AMYLASE ACTIVITY IN DIGESTIVE ORGANS OF FRESHWATER SNAIL *BELLAMYA BENGALENSIS* AGAINST TOXICITY OF COPPER SULPHATE AND *ACACIA SINUATA*

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ABSTRACT

Enzymes are highly effective and extremely specific catalysts. These are the biological polymers that catalyse the chemical reactions. The presence and maintenance of a complete and balanced set of enzymes is essential for the breakdown of nutrients to supply energy and chemical building. In this study amylase activity was noted at pre-determined LC_{50} concentration of metal copper sulphate (0.56 ppm) and pland extract of *Acacia sinuata* (232 ppm)under different exposure periodsi. e. 24 hrs., 48 hrs., 72hrs. and 96hrs. After exposure period, the amylase activity was recorded in digestive organs like salivary gland, oesophagus, intestine, stomach andhepatopacreasof freshwater snail *Bellamyabengalensis*.

The experimental results revealed that amylase activity was decreased considerably ($P< 0.001$)in all digestive organs up to 96 hrs.against metal and molluscicide. Reduced amylase activity imbalance the digestion mechanism and it may ultimately effects on nutrients and chemical building in freshwater snail *B. bengalensis*.

KEY WORDS

Amylase activity, Digestive organs, *Bellamyabengalensis*, Copper sulphateand *Acacia sinuata.*

INTRODUCTION

Industrial effluents, domestic sewage and land run-off all enter into the environmental compartments and exert their impact on living organisms. Most of the aquatic ecosystem receives excessive heavy metals from industrial effluents. In biological cycle metals are neither degraded nor metabolized, so remain highly persistent in nature. They also possess property of accumulation over a long period throughout the food chain (Subramanian, 2010). Living organisms require stress amounts of heavy metals. Iron, cobalt, copper, manganese, molybdenum and zinc are required to human life. Excessive levels can damage the cell. Their accumulation in the bodies can cause serious illness. Some of the elements that are beneficial to the organisms under certain conditions become toxic (Clayton, 1981).

Aquatic mollusks, particularly gastropods have controlled by the mechanical, chemical, biological and other methods. Despite, an integrated approach to the control of snails and has proved to be a particularly intractable problem. It has high biotic potentials and ability to disperse. Molluscs showed considerable resistance to molluscicides, which documented simple method used for control the snails (Sudiono*et al.,* 2000). Pesticides are helpful, when they used in proper way, but due to indiscriminate use of these pesticides, it gets accumulated in air, water and soil to pollute the environment. In agricultural practices, in order to take more yield, large amount of chemical fertilizers, pesticides, herbicides and

molluscicides were used to control the pests. Toxic content by runoff find its way to water bodies (Awati, 2004).

Analysis of intracellular enzymatic activity provides invaluable prognostic and diagnostic information related to health of animal. Measurement of enzyme activity is a central in research investigation.Amylase presents in saliva, which catalyses the breakdown of starch into sugar. Pancreatic amylase also hydrolyses the dietary starch into disaccharides and trisaccharides which are converted in to glucose by action of other enzymes in normal process.Digestive tract is the principle site for secretion of digestive enzymes, for digestion of food and absorption of nutrients (Pauchet*et al;* 2007). Enzyme activities in body or cells (amylase, protease, and lipases) provide information about the digestive capacity and efficiency of species related to feeding components (Buddington*et al*., 1997).But any harmful chemical enter in animal body, it effects the enzyme regulation process and its digestion mechanism.

By considering the important role of enzyme constituent of digestive organ in the normal physiological activity, work has been carried out to study comparative metal and molluscicide induced toxicity to the alimentary tract and associated digestive glands of freshwater molluscan species *Bellamyabengalensis* (Lamarck).

MATERIALS AND METHODS

Freshwater snail *Bellamyabengalensis*(L)was collected from Rajaram tank, near Shivaji University, Kolhapur, Maharashtra (India). Water from tank used for irrigation, bathing, washing and also for idol immersion. For the present investigation, freshwater Prosobranch snail *Bellamyabengalensis* (Lamark) selected. It is easily available and having population in the selected freshwater body.

1) Selected Toxicants:-

Two toxicants were used for the present study,

i) Copper sulphate

ii) Pods extract of *A. Sinuata*

2) Experimental setup:-

Experimental animals were exposed to pre-determined mean LC_{50} concentration of heavy metal copper sulphate ($CuSO_4.5H_2O$) at 0.56 ppm and pod extract of *Acacia sinuata* toxicity at 232 ppm to different exposure periods at 24 hrs, 48hrs, 72 hrs and 96 hrs respectively. Treated animals were used for the enzyme activity. Control group for both was run simultaneously and for enzyme activity digestive tissue such as salivary gland, oesophagus, intestine, stomach and hepatopancreas were selected and processed for estimation of amylase by (Ishaaya and Swirski, 1970) method..

