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Original Article

Alterations in Protein Content in three Different Vital Organs of a Freshwater Snail, *Pila globosa* Exposed to Cadmium Chloride

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Abstract

Alterations in protein content was studied in freshwater bivalve,*Pila globosa* exposed to three different concentrations (*viz.*, 0.002%, 0.005% and 0.01%) of cadmium chloride ($CdCl_2$) in mantle, foot, and liver at three different fixation intervals (*viz.*, 7d, 14d and 21d). There was a significant decrease in protein content in all the three vital organs of *P. globosa* under experimentation as the concentration of $CdCl_2$ increases. The depletion of protein content was due to the toxicity of $CdCl_2$ and this toxicological stress might have increased the proteolysis activities in the cells.

Keywards: Protein Content; *Pila globosa*; Toxicity; Cadmium Chloride.

Introduction

Increasing level of pollution and its impact on living organisms has become a subject of great concern. Heavy metals pose a serious threat to the aquatic environment because of their toxicity, persistence, tendency to accumulate in organism and undergo food chain amplification (Weis and Weis 1977 a, b).They cause severe damage to the aquatic fauna, including molluscs, fishes etc, thereby telling up on their health and population. Cadmium (Cd) is one of the most toxic and widespread heavy metal, and is a recognized carcinogen in mammals (Pruski and Dixon, 2002). Cd reaches the water bodies from combustion of fuels, and plastics, phosphate fertilizers, pesticides, domestic wastes, oil refineries and electroplating industries. Amongst the heavy metals, the chloride, sulphate and nitrates of cadmium are soluble compounds whereas carbonate and hydroxides are not. Nica et al. (2012) advocated that Cd pose serious threats to environmental health because they tend to bioaccumulate in terrestrial ecosystems. Cd has also attracted a lot of attention as a soil pollutant because of its persistence, toxicity and bioaccumulative potential along terrestrial trophic chains (Veltman et al., 2008; ATSDR, 2012). It affect the activity of biologically active molecule such as glycogen, protein and lipid of target organisms (Ghosh and Chatterjee 1985; Devaraj and Devaraj 1987).

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Among soil-dwelling invertebrates, land snails closely adhere to preconditions for serving as a pertinent bioaccumulator for soil Cd (Berger and Dallinger, 1989; Gomot, 1997; de Vaufleury et al., 2006). These molluscs are able to sequestrate and detoxify Cd via complexation to specific Cd metallothioneins (Dallinger et al., 2001), and hence can accumulate Cd far above environmental concentrations without showing any metabolic disorders (Dallinger and Rainbow, 1993). Rao Ramana and Ramamurthi (1980) have observed the effects of sumithion on biochemical constituents in *Pila globosa*, but reports on such effects of $CdCl_2$ is limited.

Even though some reports are available on the effects of Cd toxicity in different groups of animals, but a comparative study of the effect of different concentrations of $CdCl_2$on the protein content of some vital organs of *Pila globosa,* an important food chain invertebrate of fresh water body are scanty, thus the present experiment was undertaken.

Materials and Methods

Live specimens of *Pila globosa* (Phylum: Mollusca; Class: Gastropoda; Family: Ampullariidae) weighing between 20 and 25 g were collected from a local fresh water pond and acclimated in concrete vat containing fresh tap water for ten days before using them for experimentation. After that the specimens were removed into 15 L glass aquaria each containing 10 L tap water (pH 7.25±0.14, total hardness 95±2.08mgL^{-1} as $CaCO_3$, dissolved oxygen 6.56±0.75 mgL^{-1}, temperature 28 - 30^{0}C) and five number of *Pila*. Prior to experiment they were cleaned to remove the fouling algal biomass and mud. Healthy and mature snails of approximately equal size were selected. Five live specimens each kept in the aquaria with three different optimally low doses that produced fairly quantifiable changes (obtained through range-finding trials) of Cadmium chloride ($CdCl_2$) (Sigma Aldrich, Cat. No. 10108-64-2): (1) 0.002 %; (2) 0.005%; (3) 0.01%. *Pila* kept in fresh tap water was served as control. The specimens were fed with *ad libitum* with pieces of an aquatic plant *Hydrilla* till sacrificed. After 7d, 14 d and 21 days of treatment the experimental *Pila* were sacrificed and three different vital organs, namely, mantle, foot and liver were removed for protein estimation. As the foot and mantle of *Pila* directly or indirectly exposed to the waterbodies and liver is the main site of metabolism, thus taken for the experiment. For protein estimation the collected tissues were homogenized in 0.9% NaCl. After centrifugation at 3000g for 15 minutes, the supernatants were collected. For estimating the quantity of protein, the techniques of Lowrey et al (1951) using folin-ciocalteu reagent was followed. A standard curve was constructed from the different known concentration of Bovine Serum Albumin (BSA) against OD values. The amount of unknown protein (mg/gm tissue) was calculated in the routine manner (at 550 nm) against the standard curve in the U-V spectrophotometer (Simatzu, Japan).

