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Research Article

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# Impact of heavy metal induced alterations in Lipase activity of fresh water crab, *Barytelphusa guerini*

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**ABSTRACT**

*Fresh water edible crab, Barytelphusa guerini were exposed to acute dose of mercury chloride and copper sulphate. The aim of investigation was to evaluate the effect of heavy metal, $HgCl_2$ and $CuSO_4$ on the lipase activity in hepatopancreas of B. guerini. The effects were observed and studied seasonally into two groups as control and experimental. At the end of 96hrs the crabs were removed and hepatopancreatic tissue was separated to prepare enzyme extract for assay purpose. The results are reported as unit lipase activity /gm of tissue/ hr at 37°C. In the present investigation, the lipase was significantly altered and progressive decrease in activity was observed due to inhibitory action of $HgCl_2$ and $CuSO_4$. Among the exposed mercuric chloride was found to be more potent inhibitor of lipase activity as compared to control group and copper sulphate induced group. From the results, it is also observed that the enzyme activity is time dependent i.e. summer>winter>monsoon.*

**Keywords:** Acute toxicity, Hepatopancreas, Lipase enzyme, $HgCl_2$, $CuSO_4$, *B. guerini.*

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## INTRODUCTION

Enzymes are referred as highly specialized proteins. Enzymes are the reaction catalyst of biological systems. They have extraordinary catalytic power. They have a high degree of specificity for their substrates, and they functions in aqueous solutions under very mild temperature and pH. Enzymes are one of the keys to understanding how cell survive and proliferate.

The use of heavy metals such as mercury, copper, lead, cadmium etc. in agriculture and industrial areas has been increased tremendously. The reason for such studies is that, the heavy metals have specific binding affinity to sulfhydryl group of the enzymes. This would naturally alter the activity of the enzymes that may range from activation to total inhibition. The metal induced alteration in enzyme activity may be taken as more or less accurate indicator of metal toxicity. The change in enzyme activity further provides significant information about sub cellular biochemical adjustment and consequent ability of animals to adapt any environmental change.

The higher concentration of toxicants brings the adverse effect on aquatic organism at cellular level or molecular level. Ultimately it leads to disorder in biochemical composition, alteration in the functional efficiency of the

nervous system on exposure to heavy metal causes change in the enzyme activity level of carbohydrate and protein metabolism of the organism [1, 5]. Moreover, many other investigators have used enzymological techniques to evaluate the sub lethal stress induced by mercury and other metal pollutants on animals [6, 9]. Hence an attempt has been made to study the effect of mercury and copper on the enzyme secretary activity related to energy yielding processes at cellular level of freshwater crab, *Barytelphusa guerini* with respect to change in level of digestive enzyme lipase.

## EXPERIMENTAL SECTION

Experimental crab, *Barytelphusa guerini* were procured from Nathsagar dam, Paithan, near Aurangabad and brought to laboratory without any mechanical injury. The crabs were maintained for 2-4 days in plastic trough to acclimatize at laboratory condition. During acclimatization the crabs were fed with small pieces of bivalve and earthworm. Only healthy, crabs with similar size and weight were selected for experimental purpose. Crabs were divided into two groups. One group was maintained as control and second was considered as experimental.
The crabs of the experimental group were exposed to heavy metals such as $HgCl_2$ and $CuSO_4$ for 24, 48, 72 and 96 hrs short duration respectively. The water from control and experimental trough was renewed after every 24 hour and dead animals were discarded. The animals were starved a day before experimentation to avoid metabolic differences, if any, due to differential feeding. The $LC_{50}$ values of $HgCl_2$ and $CuSO_4$ for 24, 48, 72 and 96 hrs were 2.75, 2.5, 2.25 and 2.00 ppm and 14.00, 13.5, 13.00 and 12.50 ppm for monsoon season. For winter season, the concentrations were 3.00, 2.75, 2.5, 2.25 and 14.50, 14.00, 13.50 and 13.00 ppm. The concentrations were 1.75, 1.4, 1.25, 1.15 ppm and 9.00, 8.5, 8.00 and 7.5 ppm for summer season respectively.

