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SHORT COMMUNICATION

Effect of chromium chloride on serum calcium and phosphate levels of stinging catfish *Heteropneustes fossilis*

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Abstract The present study was aimed to investigate the effects of chromium chloride on the blood electrolytes of *Heteropneustes fossilis* for short- and long-term exposure. In short-term exposure the fish were subjected to 50% of 96-h LC_{50} value of chromium chloride for 96-h. In long-term exposure the experiment was performed for 30 days by using 10% of 96-h LC_{50} value of chromium chloride. Fish were killed on each time intervals from control and experimental groups after 24, 48, 72, and 96 h in short-term exposure and after 5, 10 15 and 30 days in long-term experiment. Blood samples were analyzed for calcium and inorganic phosphate levels. Acute exposure of chromium chloride caused a decrease in the serum calcium levels after 48 h in fish *H. fossilis* which persists till the close of the experiment (96 h). The serum inorganic phosphate levels remain unaffected till 48 h in the chromium chloride. At 72 h and 96 h the levels exhibit a decrease. Chronic chromium chloride caused a decrease in serum calcium levels at day 10. This decrease continues till 15 days thereafter levels tend to rise approaching control level. Serum phosphate level of the chromium treated fish decreases on day 15 and 30.

Keywords Chromium . Serum Calcium . Serum Phosphate . Catfish

Introduction

Human being uses pesticides for their benefits—for increasing crop yield and for the control of insect vectors of diseases. However, these pesticides are not always useful. The pesticides after being used ultimately find their way into a variety of different aquatic ecosystems. The pesticides may provoke their toxic effects in various forms- ranging from alteration within a single cell, whole organism or even changes in whole population. The adverse effects of toxicants become significant when they affect economically important organisms or affect those organisms which are eaten by economically important animals and human beings. After entering into the organism's body these toxicants produce stress conditions either in the form of physiological, biochemical, damage to vital organs or even death of living beings of terrestrial and aquatic environment.

Several toxicants have been reported to affect the physiological functions of aquatic organisms. For example, in fish, toxicants disturb water and ion homeostasis. Although environmental waste management system has achieved an advancement but the aquatic bio-life is still facing adverse impacts due to heavy metal discharge (Bakshi and Panigrahi 2018; Rashmi et al. 2019). Chromium is the sixth most abundant heavy metal in the earth crust (Velma and Tchounwou 2010). Chromium finds its way into the aquatic

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ecosystem through some industries mainly electroplating, polishing, paint, rubber, plastic, ceramics, fiberglass, chrome plating, chrome alloymaking, welding and foundaries. Chromium is fairly toxic to animals and human beings beyond its optimum concentration. In such instances chromium produced cytotoxic, haematological, histological, immunological disturbs and genotoxic effects to fish (Prabakaran et al. 2007; Bozcaarmutlu and Arinc 2007; Goodale et al. 2008; Tan et al. 2008; Velma et al. 2009; Palaniappan and Karthikeyan 2009; Velma and Tchounwou 2010; Mutthukumaravel and Rajaraman 2013). Ko et al. (2019) and Rashmi et al. (2019) have stated that physiological and biochemical parameters in fish blood and tissues are affected by metal exposure.

In vertebrates, calcium plays a vital role in a variety of biological processes (Srivastav et al. 2008). Although there exists several reports regarding the impact on electrolyte composition of the blood for several pollutants (Suzuki et al. 2006; Rai et al. 2009; Velisek et al. 2009; Srivastav et al. 2010; Mishra et al. 2010 a, b) but there exist no information regarding the impact of chromium chloride on blood electrolytes of fish. Hence, it was aimed in the present investigation to study the toxic effect of heavy metal chromium chloride on a freshwater teleost *Heteropneustes fossilis* in terms of serum calcium and phosphate levels.

Materials and methods

Freshwater fish *H. fossilis* (both sexes; body wt. 27-35 g) were collected and acclimatized for two weeks in 500 L plastic pools. Small-mesh dip-net of soft material was used for gentle handling of fish for experiments. Care was taken to minimize stress to the fish. Dead fish were removed immediately. Animal handling and sacrifice were carried out following the guidelines provided by Ethics Committee of the University.Fish were not fed 24 h before and during the experiment. Short-term and long-term experiments have been performed.

