± 0.47	16.31 ± 0.41	15.92 ± 0.37	15.05 ± 0.33
GH (ng/mL)	Control	0.5 ± 0.07	0.55 ± 0.08	0.51 ± 0.05	0.56 ± 0.05	0.59 ± 0.04
	Treated	0.48 ± 0.06	0.43 ± 0.08	0.39 ± 0.05	$0.39 \pm 0.02^{*}$	$0.33 \pm 0.03^{*}$
Cortisol	Control	2.60 ± 0.09	$3.31 \pm 0.13^{*}$	$3.69 \pm 0.15^{*}$	$4.33 \pm 0.23^{*}$	$5.05 \pm 0.23^{*}$
(µg/dL)	Treated	$5.04 \pm 0.14^{*}$	$6.42 \pm 0.15^{*a}$	$8.18 \pm 0.14^{*a}$	$10.60 \pm 0.16^{*a}$	$13.16 \pm 0.43^{*a}$

*= versus C (Week 1); a= versus T (Week 1); *, a = P<0.001 (By using Dunnett T3 and one way ANOVA)

Table 5. Body weight (B.W), specific growth rate (SGR) and percent increase or decrease in control and Pb acetate treated group within a period of 5 weeks in *Labeo rohita*

Groups	Initial B.W	Final B.W	SGR %	% increase and decrease
Control	15.80 ± 0.28	21.65 ± 0.31	0.391	↑ 15.62
Treated	17.70 ± 0.55	15.05 ± 0.33	-0.201	↓ 8.09

obtained showed negative correlation between GH concentration and Cortisol level (Pearson $r = -0.94$). Likewise, results of current study demonstrated that BW and cortisol are negatively correlated ($r= -3.46$). In contrast to cortisol, GH and body weight showed positive correlation ($r = 2.51$).

Discussion

In the current study control group showed increased body weight and SGR in 4th and 5th week respectively which might be due to normal feed intake. Moreover, present study showed a continuous decrease in the mean body weight, SGR and plasma GH in Pb treated group. In current study decreased SGR in treated group might be due to Pb toxicity that ultimately resulted into reduced feed intake as reported by Gharedaashi and coworkers in 2012 that chronic treatment of lead nitrate (Pb(NO$_3$)$_2$) decreases the carp growth rate and SGR. Different experiments on *Channa punctatus*, *Cyprinus and Cirrhina* spp. also revealed growth reduction by the exposure to metals mixture by inducing severe damage in intestinal epithelium, disarrangement and fragmentation of mucosal foldings (Abbas & Javed, 2016; Javed, 2015; Paul, Chakraborty, & Sengupta, 2014; Ramesha *et al.*, 2003). According to the investigation of Giguere, Campbell, Hare, McDonald and Rasmussen (2004) it was suggested that the sensitivity of fish towards different metals decreases with age due to their ability to concentrate heavy metals that exert significant impact on the tolerance limits of fish.

The present data showed that Pb exposure decreased plasma GH concentration and increased cortisol level as previously reported in a study in which fish exposed Pb (CH$_3$COO)$_2$ (Gill, Tewarz, &

Pande, 1991) because Pb acts on CRH (Cortisol releasing hormone) releasing neurons in hypothalamus to increase CRH release and, subsequently increase the cortisol level (Nair & Ajit, 2007). In the current study reduced plasma GH level in the treated group confirmed that Pb acts as a stressor. El-Shebly (2009) reported that exposing fish to Pb significantly inhibited the activity of serum GH. Various studies on the effects of metal mixtures on different fish species including *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* have proved that toxicity of the metals mixture may fluctuate, depending upon their relative concentration, particular composition and duration of fish exposure (Javed, Abdullah, & Yaqub, 2008).

Present study showed idiopathic increase in plasma cortisol level from 2nd to 5th week in control group however, control group showed a decreased cortisol level as compared to treated group that showed a dramatic increase in plasma cortisol level in all weeks respectively when compared to control group. Previous studies showed that heavy metal like Copper effected endocrine regulation in rainbow trout (*Oncorhynchus mykiss*). Moreover, exposure to heavy metals disrupts hormone signaling cellular pathways and changes secretory pattern of cortisol favoring the findings of present study (Gagnon, Jumarie, & Hontela, 2006; Marchi, Burlando, Moore, & Viarengo, 2004). Immunocytochemical and histological studies in Nile tilapia showed that animals exposed to polluted water had much smaller somatotrophs and weaker GH immune-reactivity than those in unpolluted water (Mousa & Mousa, 1999). In the present study increased plasma cortisol level in fish is analogous to the findings of Hontela (2005) who investigated that Pb (CH$_3$COO)$_2$ can induce the functional alterations in HPI (hypothalamic-pituitary-

interrenal) axis in fish. Furthermore, increased plasma cortisol level in current study might be due to increased ACTH level through Pb acetate exposure as previously been reported by Allinor (2005). Gagnon, Jumarie and Hontela (2006) reported that exposure to metals and other toxicants impaired cortisol secretion in rainbow trout *(Oncorhynchus mykiss)* and then influence social interactions and cortisol dependent processes.

Conclusion

In the present study *Labeo rohita* exposed to Pb showed increased plasma cortisol along with decreased GH concentration. These results lead to the conclusion that Pb acts as endocrine disruptor and has profound influence on the hormonal profiles and specific growth rate of carp. This study notifies that Pb poisoning may have similar effects on human health as well and can ultimately cause serious damage to human life. As major sources of Pb exposure are anthropogenic, hence concerned authorities must impose strict mitigation measures to control this hazard.

Abbreviations

GH: Growth hormone; Pb: Pb; CRH: Cortisol releasing hormone; ACTH: Adrenocorticotropic hormone; HPI: hypothalamic-pituitary-interrenal.

Acknowledgements

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