Toxic Effects of Cadmium on the Acid and Alkaline Phosphatase Activity of Female Fiddler Crab, \textit{(Uca annulipes)}

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\textbf{Abstract:} The present study was conducted to determine the toxic effects of cadmium on the quantitative aspects of alkaline phosphatase (ALP) and Acid Phosphatase (ACP) activity of intermoult female fiddler crab, \textit{Uca annulipes}. The experimental crabs were exposed to sublethal concentrations (1/3rd and 1/10th of 96h LC\textsubscript{50}) of cadmium (0.013 and 0.04 ppm) for a period of 15 and 30 days showed marked changes in enzyme composition as compared to control. Samples were taken from the ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph of representative crabs from each test and control groups on 15 and 30 days of experiment. The acid phosphatase activity was enhanced and alkaline phosphatase activity was inhibited in ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph was observed as compared to control. The data strongly suggests that cadmium is toxic to \textit{Uca annulipes} since, it seriously impairs the metabolic functions, resulting in quantitative alteration of ACP and ALP. As remedial measures need to be adopted to prevent contamination of the aquatic environment by Cd to harmless the deleterious effects on the aquatic biota.

\textbf{Key words:} Cadmium \cdot Alkaline Phosphatase \cdot Acid Phosphatase \cdot \textit{Uca annulipes} \cdot Enzymes

\textbf{INTRODUCTION}

Cadmium (Cd) is one of the most toxic heavy metals for humans. The main source of non-occupational exposure to Cd includes smoking and air by which food and water are contaminated by cadmium [1]. In addition, herbal medicine is another source of Cd. The world Health Organization (WHO) estimate that 4 billion people or 80\% of the world Population presently use herbal medicine [2]. In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural source [3, 4]. It can be accumulated in aquatic animals (e.g crabs, shrimps, oyster and muscle) after entering through different ways such as respiratory tract, digestive tract surface penetration etc [5, 6]. It is seriously harmful to the growth of aquatic life and survival resulting in decline of their populations. Cd could lead to acute or chronic intoxication of organisms and causes a variety of adverse effects, such as functional changes in the porcine renal proximal tubular epithelial cell line [7] and Cd induced hepatopancreatic cell necrosis and apoptosis in crab [8]. In addition, Cd could cause an increase of reactive oxygen species that challenge the cellular antioxidant system and lipid peroxidation in crustaceans [9-11]. At the same time, aquatic food products and animal exposed to Cd might threaten human health.

Various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may causes adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels [12]. When any aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome toxic stress. The effect of toxicants on enzymatic activity is one of the most important biochemical parameters under stress. The enzyme activity may be increased or it may be inhibited due to the active site being either denatured or distorted [13].
Heavy metals are known for their strong action on biological tissues [14]. Metal ions once absorbed into the body are capable of reacting with a variety of active binding sites and then disrupting the normal physiology of an organism which may lead to the death of the organisms. The toxic effect of heavy metals on enzyme system depend on the capacity of toxicants to react with ligands, which is essential for the normal functioning of enzymes. Thus enzyme bioassay can provide diagnostic tool to assess a change or damage caused to organism due to administration of heavy metals [15].

Stress responses occur in all animals when regulated Physiological systems are extended beyond their normal range by external stressors. Failure of all or part of the integrated homeostatic response may lead to increasing physiological disturbance and ultimately death. Indicators of such stress responses are therefore useful in assessing the short-term well-being or long-term health status of an animal and such indicators have received considerable attention in commercially important crustacean species [16]. The objective of the present study an attempt was made to study the effect of cadmium on some biomarker enzymes profiles in brackish water fiddler crab *Uca annulipes*.

### MATERIALS AND METHODS

The live of female fiddler crab *Uca annulipes* were collected from Pulicat Lake of Tamilnadu. These were collected to clayey area of the intertidal zone using a shovel and the specimens were captured during early morning hours by hand. The crabs were acclimatized to normal laboratory condition under normal day/night (12L:12D) illumination for one week in plastic troughs (18”diameter) with sufficient artificial sea water so that crabs are submerged. The water was changed periodically and crabs were fed with boiled beef liver. Water conditions during acclimatization and the experimental period were at temperature of 27± 3°C a salinity of 30 ppt, dissolved oxygen 6.8±0.22 mg/l and PH of 7.15± 0.72. Before experimentation healthy internoult stage (C-3) female crabs having equal carapace width (9.8-15.4mm) and weight (1.75-1.90g) were used for experimentation.

