TOXICITY INDUCED ALTERATION IN ENZYME PROTEASE ACTIVITY OF FRESHWATER SNAIL *BELLAMYA BENGALENSIS*

S. V. Lagade*[1], V. M. Lagade[2] and N. A. Kamble[3]
[1]Department of Zoology, Rajarshi Chhatrapati Shahu College,
Kolhapur (Maharashtra), India 416005.
[2]Department of Zoology, Shri. Yashwantrao Patil Science College, Solankur,
Kolhapur, (Maharashtra), India 416 212.
[3]Department of Zoology, Shivaji University Kolhapur, (Maharashtra), India 416004.
*Corresponding author E-mail: drsawpanajalagade@gmail.com

Abstract:

Enzymes as biocatalyst regulate the rate of physiological process and occupy central role in health and also has critical role in diseased conditions. Enzymes are involved in number of physiological reactions from simple digestion of food proteins to highly regulated cascades. Digestive enzyme like proteases can either break specific peptide bonds, depending on the amino acid sequence of a protein or break complete peptide to different amino acids. In the present investigation, the digestive enzyme i.e protease activity was recorded at different exposure periods (24h, 48h, 72h. and 96h) after exposure to metal copper sulphate and plant pod extract of *A. sinuate* along with control group. All experimental sets were calculated for pre-determined mean LC_{50} concentration of metal copper sulphate (0.56 ppm) and pod extract of *Acacia sinuata* (232 ppm).

In this study, the proteases activity was detected after exposure to copper sulphate and plant pod extract *A. sinuata* in different digestive tissues like salivary gland, oesophagus, intestine, stomach, hepatopacreas of freshwater snail *Bellamya bengalensis*. After exposure periods, the proteases activity was highly decreased in digestive tissue at both exposures i.e. copper sulphate and *Acacia sinuata*. Obtained results conclude that, the digestive system play important role in enzyme activity however, any toxic metal enter into the body, the enzyme activity was significantly altered and it may produce detrimental impact on physiological system of organism.

Keywords: *Bellamya bengalensis*, Copper sulphate, *Acacia sinuata*, Digestive tissue, Proteases activity.

Introduction:

Almost all human activities generate potential pollutants. A developmental process has released organic and inorganic wastes in the environment. Contamination in the media is by biological, physical and chemical mixing of toxicants. If concentrations of contaminant in the environment increased at higher levels, causes severe ecological damages. Over 10 million of organic compounds were synthesized and perhaps 10,000 found in regular domestic and industrial use. The nature of toxic chemical depends on the concentration of substances and it's time duration for which, the organism are exposed to it. Toxic impact may bring physiological, biochemical or pathological alterations in organism (Subramanian, 2010).

Proteases occur naturally in all organisms. These enzymes are involved in number of physiological reactions from simple digestion of food proteins to highly regulated cascades. The protease activity can be a destructive change, which abolishing digestive function or digesting it to its principal components. Proteases belong to the class of enzymes known as hydrolases, which catalyse the reaction of hydrolysis of various bonds with the participation of water molecule.

In present scenario, large numbers of toxicants are entered in environment and produced impact on ecosystem. At Present, aquatic habitats are enormously polluted by adding various industrial toxic components regularly. Results of this increase the health risks to all aquatic organisms (Pandey et. al., 2005). In ecological health assessment of aquatic habitat, the molluscs as an invertebrate are good bio-monitoring agents because they can accumulate pollutants considerably higher than other aquatic animals (Hamed and Emara, 2006).

Through extensive reviewing the literatures, it came to know that there is no any documentation or study on comparative effect of metal and plant extract on freshwater snail *Bellamya bengalensis*. Hence, the present work has been undertaken to study the toxicity of metal Copper sulphate and pod extract of plant *Acacia sinuate* on digestive enzyme i.e. protease from different tissues of digestive tract.

