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Effect of Heavy Metal ($CuSo_4$) on Digestive Enzymes Amylase on Fresh Water Bivalve *Lamellidens Marginalis*

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ABSTRACT

The Freshwater bivalve Lamellidens marginalis from the Godavari river at Paithan were exposed to lethal and sublethal concentration (1.9 ppm and 1.0 ppm) of Copper Sulphate as acute and chronic treatment. The amylase activity in the control bivalves L. marginalis was 7.1281 mg. of maltose/mg protein/hr. of room temperature. In the present investigation heavy metal $CuSo_4$ showed decrease in amylase activity 2.223 mg. at maltose/mg protein/hr at the end of 72 hrs.

Key word's : Heavy metals, Lamellidens, Marginalis amytase.

Introduction

Enzymes are referred as biological, catalysts which obey certain general rules. The enzymes catalyzed reactions take place at physiologically low temperature (37°C) and required extremely small amount of enzymes. Chemically, enzymes are complex protein molecules synthesized in the cells where they are as biocatalysts in carrying out various physico-chemical reactions. There proteins have their own specificity and kineties. They enzymes help in attaining a reaction in a state of equilibrium. An enzyme recognizes its specific substrate and reacts with it to form products and gets regenerated at the end of the reaction Mukherji and Kanungo, 1954 have investigated the digestive enzymes of pelecypods. They were reported from digestive diverticula and digestive gland of several lamellibranchs.

Studies on the digestive enzyme of lamellibranchs were first initiated by Young (1972) in oyster. Mansour, (1946), Zaki (1951) reported the presence of proteases, peptidases and lipases, from the stomach juice as well as digestive diverticula of *Unio Prasedens*. But among the fresh water musscls little work has been done on the physiology of digestion and digestive enzymes. Hence to study the changes in enzymatic pathway it is potent approach to possess to toxicity of the heavy metals.

Ghosh (1961) reported amylase lipase and protease from the Salivary gland and digestive diverticula in *Lamellidens stagnalis*. Few workers have studied digestive enzymes of lamellibranch molluscs. The literature shows investigation with regard to effects of heavy metal on digestive processes of aquatic animals especially where fresh water molluscs *Lamellidens marginalis* are concerned with respect to changes in the level of digestive enzymes.

Materials And Methods

The bivalvesl *L. Marginalis* were collected from the Godavari river at Paithan. They were acclimatized to the laboratory conditions for 5 to 6 days. The bivalves were exposed to median lethal concentration 1.6 ppm $CuSo_4$ as acute and 0.8 ppm $CuSo_4$ as chronic treatment. The control and treated bivalves were, fed on fresh water crushed algae and Hydrilla during exposure period.

The digestive glands from five to ten mussels were separated and washed in distilled water, These

Table 1. Changes in Amylase activity in the bivalve L. Marginalis after acute exposure of heavy metal Copper Sulphate.

Sr. No.	Control	Copper Sulphate		
		24 hrs.	48 hrs.	72 hrs.
1)	7.1281±	3.212±	2.678±	2.223±
	0.216	0.009	0.02	0.021
		P 0.05	P 0.05	P 0.05
		*- 62.12	- 80.70	- 87.60

Enzyme activity in expressed as mg. of maltose/mg of protein/hr. at room temp.
Each value is the mean of five observations ± S.D.
Values are significant at p 0.01. p 0.001, p 0.05.
* These value indicates % inhibition (-ve)

digestive glands were then dried between the folds of muslin cloth, dehydrated and defatted by treatment with ice cold acetone (Summer and Summer. 1947). The material was ground in a clean ice chilled glass morter and repeatedly washed in acetone and filtered till the filtrate was colourless. The powder thus obtained was dried under fan and stored in a clean bottle in freezer at 3 to 5^0 c. In all the experiments. 1%. homogenate at digestive gland prepared in glass dist. water was used. Half of this extract was boiled for half an hour to destroy the enzyme activity and this boiled extract was used as control for all the experiments.

Test for amylase

The drop of homogenised tissue were taken in microtubes. Boiled homogenate was taken in a separate tube. In each of control and experimental tubes two drops of 0.5% boiled starch. Solution were added A few drops of toluene were added to cover this reaction mixture which was allowed to incubate for 24 hrs. at room temperature. After incubation potassium iodide, iodine test was performed. The solution does not turn blue indicating the presence of amylase.

Estimation of Amylase

The invertase activity was determined as described by Noelting and Bernfold (1948). The reaction mixture consists of 0.5ml substrate (2%) 1.5ml Phosphate buffered (pH 7.5) and 0.5 ml tissue homogenate (16% w/v). After 1 hr of incubation at 37^0C the enzyme activity was terminated by adding 2ml of 3.5 denitrosalieylic acid reagent, then heated on boiling water bath for 5 minutes, allow to cool and the optical density was recorded at 5.30µm. For invertase activity sucrose solution was used as a substrate. A known amount of maltose, and glucose with the same procedure gave the calibration curve for estimating invertase activity.

Observation and Results

The amylase activity in the control bivalves, *L. Marginalis* was 7.1281 mg of maltose/mg protein/hr. of room temp. The acute exposure to $CuSo_4$ showed amylase activity of 2.223 mg of maltose/mg protein/hr. at the end of 72 hrs. The decrease in amylase activity at 24.48 & 74 hrs. of exposure in $CuSo_4$ is satistically significant at $p<0.001$, $p < 0.05$ and $p < 0.001$ levels.

Changes in amylase activity of the bivalve *Lamellidens marginalis* after chronic exposure to heavy metals $CuSo_4$ is control 6.700 mg of maltose/mg protein/hr. at the end at 20 days to 2.530 mg of maltose/mg protein/hr. The activity decreased in all

Table 2. Changes in Amylase activity in the bivalve L. Marginalis after chronic exposure of heavy metal mercuric chloride.

Sr. No.	Enzyme	Control	Copper Sulphate			
			5 days	10 days	15 days	20 days
1)	Amylase	6.700±	3.798±	3.750±	3.709±	2.530±
		0.009	0.031	0.0051	0.0052	0.0043
			P 0.001	P 0.05	P 0.05	P 0.001
			*- 57.80	- 70.33	- 82.30	- 82.40

Enzyme activity in expressed as mg. of glucose/mg of protein/hr. at room temp.
Each value is the mean of five observations ± S.D.
Values are significant at p 0.05. p 0.001 level.
*These value indicates % inhibition (-ve)

the cases as the time period increased. All the values are statistically significant at $p < 0.05$ and $p < 0.01$ levels.

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