(i) Short-term exposure: The fish were subjected to 50% of 96 h LC_{50} value of chromium chloride (i.e. 31.63 mg L^{-1}). The LC_{50} value for chromium chloride for *Heteropneustes fossilis* has been reported by Srivastav et al. (2015). Fish were kept in groups of 10 in 30 L media. Six fish were killed on each time intervals from control and experimental groups after 24, 48, 72, and 96 h of exposure period. Blood was collected and analyzed for calcium and phosphate levels.

(ii) Long-term exposure: The fish *H. fossilis* were acclimatized for two weeks to the laboratory conditions. The experiments were performed for 30 days by using 10% of 96 h LC_{50} value of chromium chloride (6.32 mg L^{-1}). Simultaneously, a control group was also used for comparison. Six fish from the control and experimental groups were sacrificed after 5, 10, 15 and 30 days of the toxicant treatment.

The physicochemical characteristics of the tap water used in the experiment were- photoperiod 11:42–12:54; temperature 26.74 ± 2.11 ºC; pH 7.26 ± 0.09; hardness 135.25 ± 5.69 mg L^{-1} as $CaCO_3$; dissolved oxygen 7.85 ± 0.36 mg L^{-1} and no free chlorine. No aeration was given as the fish is air breathing.

Blood samples were collected under slight anesthesia (MS 222) by sectioning of the caudal peduncle in both short-term and long-term experiments. The sera were separated by centrifugation at 3500 rpm and analyzed for calcium (calcium kit, Sigma-Aldrich) and inorganic phosphate levels (inorganic phosphorous reagent kit, Pointe Scientific, Inc. USA). All determinations were carried out in duplicates for each sample.

Each data represents mean ± S.E. of six specimens and Student's t test was used to determine statistical significance between the experimental group and its specific time control group.

Results

Short-term exposure

The serum calcium levels exhibit significant decrease at 48 h in chromium chloride treated *H. fossilis* which persists till the close of the experiment (96 h) (Figure 1). The serum inorganic phosphate levels remain unaffected till 48 h in the chromium chloride exposed fish. After 72 and 96 h the levels exhibit a significant decrease (Figure 2).

Long-term exposure

The serum calcium level of the chromium chloride treated fish decreases significantly on day 10 and day 15.

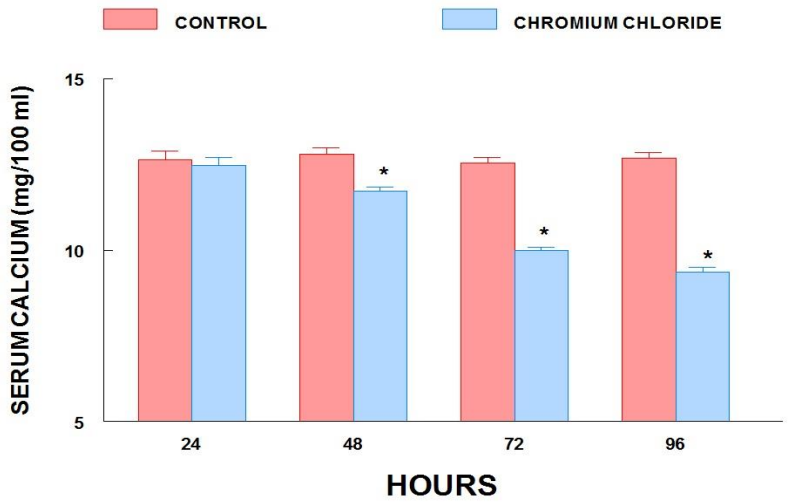

Fig. 1 Serum calcium levels of short-term chromium chloride treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

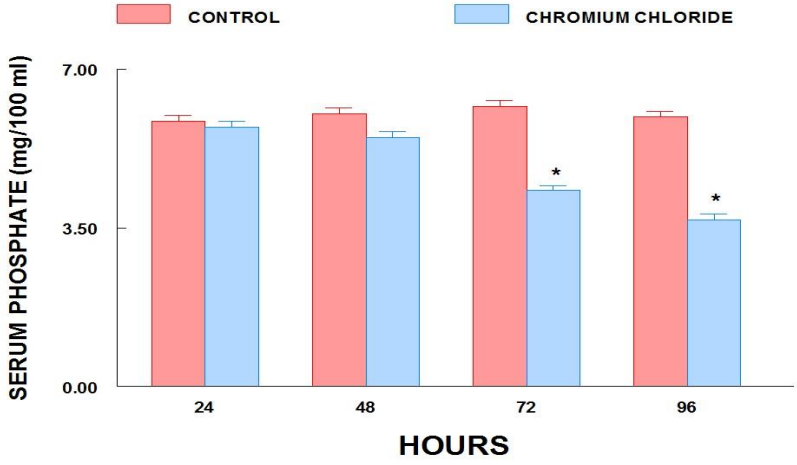

Fig. 2 Serum phosphate levels of short-term chromium chloride treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

