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Effect of Heavy Metal Cadmium on Total Protein Content, Amino Acid Content and Alanine Aminotransferase Activity of Freshwater Snail, *Pila globosa*

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ABSTRACT

Adult freshwater snails *Pila globosa* (Gastropod, Ampullariidae) were exposed for a six-day period in laboratory conditions to cadmium toxicity. The effect on the total protein content, amino acid content and activity of alanine aminotranferase (ALT) with increase in the treatment period were calculated. A remarkable decrease in the protein content (Control 74.01; 48hrs 59.99; 96hrs 56.49; 144hrs 50.03 mg/ml/gm wet wt. tissue) and a significant increase in amino acid content have been recorded for each treatment period (48hrs 18.01; 96hrs 25.76; 144hrs 31.36 mg/ml/gm wet wt. tissue) in comparison to control (8.43 mg/ml/gm wet wt. tissue). Cadmium was found to have a synergistic effect on activity of ALT. The activity of ALT significantly increases with increase in the treatment period (48hrs 0.0007; 96hrs 0.0016; 144hrs 0.0035 unit/ml/min) as compared to the control (0.0004 unit/ml/min). The results clearly suggested that under the toxicity stress of the heavy metal cadmium, proteins are gradually degraded into their corresponding amino acids. The increased ALT activity with increase in amino acid content is a clear indication of transamination reaction where corresponding keto acids are formed from amino acids that ultimately enters the citric acid cycle in which electrons are abstracted. Electrons are carried by NADH and $FADH_2$ which are funnelled into a chain of mitochondrial electron carrier reducing O_2 TO H_2O. This electron drives the production of ATP which is required by the treated snails to meet their high energy demand during the heavy metal stress.

Keywords: *Pila globosa*, Protein, Amino acid, Alanine Aminotranferase, Cadmium, Keto acids.

I. INTRODUCTION

The aquatic environment is highly fragile, complex and diverse. Aquatic toxicology has been defined as the study of the effects of chemicals and other toxic agents on aquatic organisms with special emphasis on adverse or harmful effects. A toxicant or foreign substance (i.e., xenobiotic) may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of the water and making it unfavourable for aquatic life. Toxicity is a relative property reflecting a chemicals potential to have a harmful effect on a living organism. Aquatic ecosystems are progressively coming under permanent pressure of anthropogenic pollutants and heavy metal contamination of aquatic ecosystems is a worldwide problem posing health hazards not only to the inhabitant organisms but also to the non-target populations including human beings through food chains and food webs.

Many chemicals released into the environment are able to generate toxicity in aquatic organisms. Heavy metals constitute one of the major contaminants, which regularly find their way into the aquatic ecosystem. Metals, being elements, cannot be broken down or destroyed by degradation; instead they accumulate within the environment in different forms. In aquatic systems, the heavy metals of greatest concern are copper, zinc, cadmium, mercury and lead. Many of them are essential for metabolism at lower concentration and are vital for enzymatic activity and as respiratory pigments in organisms. However, all essential trace metals become toxic when their concentration exceeds a threshold value. Managing metal contamination requires an understanding of the concentration dependence of toxicity. Toxicity tests are, therefore, used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions that permit

comparison with other chemicals or species tested, and comparison of similar data from different laboratories.
The class bivalvia is of interest in pollution studies, as it comprises sedentary filter feeding invertebrates, which are likely to accumulate pollutants from the environment. Bivalves have received extensive attention in the literature owing to their reported ability to reflect environmental levels of trace metal contaminants in marine and estuarine ecosystems. Bivalves exhibited several characteristics of an ideal indicator species [1, 2] including the following: ability to accumulate high concentration of contaminants without dying; a sedentary life history; high numerical abundance; sufficient life span to permit sampling of more than one year and throughout the monitoring period; large size so that ample tissue is available for analysis; and good adaptation to laboratory conditions. Since mussels are a group of major fouling organisms, they have been the subject of many toxicological investigations. The ability of mollusc to concentrate high amount of heavy metals without any apparent bad effects makes these animals very dangerous to their predators [3]

The freshwater snail, *Pila globosa* is common in freshwater canals and ponds. It is also a favourite food of some tribes. In a freshwater ecosystem, *Pila globosa* may be used as bioindicator of heavy metal contamination freshwater resources for several reasons. First, it lives in static water, and is therefore easy to use as an experimental animal. Second, it is a filter feeder and accumulates pollutants including heavy metals in its tissues. Third, it is abundant and plays important roles in the environment. Keeping the above facts in view, the present study was conducted to determine the acute toxicity of cadmium to the freshwater snail *Pila globosa* with relation to total protein and amino acid content and ALT activity.