Stock solution of cadmium chloride (Merk) was prepared by dissolving appropriate amount of distilled water.

#### Toxicity Bioassay:

Acute toxicity test was performed to determine the potency of cadmium for static but renewal type of bioassay was adopted in the present investigation to estimate the LC$_{50}$ values (Table 1). The experiment was carried out to find the range of concentrations for confirmatory evaluation. The LC$_{50}$ was calculated by using probit analysis [17]. The mortality was recorded for the crab 24, 48, 72 and 96 h exposure to cadmium were corrected for natural response by abbot’s formula [18]. The crabs were exposed to sublethal concentrations (1/10$^6$ and1/3$^3$)  96 h LC$_{50}$ 0.013 and 0.04 ppm of cadmium for 15 and 30 days. Simultaneously control group of crabs were also maintained. The test media was changed daily to maintain cadmium concentration. The crabs were fed during chronic exposure with boiled beef liver twice a week.

#### Estimation of Enzymes (ACP and ALP) Activity:

At the and of exposure period, the ovary, spermatheca, hepatopancreas, muscle, gill were removed, weighed before homogenization using 10ml, chiled distilled water and centrifuged at 3000 rpm for 15 minutes. The supernatant was then used for enzymatic studies. The haemolymph was collected by using sterilized hyperdermic needle rinsed with 0.2% EDTA to avoid coagulation [19]. Acid phosphatase (ACP) and alkaline phosphatase (ALP) was analyzed according to the method of Tennis wood [20]. The optical density was measured at 415 nm in Baush and spectronic 21 spectrophotometer. The values are expressed µg PNPP to PN/P/ mg wet tissue and µg PNPP/ml haemolymph.

#### Statistical Analysis:

The obtained data are statistically analyzed by one-way analysis of variance (ANOVA) was performed based on the method of devised by Winer [21].

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Upper confidence limits (UCL) (ppm)</th>
<th>Lower confidence limits (LCL) (ppm)</th>
<th>Regression results</th>
<th>Slope function (S)</th>
<th>Correlation co-efficient square ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.055</td>
<td>0.061</td>
<td>0.049</td>
<td>Y=-2.153x + 9.546</td>
<td>1.276</td>
</tr>
<tr>
<td>48</td>
<td>0.048</td>
<td>0.053</td>
<td>0.042</td>
<td>Y=-1.485x + 9.546</td>
<td>1.277</td>
</tr>
<tr>
<td>72</td>
<td>0.044</td>
<td>0.050</td>
<td>0.038</td>
<td>Y=-0.524x + 8.605</td>
<td>1.306</td>
</tr>
<tr>
<td>96</td>
<td>0.038</td>
<td>0.044</td>
<td>0.032</td>
<td>Y=0.670x + 7.43</td>
<td>1.339</td>
</tr>
</tbody>
</table>

Table 1: The LC$_{50}$ values and regression equation for *U.annulipes* exposed to cadmium.
RESULTS

ACP Level and Dependent Enzyme Activities in Response to Cadmium Exposure

Ovary: The acid Phosphatase activity (ACP) found in control crab was 8.51 and 8.53 µg PNPP to PNP/100mg, where after exposed to lower sublethal concentration of cadmium ACP activity was 10.60 and 11.16 µg PNPP to PNP/100mg and for higher concentration was 11.02 and 12.01 µg PNPP to PNP/100mg at 15 and 30 days exposure respectively. However, the ACP activity increased 2.63% and 3.48% was observed higher sublethal concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

Spermatheca: The level of ACP found in the control crabs was 4.35 and 4.38 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ACP level was 5.11 and 5.18 µg PNPP to PNP/100mg and for higher concentration was 5.15 and 5.94 µg PNPP to PNP/100mg at 15 and 30 days of exposure respectively. However, the increased 0.80% and 1.56% ACP activity was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

Hepatopancreas: The activity of ACP found in the control crab was 5.08 and 5.10 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ACP activity was 6.62 and 7.01 µg PNPP to PNP/100mg and for higher concentration was 6.84 and 7.42 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the increased 1.05% and 1.52% was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

Muscle: The level of ACP found in the control crabs was 3.22 and 3.25 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ACP level was 3.87 and 4.10 µg PNPP to PNP/100mg and for higher concentration was 4.05 and 4.46 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the increase in the ACP level 0.49% and 0.79% was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