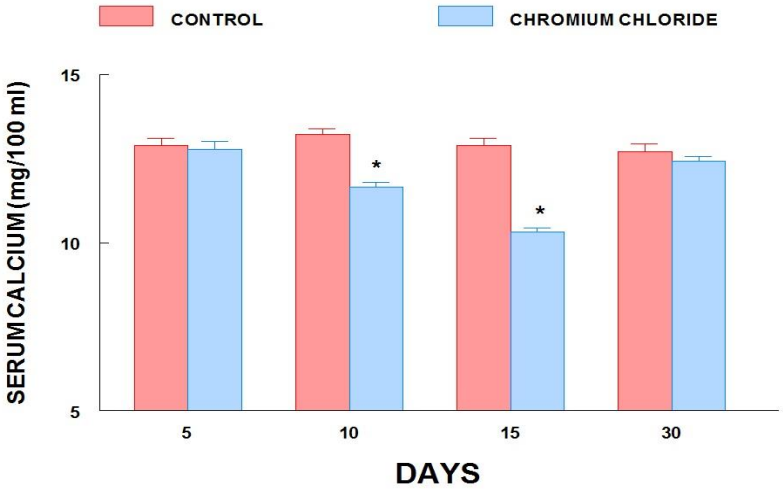

Fig. 3 Serum calcium levels of long-term chromium chloride treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

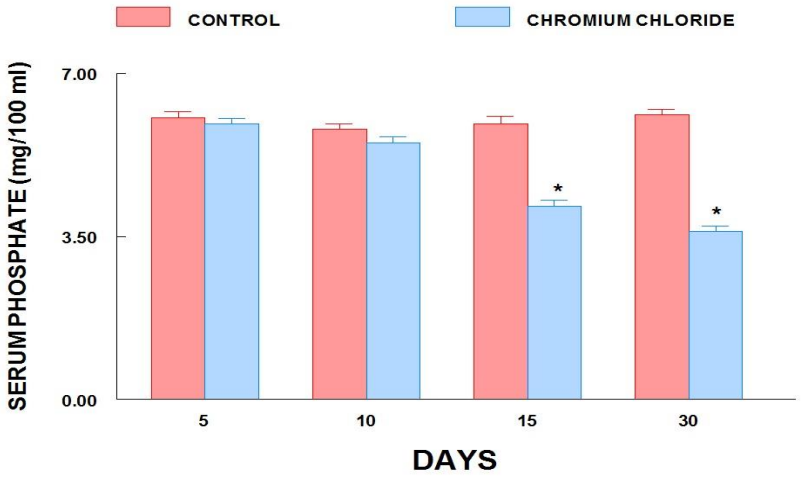

Fig. 4 Serum phosphate levels of long-term chromium chloride treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

However, on day 30 the levels become close to the normal values (Figure 3). Chromium chloride treatment provokes a significant decrease in serum phosphate levels at day 15 (Figure 4). This decrease progresses till the end of the experiment (Figure 4).

Discussion

Exposure of chromium chloride provoked hypocalcemia in the freshwater catfish, *H. fossilis*. There exists no study regarding the effect of chromium on the blood calcium content of fish, hence this study is the first report. In the past decreased blood/serum calcium content has been reported in teleosts exposed to various toxicants- cypermethrin (Mishra et al. 2001, 2010 a,b; Pandey et al. 2009), deltamethrin (Srivastav et al. 1997, 2010), aldrin (Bano 1982; Singh et al. 1996), cadmium (Larsson et al.1981; Pratap et al.1989; Rai and Srivastav 2003; Rai et al. 2009), propoxur (Singh et al. 1997), formithion (Singh et al. 1997), azadirachtin (Kumar et al. 2011a), *Euphorbia tirucalli* (Kumar et al. 2011b), *Euphorbia royleana* (Prasad et al. 2011a) and *Nerium indicum* (Prasad et al. 2011b). In contrast increased blood calcium levels of fish have been noticed after exposure to pesticides (Sastry and Sharma 1978; Bansal et al. 1979; Dalela et al. 1981; Sharma et al. 1982; Suzuki et al. 2006). No effect has been noticed in blood calcium content of DDT treated flounders *Platichthys flesus* (Haux 1979), methoxychlor treated northern puffer *Sphaeroides maculatus* (Eisler 1967), cadmium exposed *Oncorhynchus niloticus* (Oner et al. 2008) and bifenthrin treated rainbow trout *Oncorhynchus mykiss* (Velisek et al. 2009).