II. METHODS AND MATERIAL

The snails were cultured in a glass tank of size under proper conditions of aeration and care. The standard treatment solution (2mg/kg) of cadmium was prepared in 500ml distilled water from analytical grade metallic slats of $CdCl_2$. $5H_2O$. Toxicity experiments were performed for a six-day period using adult snails (shell length approximately 2.2-3.8cm) obtained from stocking tanks. A control with dechlorinated tank water only was also used. A total of 5 animals per 48 hrs treatment were used in the experiment and a total of 100 animals (50 snails were kept as control and 50 in the experimental set up) were employed in the investigation. The snails in the experimental tank were kept in stressed condition for 48 hour, 96 hour and 144 hour respectively. Any dead animals were removed immediately. After obtaining the tissues from respective hours of treatment, it was washed with cold normal saline water, dried in blotting paper, and then stored in deep freeze till use.

A 20% homogenate of all the tissues (control, 48hrs, 96hrs and 144hrs of cadmium treatment) were prepared in ice cold 0.25M sucrose solution at $\pm 2°$ C using potter elvehjem homogenizer fitted with Teflon pestle. The homogenate thus obtained were centrifuged at 5000g for 10 minutes to remove tissue debris. The volume of the supernatant was measured and preserved as the source of enzyme at <4º C for assay of enzyme activity and Km. Protein content of each fraction were determined by Lowry's method [4]. Ninhydrin test was used to determine the content of amino acids in both control and treated tissues [5]. Separate pairs of test tubes were labelled and taken for enzyme assay. The enzyme assay for Alanine Aminotransferase was estimated using Dinitrophenyl Hydrazine method of aminotransferase assay [6]. The assay is based on the formation of pyruvate in the transamination reaction of alanine and alpha-keto gluterate catalysed by ALT. The optical density of the colored complex was measured in a spectrophotometer at 520nm.

The data generated were analyzed by using student's t-test to examine the validity of hypothesis which was to observe whether there is any significant difference in protein content, amino acid content and enzyme activity of alanine aminotransferase in treated group as compared to control. The t- test is performed at $P<0.05$ confidence level.

III. RESULT AND DISCUSSION

The results of Lowry method for protein estimation showed a significant decrease ($P<0.05$) in the protein content of soft tissues of the treated animals in comparison to that of the control (Fig.1). The highest protein content has been recorded for the control, 74.01mg/ml/gm wet wt. tissue followed by 48hrs

(59.99mg/ml/ gm wet wt. tissue) and 96hrs (56.49mg/ml/gm wet wt. tissue) and the lowest level was recorded in 144hrs (50.03mg/ml/gm wet wt. tissue) of cadmium exposure.

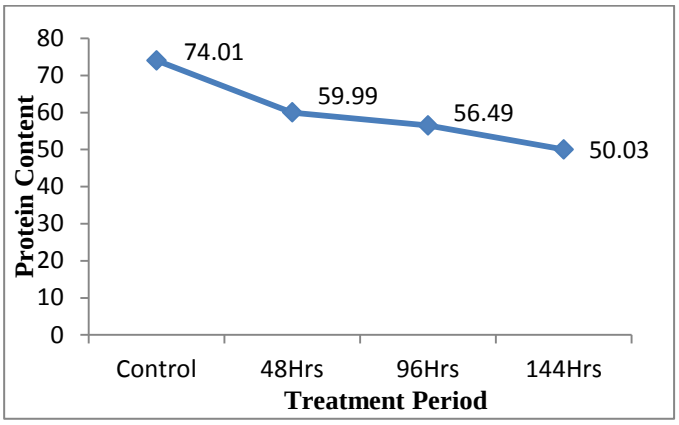

Figure 1. Line diagram showing effect of cadmium on the total protein content (mg/ml/gm wet wt. tissue)

Ninhydrin test for amino acid estimation showed a significant increase ($P<0.05$) in amino acid content in response to cadmium toxicity in the treated groups (Fig.2). In control, the level of amino acid was recorded 8.43mg/ml/gm wet wt. tissue. In the treated group, the amino acid content was observed to be gradually increased with increase in treatment period of cadmium toxicity and the highest level was recorded in 144hrs (31.36 mg/ml/gm wet wt. tissue) followed by 96hrs (25.76 mg/ml/gm wet wt. tissue) and 48hrs (18.01 mg/ml/gm wet wt. tissue) respectively.