Gill: The ACP activity found in the control crabs was 3.22 and 3.25 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ACP activity was 3.87 and 4.10 µg PNPP to PNP/100mg and for higher concentration was 4.05 and 4.46 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the increase in the ACP activities 1.50% and 1.85% were found at higher concentration after 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

Haemolymph: The ACP activity found in the haemolymph of the control crabs was 2.04 and 2.06 µg PNPP to PNP/ml, where after exposed to lower concentration of cadmium ACP activity was 2.80 and 3.11 µg PNPP to PNP/ml and for higher concentration was 3.08 and 3.58 µg PNPP to PNP/ml at 15 and 30 days respectively. However, the increase in the ACP activity 1.05 % and 1.52 % was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 a-d).

ALP Level and Dependent Enzyme Activities Response to Cadmium Exposure

Ovary: The Alkaline Phosphatase (ALP) activity found in the ovary of the control crab was 6.30 and 6.32 µg PNPP to PNP/100mg. Where after exposed to lower sublethal concentration of cadmium ALP activity was 5.48 and 4.98 µg PNPP to PNP/100mg and for higher concentration was 5.20 and 4.65 µg PNPP to PNP/100mg at 15 to 30 days respectively. However, the ALP activity decreased 1.10 % and 1.67 % was observed at higher sublethal concentration at 15 and 30 days exposure respectively as compared to control (Fig-2 e-h).

Spermatheca: The ALP activity in the spermatheca of the control crab was found to be 2.55 and 2.56 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ALP activity was 2.28 and 2.16 µg PNPP to PNP/100mg and for higher concentration was 2.22 and 2.13 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the ALP activity decreases 0.33 % and 0.43 % were found at higher concentration at 15 and 30 days exposure respectively as compared to control (Fig-2 e-h).

Hepatopancreas: The activity of ALP found in the control crabs was 4.83 and 4.84 µg PNPP to PNP/100mg, were after exposed to lower concentration of cadmium ALP activity was 3.60 and 2.82 µg PNPP to PNP/100mg and for higher concentration was 3.60 and 2.82 µg PNPP to PNP/100mg at 15 to 30 days respectively. However, the ALP activity decreased 1.23 % and 2.02 % was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig-2 e-h).
Fig. 2(a) ACP activity in the different tissues of *U. annulipes* exposed to lower sublethal concentration of cadmium for 15 days

Fig. 2(b) ACP activity in the different tissues of *U. annulipes* exposed to lower sublethal concentration of cadmium for 30 days

Fig. 2(c) ACP activity in the different tissues of *U. annulipes* exposed to higher sublethal concentration of cadmium for 15 days

Fig. 2(d) ACP activity in the different tissues of *U. annulipes* exposed to higher sublethal concentration of cadmium for 30 days
Fig. 2(e) ALP activity in the different tissues of *U. annulipes* exposed to lower sublethal concentration of cadmium for 15 days.

Fig. 2(f) ALP activity in the different tissues of *U. annulipes* exposed to lower sublethal concentration of cadmium for 30 days.

Fig. 2(g) ALP activity in the different tissues of *U. annulipes* exposed to higher sublethal concentration of cadmium for 15 days.

Fig. 2(h) ALP activity in the different tissues of *U. annulipes* exposed to higher sublethal concentration of cadmium for 30 days.
**Muscle:** The ALP activity found in control crabs was 4.37 and 4.39 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ALP activity was 3.88 and 3.60 µg PNPP to PNP/100mg and for higher concentration was 3.64 and 3.36 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the ALP activity inhibited 0.73 % and 1.03 % was observed at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig.2 e-h).

**Gill:** The level of ALP found in the control crabs was 4.73 and 4.78 µg PNPP to PNP/100mg, where after exposed to lower concentration of cadmium ALP level was 3.94 and 3.50 µg PNPP to PNP/100mg and for higher concentration was 3.70 and 2.99 µg PNPP to PNP/100mg at 15 and 30 days respectively. However, the decreased 1.03 % and 1.79 % was observed at higher sublethal concentration at 15 and 30 day of exposure respectively as compared to control (Fig-2 e-h).

**Haemolymph:** The ALP activity found in the haemolymph of the control crabs was 2.85 and 2.88 µg PNPP to PNP/ml, were after exposed to lower concentration of cadmium ALP activity was 2.31 and 2.16 µg PNPP to PNP/ml and for higher concentration was 2.22 and 1.90 µg PNPP to PNP/ml at 15 and 30 days respectively. However, the ALP activity decreases 0.63 % and 0.98 % were found at higher concentration at 15 and 30 days of exposure respectively as compared to control (Fig.2 e-h).