Chromium exposed *H. fossilis* exhibit a decrease in blood phosphate content. The effects of chromium have not been evaluated on the serum phosphate level of the fish, hence the results of the present study could not be compared with other reports. The observed hypophosphatemia in chromium exposed fish derives support from the studies of earlier investigators who have also recorded a decrease in blood phosphate content after exposure of the fish to synthetic pyrethroids (deltamethrin- Srivastav et al. 1997; cypermethrin- Mishra *et al*. 2002), heavy metal (cadmium- Rai and Srivastav 2003) and botanical pesticides (azadirachtin- Kumar et al. 2011a; *Euphorbia tirucalli*- Kumar et al. 2011b; *Euphorbia royleana*- Prasad et al. 2011a; *Nerium indicum*- Prasad et al. 2011b). Contrary to it, hyperphosphatemia has been reported after exposure of fish to various toxicants- cypermethrin (Pandey et al. 2009), eldrin (Colvin and Philips 1968), cadmium (Larsson et al. 1976), endosulfan (Gill et al. 1991), aldrin (Singh et al. 1996), propoxur (Singh et al. 1997) and formothion (Singh et al. 1997). No change has been noticed in the plasma phosphate levels of fish exposed to cadmium in water (Pratap et al. 1989). Increased mucus secretion which forms a coating on gill surface has been noticed in the chromium exposed *H. fossilis*. It has been reported that mucus is not a barrier to Ca^{+2} (Part and Lock 1983; Mayer-Gostan and Noan 1992). The observed mucus coating on gill may not be possible cause for decreased gill calcium influx resulting into hypocalcemia. Hypocalcemia in chromium exposed *H. fossilis* could be attributed to the impairment of net electrolyte

influx either at the gill and/or renal function. Gills have been noticed to undergo degeneration after pesticide exposure to fishes (Wedmeyer and Yasutake 1974; Voyer et al. 1975; Engelhardt et al. 1981; Tuurala and Soivio 1982; Woodward et al. 1983; Nath 1985; Srivastava et al. 1989; Karan et al. 1998; Erkman et al. 2000; Cengiz and Unlu 2002, 2003; Fanta et al. 2003; Ortiz et al. 2003; Cengiz 2006; Camargo and Martinez 2007; El-Sayed and Saad 2007; Velmurugan et al. 2007; Adeyemo 2008; Tennyson et al. 2008; Jayachandran and Pugazhendy 2009; Kumar et al. 2010). The surface for ionic permeability is decreased due to gill degeneration which caused reduction in blood electrolyte levels. Kidney of fish exposed to various pollutants has been reported to show degenerative features by several investigators (Verma et al. 1975; Goel and Garg 1977; Sukumar and Karpagaganapathy 1986; Gill et al. 1988; Srivastava et al. 1990, Akram et al. 1999). In the present study hypocalcemia and hypophosphatemia noticed in chromium exposed *H. fossilis* could be linked to the damage caused to the kidney. Due to kidney degeneration reabsorption of these ions is reduced hence enhanced efflux of these ions may have resulted into hypocalcemia and hypophosphatemia in chromium treated *H. fossilis*. Earlier Koyama and Itazawa (1977), Roch and Maly (1979), Haux and Larsson (1984) and Akram et al. (1999) have also suggested kidney damage to be the possible cause for hypocalcemia noticed in cadmium exposed fish.

Conclusion

Based on the findings of this study, we can conclude that exposure to chromium chloride adversely affected the serum calcium and phosphate of the fish. The disturbances in these vital electrolytes could affect the general physiology of the fish as these ions are necessary for vital functions in organisms.

Conflict of interest The authors declare no conflicts of interest.

Authors' contribution (Ajai) coordinate of project, supervision, review, text edition and draft the paper; (Agarwal) conducted the bioassay and laboratorial analysis; (Abhishek) conducted the bioassay and laboratorial analysis; (ManiRam) conducted the bioassay and laboratorial analysis; (Sunil) review and statistic; (Suzuki) text edition and draft the paper.

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