All results of the enzyme analysis are given as the mean of three readings with ± standard deviation (SD). In the statistical analysis, the one-way analysis of variance (ANOVA) was used to test.

RESULTS

A)Amylase activity in different digestive organs of control group:-

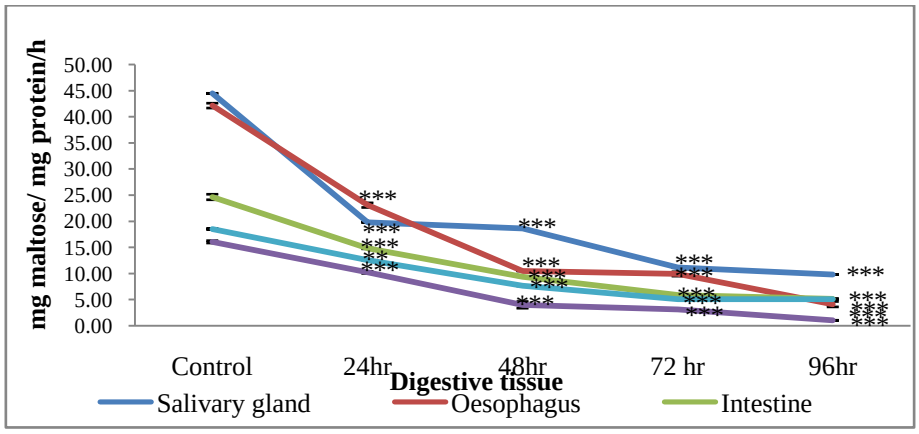
Amylase activity of control group in snail *B.bengalensis* of different digestive organs, where salivary gland showed 44.47 mg maltose/mg protein/hrs, oesophagus contained 42.13 mg maltose/mg protein/hrs,

intestine showed 24.65 mg maltose/mg protein/hrs, stomach has 16.07 mg maltose/mg protein/hrs and hepatopancreas was with 18.53 mg maltose/mg protein/hrs. Results showed that, salivary gland and oesophagus has maximum amylase activity as compared to other tissues. Above results of enzymatic activity was compared to the metal and molluscicide induced experimental group.

a) Effect of Copper sulphate on activity of amylase:

Intoxication of coppersulphatehaschanged amylase activity after 24 hrs, it was 19.81 mg maltose/mg protein/hrs, up to 96 hrs reduced significantly in 9.83 mg maltose/mg protein/hrs. The amylase activity in oesophagus after 24 hrs was 23.09 mg maltose/mg protein/hrs, after 48 hrs 10.53, at 72 hrs 9.93 and 4.41after 96 hrs exposure. Intestine has 14.82 after 24 hrsand after 96 hrs 5.15 was found. The stomach showed 10.23 amylase activity after 24 hrs, 4.02 and after 96 hrs 1.06 was noted. The hepatopancreas has amylase activity as 12.6 after 24 hrs, 5.11 at 96 hrs. The enzymatic data was represented in graph No. 1.

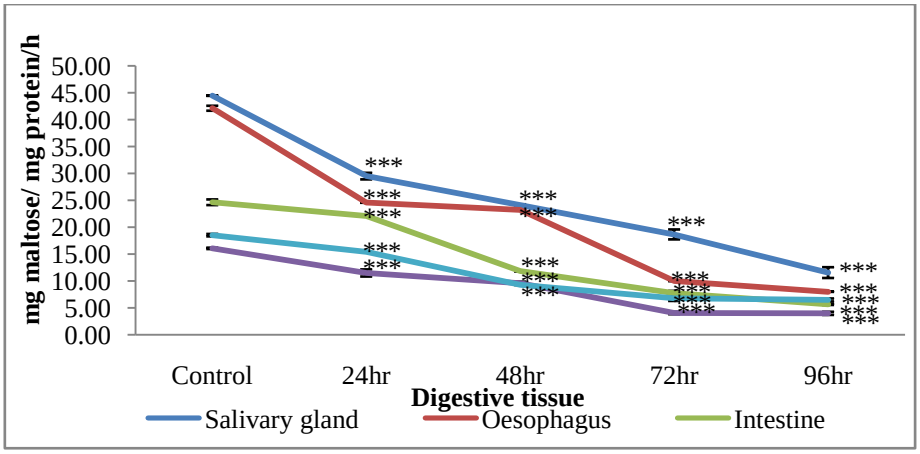
Graph No. 1: Changes in the Amylase activity after exposure to copper sulphate



b) Effect of *A. sinuata* on activity of amylase:

Intoxication of *A. sinuata* to the experimental snail, the amylase activity was decreased as compared to control group. Salivary gland found 29.51 mg maltose/mg protein/hrs at 24 hrs, 24.10 mg maltose/mg protein/hrs after 48 hrs, 18.67 mg maltose/mg protein/hrs at 72 hrs and 11.57 mg maltose/mg protein/hrs decreased amylase activity at 96 hrs. In oesophagus, at 24 hrs 24.57 and 96 hrs found 8.01 mg maltose/mg protein/hrs. Intestine showed 22.08 after 24 hrs, and 5.67 at 96 hrs. Stomach recorded 11.50 amylase activity at 24 hrs, and 3.99 mg maltose/mg protein/hrs at 96 hrs respectively. In hepatopancreas amylase activity was 15.42 at 24 hrs, and at 96 hrs6.48 amylase activity was reduced. The enzymatic data was represented in graph No. 2.