Statistical Analysis

For calculation of the differences between control and treated series, Student's t-test was conducted and the level of significance determined by using the Fisher and Yates statistical tables (Fisher and Yates, 1963).

Results and Discussion

Heavy metals, particularly Cd has been identified as the major source of aquatic pollution and detected in alarming quantities in many water bodies, particularly at or near industrial localities where effluents are routinely discharged. Tumorigenic, carcinogenic, mutagenic, teratogenic, and other cytotoxic effect of $CdCl_2$ have been extensively studied in both microorganisms and higher animals, particularly in mammals (Kalinina and Ploukhina. 1977; Felten, 1978; Bruce and Heddle, 1979; Deknudt and Gerber, 1979 Leonard, 1979), but such data are extremely limited for molluscs (Shuhaimi-Othman et al., 2012; Nica et al., 2012; Venkata Chandrudu and Radhakrishnaiah, 2008, 2013).

In the present experiment alterations of total protein content of *P. globosa* was found in all the three tissues examined (*i.e.*, foot, mantle and liver) at three different concentrations (*i.e.*, 0.002%, 0.005% and 0.01%) of $CdCl_2$. A critical analysis of the data reveals that the protein content was maximum in the liver tissues as compared to foot and mantle (Table-1, 2 and 3). It was found that in foot the amount of protein was decreased as the concentrations of the $CdCl_2$ increases in all the consecutive fixation intervals (*viz.*, 7d, 14d and 21 d), and the results was found to be significant ($p < 0.05$; 0.01; 0.001) for all the doses (see table-1). More or less similar trend of result was found for mantle and liver tissues (see Table-2 and 3). Previously, the significant decrease in total protein content in foot, hepatopancreas and gills of the fresh water mussel, *Lamellidens corrianus* on exposure to organochlorine insecticide, hildan have been advocated by Kulkarni, et al (2005). In fact, Hightower (1991)

Table 1: Showing the amount of protein (mg/gm) in the foot of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals

Days intervals	% of doses of $CdCl_2$ in respect of control			
	Control	0.002%	0.005%	0.01%
7 days	23.87±0.79	22.78±0.62	21.62±0.19^a	17.87±0.61^c
14 days	25.90±0.40	23.20±0.49^b	24.29±0.11^b	20.09±0.47^c
21 days	22.69±0.72	22.08±0.39	20.95±0.41	18.34±0.52^b

Number of individuals examined in each series/fixation intervals =5; ^{a} $p < 0.05$; ^{b} $p < 0.01$; ^{c} $p < 0.001$.

Table 2: Showing the amount of protein (mg/gm) in the mantle of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals.

Days intervals	Control	% of doses of $CdCl_2$ in respect of control 0.002%	0.005%	0.01%
7 days	14.66±0.36	12.73±0.57[a]	12.43±0.59[a]	11.79±0.60[b]
14 days	15.75±0.48	13.86±0.30[a]	11.82±0.28[c]	11.63±0.45[c]
21 days	15.03±0.25	13.22±0.41[b]	12.50±0.57[b]	10.10±0.41[c]

Number of individuals examined in each series/fixation intervals =5; [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

Table 3: Showing the amount of protein (mg/gm) in the liver of *P. globosa* in three different doses of $CdCl_2$ in respect of control at three different fixation intervals

Days intervals	Control	% of doses of $CdCl_2$ in respect of control 0.002%	0.005%	0.01%
7 days	35.23±0.08	34.71±0.22	33.62±0.52[a]	30.96±0.31[c]
14 days	36.67±0.04	35.98±0.45	35.53±0.80	33.91±0.38[c]
21 days	34.12±0.39	32.33±0.19[b]	32.10±0.39[b]	31.71±0.57[b]

Number of individuals examined in each series/fixation intervals =5; [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

introduced the term "proteotoxicity" as a central aspect of toxicity, which takes place at the level of protein. A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein due to the proteotoxic effect of $CdCl_2$. On the other hand, the decrease in average total protein content of tissue after treatment suggests enhancement of proteolysis to meet the high energy demands under Cd or other stress (Patil, 2011). The depletion of tissue protein content was might be due to diversification of energy to meet the impending energy demand under toxic stress (Vincent et al., 1995). The result of the present experiment clearly demonstrated that the depletion of protein content in the vital organs of *Pila* was due to the toxicity of $CdCl_2$.

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