**Table -1: Lipase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to $HgCl_2$ and $CuSO_4$ in monsoon season**

| Treatment | Control | 24 hrs | 48 hrs | 72 hrs | 96 hrs |
|---|---|---|---|---|---|
| **$HgCl_2$** | 2.990 ±0.0005 | 2.646 ±0.025 (11.50%) *** | 2.160 ±0.02 (27.75%)*** | 1.643 ±0.0015 (45.05%)*** | 1.214 ±0.002 (59.39%)*** |
| **$CuSO_4$** | 2.990 ±0.0005 | 2.760 ±0.02 (7.69%)*** | 2.410 ±0.01 (19.39%)*** | 1.920 ±0.0015 (35.78%)*** | 1.360 ±0.01 (54.51%)*** |

*Enzyme activity is expressed as unit lipase activity /gm of tissue/ hr at 37$^oC$.*
*Each value is the mean of three observations ± S.D.*
*Values are significant at *** P< 0.001*
*Bracket values indicate percent variation over control*

**Table-2: Lipase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to $HgCl_2$ and $CuSO_4$ in winter season.**

| Treatment | Control | 24 hrs | 48 hrs | 72 hrs | 96 hrs |
|---|---|---|---|---|---|
| **$HgCl_2$** | 2.850 ±0.01 | 2.510 ±0.01 (11.92%)*** | 2.066 ±0.057 (27.50%)*** | 1.500 ±0.02 (47.36%)*** | 1.110 ±0.01 (61.05%)*** |
| **$CuSO_4$** | 2.850 ±0.01 | 2.625 ±0.002 (7.89%)*** | 2.180 ±0.01 (23.50%)*** | 1.760 ±0.002 (38.24%)*** | 1.270 ±0.002 (55.43%)*** |

*Enzyme activity is expressed as unit lipase activity /gm of tissue/ hr at 37$^oC$.*
*Each value is the mean of three observations ± S.D.*
*Values are significant at *** P< 0.001*
*Bracket values indicate percent variation over control*

The lipase activity was determined by the method of Sinha [10] based on titrimetric estimation of liberated fatty acids from substrate during enzyme action. The reaction mixture consisted of 1 ml olive oil as substrate, 1 ml of phosphate buffer of pH 8 and 1 ml of tissue homogenate (10% w/v). The reaction mixture was incubated for 1 hr at $37^0$ C with frequent shaking. The enzyme activity was terminated by boiling reaction mixture in water bath. Lipolytic activity was determined by titrating the reaction mixture with NaOH (0.1N) solution after adding 3ml of alcohol (95%) using 0.5% alcoholic phenolphthalein as an indicator. The difference between the volume of NaOH (0.1N) solution utilized in unboiled and boiled homogenate containing reaction mixture indicated the lipase activity.

**Table-3: Lipase activity in hepatopancreas of *Barytelphusa guerini* during acute exposure to $HgCl_2$ and $CuSO_4$ in summer season.**

| Treatment | Control | 24 hrs | 48 hrs | 72 hrs | 96 hrs |
|---|---|---|---|---|---|
| **$HgCl_2$** | 3.110 ±0.015 | 2.670 ±0.02 (14.14%)*** | 2.370 ±0.0015 (23.79%)*** | 2.120 ±0.012 (31.83%)*** | 1.880 ±0.015 (39.54%)*** |
| **$CuSO_4$** | 3.110 ±0.015 | 2.825 ±0.002 (9.16%)*** | 2.490 ±0.01 (19.93%)*** | 2.320 ±0.015 (25.40%)*** | 2.160 ±0.06 (30.54%)*** |