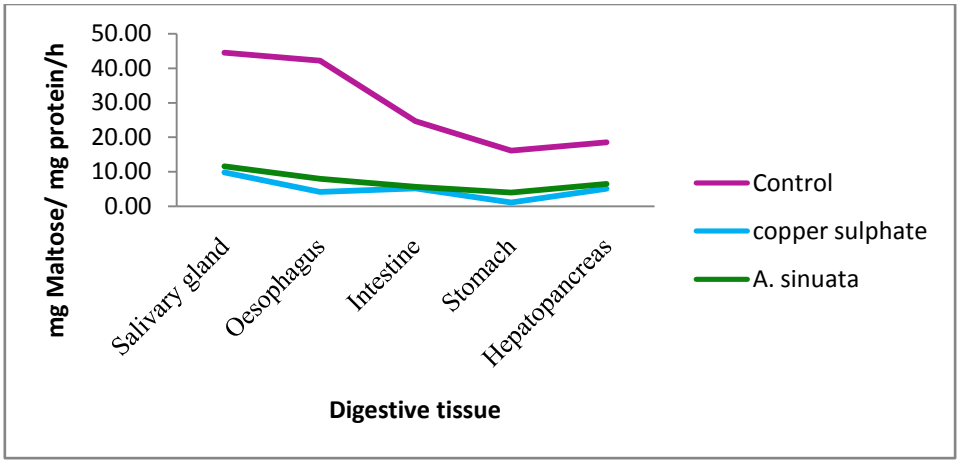
Graph No. 2: Changes in the Amylase activity after exposure to *Acacia sinuata*



DISCUSSION

In the present study, effect of metal copper sulphate and pod extract of *Acacia sinuata* was assessed for enzymological alterations in the amylase activity. Recently, Al Daihan (2008) noted that, activity of α-amylase and lipase were significantly reduced in *S. nigrem* in treated snails, inhibition of these two enzymes activity has affected the development of *Schistosome parasite*. Alteration in enzyme activity can be correlated with molluscicide induced changes and altered structure of the cell (Triebskorn, 1991). Exposing *Cnaphalocrocismedinalis* (Guenee) invertebrate to sub-lethal doses of *Bacillus thuringiensis* (Kurstaki) in the laboratory reduced digestive enzyme activities (Senthil Nathan *et al*; 2005 and 2006).Some workers, found the biomechanisms of digestive enzymes constitute a physiological parameter affecting the digestive capacity (Ibarrolai*et al*., 2000), the control of such a parameter may be important elevate assimilation rate and consequently the level of energy gain (Grizwi and Herral,, 1991).

Graph No. 3: Comparative account of Amylase activity in digestive organs after intoxication of $CuSO_4$ and *A. sinuata*



In the present investigation, we found that copper induced amylase activity was decreased up to 78% in salivary gland, oesophagus has 90%, intestine with 79%, stomach 93% and 72% reduction was found in hepatopancreas. Intoxication of *A. sinuata* showed reduction of amylase activities approximate

74% in salivary gland, 81% in oesophagus, 76% in intestine, 75% in stomach and 65% in hepatopancreas respectively(Graph No. 3).

In molluscan species the structure of α-amylase cDNA was determined in *Pectin maximus* (LE Moine*et al*., 1997); in the oyster, from a digestive gland cDNA library, only one cDNA was recovered and other isoforms were determined by electrophoretic seperations (Moal*et al;* 2000). Gene B preferentially expressed in labial palps (to be compared to mammalian salivary α-amylase) and at a lower level, in the other tissues. In the tropical shrimp *Liptopenaeusvanname*; the three genes were found to be expressed only in the digestive gland (LE moulla*et al;* 1996). Amylase activity promoted carbohydrate digestion in animals (Areekijseree*et al.,* 2006; Supannapong*et al.,* 2008) and used indicator for carnivorous feeding habit (Hofer and Schiemer, 1981). Results were comparatively interpreted in which maximum amylase reduction was found in stomach and oesophagus by both intoxicants of freshwater snail *B. bengalensis*.

CONCLUSION

Present investigation concludes that, any toxic content was responsible for reduction of physiological and enzymatic activity. So, the copper sulphate and pod extract of *Acacia sinuata* has significantly decrease the amylase activity in the freshwater snail *B. bengalensis.*

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