**DISCUSSION**

Toxicants causes a disturbance in the physiological state of the animal which affects enzyme activity. Toxicants bring about distortions in the cell organs, which may bring about elevation or inhibition in the activity of various enzymes. The toxic effect of heavy metals on enzyme system depends on the capacity of toxicants to react with ligands, which is essential for the normal functioning of enzymes. Thus enzyme bioassay can provide diagnostic tool to assess a change or damage caused to organism due to administration of heavy metals [22].

In the present study, we evaluate the possible impact of cadmium showed that ACP and ALP activity altered significantly in various tissues of *Uca annulipes* under stress. The acid phosphatase activity was increased in all the tissues through out exposure periods in both concentrations and its time dependent. The highest increase observed in hepatopancreas (2.32%) followed by ovary (Fig.2 d). Acid phosphatase is a lysosomal enzyme that hydrolyse the phospho-esters in the acid medium. The intracellular distribution patterns of enzymes in the rat liver tissue and reported that generally the decreased activity of ACP activity attributed to the activation of enzyme, which was kept in latent state inside the membrane of lysosomes [23]. Phosphatase play an important role in carbohydrate metabolism [24]. The increase in acid phosphatase activity due to accumulation of mercury in the lysosomes and blockage in the release of enzymes and carbohydrate forms the reverse of many crustaceans accumulated in the hepatopancreas [25].

Increased ACP activity in different tissues of food fish *clarias batrachus* exposed to chlorpyrifos was also reported [26]. Similar reports observed in fresh water crab, *Spiralothelphusa hydromora* treated with the pesticides, cypermethrin [27]. In the present study, the increased ACP activity was observed in lower and higher sublethal concentrations of cadmium in both 15 and 30 days of treatment. The increased ACP activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphatase to release energy in view of impaired ATPase system during cadmium stress.

Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden [28]. Increased Acid Phosphatase activity was reported earlier [29] in another freshwater crab, *Oziotelphusa sensex sensex* under sumithion stress.

The Alkaline phosphates activity was decrease in all the tissues throughout exposure period in both concentrations. The highest decrease observed in Hepatopancreas (2.02%) followed by gill and ovary (Fig. 2 h). ALP is a non-specific enzyme in the tissue especially liver that plays an important role in dephosphorylation of organic compounds. In higher animals this enzyme is involved in bone formation and in membrane transport [29]. Further more, ALP is involved in the synthesis of nuclear protein, nucleic acid and phospholipids [30]. ALP is involved in carbohydrate metabolism, growth and differentiation, protein synthesis, Synthesis of certain enzymes secretion activity and transport to phosphorylated intermediates across the cell membrane [31]. Thus, any alteration in the activity of ALP affects the organisms.

Water is one of the basic requirements of all aquatic as well as terrestrial lives for their growth and survival. Aquatic systems are contaminated by disposal of various a biotic factors. Heavy metals pesticides by virtue of their
design and application induce a broad spectrum biocidal effect influencing most of the organisms [32]. These pollutants also destroy the quality of aquatic ecosystems and render it unfit for various aquatic organism, particularly freshwater crabs. Among all the heavy metals lead, cadmium and mercury are known to be extremely toxic once dispersed in biosphere, these metals cannot be recovered or degraded. Hence environment effects of metal contamination tend to be permanent [33, 34].

In the results of the present study also indicate that there is another possibility for the decrease in ALP activity in all tissues might be due to inhibition of this enzyme on protein directly or indirectly by cadmium intoxication. The inhibition of ALP activity found in the liver, gall and brain by phenylmercuric acetate could be due to the interaction of chemical with cofactors and regulators of the enzyme [35]. Further, he stated that the observed decreases of ALP enzyme activity probably would facilitate the increased activity of phosphorylation enzyme in the tissue of fish and cause subsequent break down of glycogen for energy release during toxic stress. Similarly in the present investigation, the activity of ALP was found to decrease in the experimental crabs, as compared to control. So it was concluded that toxic effect of cadmium causes disturbance in the physiological status of the crab which leads to the metabolic abnormalities.

ACKNOWLEDGEMENT

Author are thankful to the principal, sir Theagaraya College, Chennai-600 021 for providing necessary facilities in the work.

REFERENCES