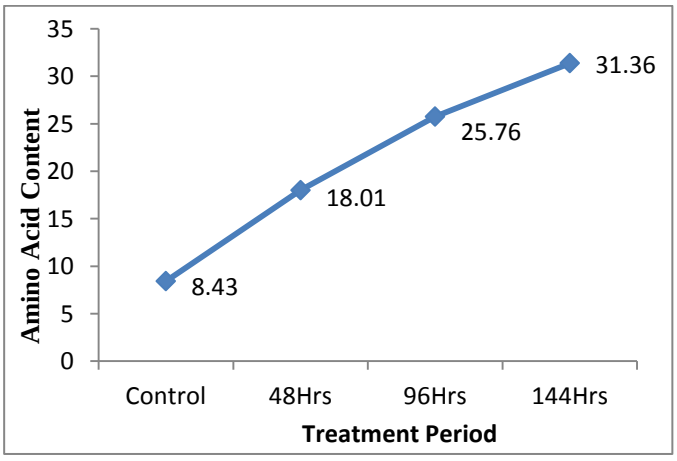

Figure 2. Line diagram showing effect of cadmium on the amino acid content (mg/ml/gm wet wt. tissue)

The effect of cadmium on activity of ALT was found to be synergistic. The activity of ALT significantly increases ($P<0.05$) with increase in the treatment period as compared to the control (Fig.3). The lowest activity was observed in the control tissues (0.0004unit/ml/min) followed by a significant increase in 48hrs (0.0007unit/ml/min), 96 hrs (0.0016unit/ml/min) and 144hrs (0.0035unit/ml/min) respectively.

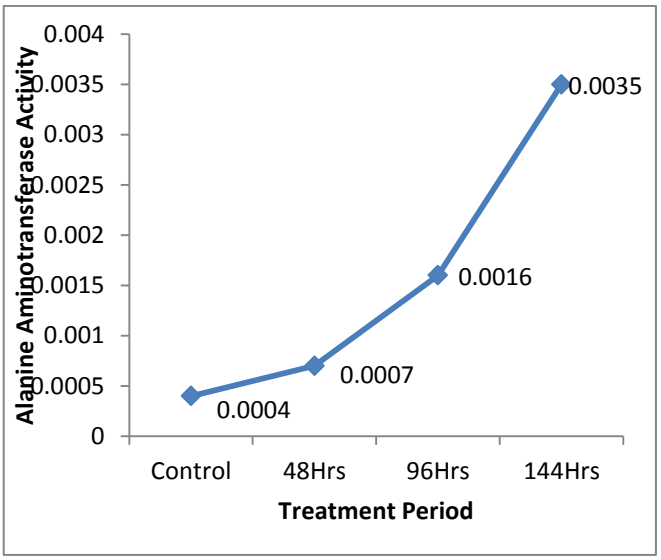

Figure 3. Line diagram showing effect of cadmium on Alanine Aminotransferase Acivity (unit/ml/min)

Proteins, the essential biomolecules are made up of 20 amino acids. Under stress condition of acute cadmium toxicity, the level of protein decreases while amino acid level increases in the soft tissues of snails treated with heavy metal in comparison to its counterparts in the control. This might happen because under stress condition proteins are broken down to amino acid to meet their metabolic need. Another reason for decreasing level of protein is due to eliminate harmful protein that might accumulate during stress condition which might be toxic to the cell. Under stress condition of starvation and heavy metal treatment, increase in amino acids and glucose level in the edible tissues of snails treated with heavy metal in comparison to its counterparts in the control tank indicates that they are converted to acetyl coA through transamination and glycolysis which enters citric acid cycle in which electrons are abstracted. Electrons are carried by NADH and $FADH_2$ which are funnelled in to a chain of mitochondrial electron carriers reducing O_2 to H_2O. This

electron drives the production of ATP which is required by the treated snails to meet their high energy demand.

Decrease or increase in enzyme activity represents stress in any organism that results in metabolic burden. Aminotransferase activity has been reported to be sensitive to heavy metal pollutants [7]. In the present investigation, the activity of alanine aminotransferase was found to increase in the experimental snails when compared with that of the control snails. The data observed during the research work clearly suggested a significant effect of cadmium not only on total protein and amino acid content but also on ALT activity of the organism. The higher activities of ALT as observed on 144hrs of exposure to cadmium suggest that an extended period of contact can lead to stress in organisms.

IV. CONCLUSION

Exposure to heavy metals evokes several behavioral, physiological, and biochemical changes that appear to be closely related. To counteract any stress, energy reserves, which might otherwise be utilized for growth, and reproduction will have to be diverted towards enhanced synthesis of detoxifying ligands (metal binding proteins, granules), or expended in order to maintain an elevated efflux of metal. Consequently, various enzymes related to energy metabolism alter their activity pattern depending on the nature of stress. Excess energy is required to carry out defensive behavioral responses that help animal to adapt and survive. This confers some confidence in quantifying metabolic changes in the energy parameters, and related enzyme activities as integrated markers of healthy physiological status.

Proteins are the most abundant organic molecules of living system and form the basis of structure and function of life. Proteins have many different physiological functions in bivalves. They are associated with enzymes, transport, and regulation of metabolism, defense, structural elements, and storage, and hence represent an important biochemical constituent in mussel haemolymph. The apparent sensitivity of ALT suggests that analysis of enzyme activity in body tissue can be used as biomarker in metal pollution monitoring. This base line data together with a comparison of other enzyme levels from field samples in pollution monitoring studies endow on them the status of a promising biomarker in aquatic toxicology.

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