Material and Methods:

The toxicity induced alterations in protease activity of different digestive tissues of freshwater snail *B. bengalensis* (L) was carried out after intoxication of heavy metal copper sulphate and pod extract of *A. sinuata*. In this study, mollucan species i.e. freshwater snail (*B. bengalensis*) is used as an animal model to assess the toxic effects of exposures.

a. Collection site:

Freshwater snails *Bellamya bengalensis* were collected from 'Rajaram tank' near Shivaji University, Kolhapur. Snails were brought to the laboratory in polythene bags. The shells of experimental snails were cleaned to remove fouling algal mass and mud. Snails were brought to the laboratory, acclimatized for a week. During laboratory acclimatization, they were feed by plant *Pistia, Hydrilla* and provided with proper ventilation.

b. Experimental design:

In the present study, the digestive tract of freshwater snail *B. bengalensis* is the main site for study the protease activity against exposure of metal copper sulphate and pod extract of *A. sinuate.* Concentration of metal i.e. copper sulphate was prepared at different concentration i.e. 2, 3, 4 and 5-ppm and concentration of plant pod extract was prepared 200, 300, 400 and 500 ppm. The experimental animal, freshwater snail *B. bengalensis* was exposed to four different concentrations of copper sulphate and pod extract along with control group for 96 hours.

After exposure to both exposures, the enzyme protease activity was tasted in digestive tissues like salivary gland, oesophagus, intestine, stomach, hepatopacreas at 24 hours interval upto 96 hours. The protease activity from each of intoxicated group as per exposure periods (24 hrs, 48 hrs, 72 hrs, and 96 hrs.) was determined by applying methods of Egauche and Iwamoto, (1982).

c. Statistical analysis:

All results of the enzyme protease analyses are given as the mean of five separate analyses with ± SD (Standard Deviation). In the statistical analysis, the one-way analysis of variance (ANOVA) with the Bonferroni post-test to compare replicate means was carried out with the aim of uncovering any significant changes in enzyme activity.

Results:

Protease activity in different digestive organs of control group:

The protease activity of control group in different digestive organs such as 2.26 mg, oesophagus 1.92 mg intestine 2.29 mg, stomach 3.11 mg and hepatopancreas 3.84 mg Tyrosine/ mg protein/hrs were recorded respectively. (Fig. 1 and 2).

a. Effect of Copper sulphate on activity of protease:

Intoxication of copper sulphate in experimental groups, the protease activity was significantly altered as compared with control group at different exposure periods in selected digestive organs. The altered protease activity at 24h was 1.77 mg in Salivary gland, at 48 hrs 1.05 mg, at 72 hrs 0.50 mg and at 96 hrs 0.44 mg Tyrosine/ mg protein/hrs activity was recorded. In oesophagus, the protease values were 1.52 mg, 0.97 mg, 0.90 mg and 0.81 mg Tyrosine/ mg protein/hrs at 24h, 48h,72h and 96h respectively. In stomach, the protease activity was 0.94 mg at 24h where at 48 hrs 0.89 mg, at 72 hrs 0.88 mg and at 96 hrs 0.51 mg Tyrosine/ mg protein/hrs were protease activity recorded respectively. Intestine organ showed 1.32 mg at 24 hrs, 0.54 mg at 48 hrs, 0.46 mg at 72 hrs and 0.31 mg Tyrosine/ mg protein/hrs at 96 hrs protease activities.

Hepatopancreas organ showed 1.15 mg at 24hrs, 0.84 mg 48 hrs, 0.80 mg 72hrs and 0.18 mg Tyrosine/ mg protein/hrs at 96 hrs protease activity recorded. (Table 1 and Fig. 1).

Above values of protease activity was significantly ($p<0.001$) declined from 24h to 96h of in all digestive organs after exposure to metal copper sulphate. (Table 01). The protease activity after exposing to copper sulphate at 96 hours as compared to control group, the salivary gland was recorded (-80%) declined in protease activity. While, esophagus showed (-58%) decline followed by (-83%) in stomach, (-86) in intestine and (-95%) in hepatopancreas respectively.

b. Effect of *A. sinuata* on activity of protease:

Similarly, as metal copper sulphate the protease activity was recorded after exposing to plant pod extract of *A. sinuata* the in various digestive organs of *B. bengalensis* upto 96 hours.