*Enzyme activity is expressed as unit lipase activity /gm of tissue/ hr at 37°C.*
*Each value is the mean of three observations ± S.D.*
*Values are significant at *** P< 0.001*
*Bracket values indicate percent variation over control*

## RESULTS

The seasonal effects of mercuric chloride and copper sulphate on lipase activity in digestive gland of *Barytelphusa guerini* was studied after acute exposure and results are summarized in table no. 1-3. In monsoon season the lipase activity of control, crabs showed (2.990 ± 0.0005) unit lipase/gm of tissue/ hr. In monsoon season the lipase activity of the crabs exposed to mercuric chloride after 24, 48, 72 and 96 hrs showed (2.646 ± 0.025; P<0.001); (2.160 ± 0.02; P<0.001); (1.643 ± 0.0015; P<0.001) and (1.214 ± 0.002; P<0.001). Similarly lipase activity of the crabs exposed to copper sulphate showed (2.760 ± 0.02; P<0.001); (2.410 ± 0.01; P<0.001); (1.920 ± 0.0015; P<0.001) and (1.360 ± 0.01; P<0.001).

In winter season the lipase activity of control crabs showed (2.850 ± 0.01) unit lipase/ gm of tissue/ hr. In winter season the lipase activity of the crabs exposed to mercuric chloride after 24, 48, 72 and 96 hrs showed (2.510 ± 0.01; P<0.001); (2.066 ± 0.057; P<0.001); (1.50 ± 0.02; P<0.001) and (1.110 ± 0.01; P<0.001). Similarly lipase activity of the crabs exposed to copper sulphate showed (2.625 ± 0.002; P<0.001); (2.180 ± 0.01; P<0.001); (1.760 ± 0.002; P<0.001) and (1.270 ± 0.002; P<0.001).

In summer season the lipase activity of control, crab showed (3.110 ± 0.015) unit lipase/gm of tissue/ hr. In summer season the lipase activity of the crabs exposed to mercury chloride after 24, 48, 72 and 96 hrs showed (2.670 ± 0.02; P<0.001); (2.370 ± 0.0015; P<0.001); (2.120 ± 0.012; P<0.001) and (1.880 ± 0.015; P<0.001). Similarly lipase activity of the crabs exposed to copper sulphate showed (2.825 ± 0.002; P<0.001); (2.490 ± 0.01; P<0.001); (2.320 ± 0.015; P<0.001) and (2.160 ± 0.06; P<0.001).

## DISCUSSION

The analysis of the results revealed that the mercuric chloride is highly toxic than copper sulphate and found to be more potent enzyme inhibitor in all seasons. [11] Studied the effect of copper chloride on the enzyme activity of the crab, *Sesarma qudratum*. [12] Reported decrease in amylase activity in *L. marginalis* and explained that mercury chloride and copper chloride are potent inhibitors of amylase activity. [13] Studied the lipase activity level in the freshwater crab, *Barytelphusa guerini* after exposure to two pesticides (Sevin and DDT), reported decrease in the activity level of lipase when compared with the control.

From the above results it was concluded that the decrease activity was time dependent. Similar results have been reported by [14, 15] observed decreased amylase activity due to heavy metal in freshwater bivalve, *Parreysia favidens*. Similar results have been reported by many workers [16] in *Parreysia corrugata*; [17] in *Lamellidens marginalis* and [17] in *Corbicula striatela*. [19] Studied the alterations in the level of dehydrogenase in a freshwater fish, *Tilapia mossambica* exposed to arsenic toxicity.

During the study of enzyme activity in the hepatopancreas of freshwater crabs, *Barytelphusa guerini*, observed significant decrease in lipase activity by the exposure of $HgCl_2$ and $CuSO_4$. The continuous decrease was seen in lipase activity after acute exposure of $HgCl_2$ and $CuSO_4$. It is observed that mercuric chloride is highly toxic than

copper sulphate and found to be more potent enzyme inhibitor. Further, it was concluded from the statistical data given in table 1-3 the decrease activity was time dependent.

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