In salivary gland, the protease activity was recorded as 2.23mg, 1.76 mg, 0.96 mg and 0.85 mg Tyrosine/ mg protein/hrs at 24h, 48h, 72h and 96h respectively. In oesophagus, the protease values 1.88 mg after 24 hrs, 1.47 mg after 48 hrs, 1.31 mg after 72 hrs and 1.29 mg Tyrosine/ mg protein/hrs after 96 hrs were recorded. In stomach, the protease values were 2.93 mg, 2.66 mg, 2.47 mg and 1.32 mg Tyrosine/ mg protein/hrs at 24h, 48h, 72h and 96h recorded respectively. The intestine organ showed 2.14 mg Tyrosine/ mg protein/hrs at 24 hrs. Whereas, at 48 hrs, 72h and 96h the protease activity was noted 1.48 mg, 1.47 mg 1.44 mg Tyrosine/ mg protein/hrs correspondingly. The protease values in hepatopancreas organ were noted as 3.69 mg, 1.81 mg 1.60 mg and 1.50 mg Tyrosine/ mg protein/hrs at 24h, 48h, 72h and 96h respectively. (Table 2 and Fig. 2).

After exposing to plant pod extract of *A. sinuate*, the enzyme protease was significantly decreased ($p<0.001$) in all digestive organs like salivary gland, oesophagus, stomach, intestine and hepatopancreas respectively. After exposing to *A. sinuate* at 96 hours the salivary gland showed (-62%) decline of protease activity followed by (-33%) in esophagus,(-58%) in stomach, (-37) in intestine and (-61%) in hepatopancreas correspondingly.

c. Comparative account on Toxicity of copper sulphate and *A. sinuata* on protease activity:

In context of comparative effect of both intoxicants, the freshwater snail *B. bengalensis* shows different protease activity in selected digestive organs. The protease activity in all selected digestive organs showed significant ($p<0.001$) high decreased in snail after exposing to copper sulphate than pod extract of *A. sinuata*. Almost two-three fold decline in protease activity was recorded after exposed to metal copper sulphate then protease values were observed in exposure of plant extract *A. sinuata.* (Fig. 3).

Discussions:

Protease activity in the digestive tract found key determinant of digestion and assimilation efficiency of ingested proteins for animals. Generally pepsin in the stomach and trypsin, chymotrypsin, carboxy and aminopeptidases in the intestine were responsible for the hydrolysis of ingested proteins (Whitaker, 1994). Digestive tract is the principle site for secretion of digestive enzymes, for digestion of food and absorption of nutrients (Pauchet *et al;* 2007). Serine proteinase, such as trypsin and chymotrypsin, are predominant in the gut of lepidopterans. Gut proteases further release amino acids from peptides, produced by endopeptidases (Tetra and Ferreira, 1994).

In the present investigation, both intoxicants i.e. copper sulphate and pod extract of *A. sinuata* were significantly inhibited the protease activity in different organs of digestive organs of freshwater snail *B. bengalansis*. In the context of acute exposure of both toxicants produced detrimental impact on metabolic process of freshwater snail. Similar results were recoded in protease values of *L. marginalis* after exposure to heavy metals $CuSO_4$, $HgCl_2$ and $CdCl_2$. According to literature, Abdel-Kader *et al.,* (2005) the digestive enzymes like carboxylase, lipase and protease were mainly targeted in molluscan species under influence of molluscicide.

After exposing to pod extract of *A. sinuate,* the protease values in digestive organs were decline upto 33-62%. Whereas, intoxication of copper sulphate the experimental animal *B. bengalansis* shows significant decline in protease activity as compared to exposure of pod extract. Under influence of copper sulphate, the protease values were decreased up to 58-95% in selected digestive organs. Sultana and Lomate, (1997) reported the decreased amylase activity in *L. marginalis* under effect of mercury chloride and copper chloride. Similarly, in recent past the few

literatures such as Sultana (1995); Deshmukh (1995); Mahajan and Zambare (2001); Al-Daihan, (2008); Sonawane (2017) have been reported the decline in digestive enzyme activity and inhibition of digestive enzymes under influence of stress in Molluscan species

Conclusion:

In context of metal exposure and one of mollucide i.e. pod extract *A. sinuata* the digestive enzyme protease is significantly inhibited and protease values were decline up to 95% in digestive organs at exposure of copper sulphate. On the other hand, under influence of pod extract *A. sinuata* the protease values decreased up to 62%. Obtained values conclude that, the both intoxicants are very harmful for metabolic process of freshwater snail *B. bengalensis* due to decrease in secretion of digestive enzyme at short-term exposure of copper sulphate and pod extract. Comparatively, the metal copper sulphate is more detrimental than pod extract *A. sinuata.*

References:

1. Abdel Kader, A., Hamdi, S.A, and Rawi, S. M. (2005): Biological and biochemical studies on *Biomphalaria alexandria* snail, treated with low concentration of certain molluscide (Synthetic of plant origin) certain Molluscides (synthetic of plant origin) *J. Egypt. Soc. Parasitol*. 3; 841-858.
2. Al-Daihan, S. (2008): Measurement of selected enzymatic activate in *Solanum nigrum*- treated *Biomphalaria arabica* snails. Appl. Sci. 8: 881-885.
3. Deshmukh, C. P. and Tembhare, D. B. (1998):Effects of two organophosphates on the midgut epithelium and digestive enzymes in the larva of the fruit sucking moth, *Othreis maternal L.* (Lepidoptera: Noctuidae). *J. Adv. Zool*.19: 107-114.
4. Egauchi, M. and Iwamoto, A. (1982): Comparison of three alkaline proteases from digestive fluid of the silkworm, *Bombyx mori* L. Comp. Biochem. Physiol. 72 (A): 359-363.
5. Hamed, M.A. and Emara, A.M. (2006): Marine molluscs as bio-monitors for heavy metal levels in the Gulf of Suez, Red Sea, Journal of Marine systems. 60: 220-234.
6. Hart, W. B., Daudoroff, P. and Greenbank, J. (1945): The evaluation of toxicity of industrial wastes, chemicals and other substances to freshwater fish, waste control Laboratory, Atlantic Refining Co, Philadelphia. 317, 1147.
7. Kuz'mina V.V., and Kuz'mina Y.G (1991): Characteristic of some digestive tract enzymes in the street, *Ascipenser Ruthenus*. J. Ichthyol. 31: 120-129.
8. Mahajan, P. R., and Zambare, S. P. (2001): Ascorbate effect on copper sulphates and mercuric chloride induced alternations of protein level in freshwater bivalve, *Corbicula strialetta*. Asian *J. Micro. Biotech. Env. Sci*. 3(2-1): 95-100.
9. Pandey, K.J., Shukla S., and Trivedi (2005): Fundamental of Toxicology, New Central book agency (p) Ltd. 8/1 Chintamoni Das Lane, Kolkatta, 700 009.
10. Pauchet, Y., Muck, A., Svatos A., Heckel, D. G., Preiss, S. (2008): Mapping the larval midgut lumen proteome of Helicoverpa armigera,a generalist herbivorous insect. J.Proteome Res. 7(4):1929-1639.
11. Subramanian, M. A. (2010): Book of Toxicology (Principle and Methods), 2nd Revised Edition. MJP Publishers, A Unit of Tamilnadu Book House 47, Street Triplicane, Chennai.
12. Sultana M. And Lomte V.S. (1997): Environment and Ecology, 15 (4), 803-809.
13. Sultana M.,(1995): Impact of pollutants on some physiological aspects of freshwater bivalve, *Lamellidens marginalis*. Ph.D. Thesis, Dr. B.A.M. University Aurangabad. M.S.,.
14. Tetra, W. R. and Ferreira, C. (1994): Insect digestive enzymes: properties, compartmentalization and function. Comparative Biochemistry and Physiology 109B: 1-62.
15. Whitaker J.R. (1994): Principles of Enzymology for the Food Sciences. Mareel Dekker, New York, 625 pp.

Figure 1: Alterations in the protease activity after exposure to copper sulphate

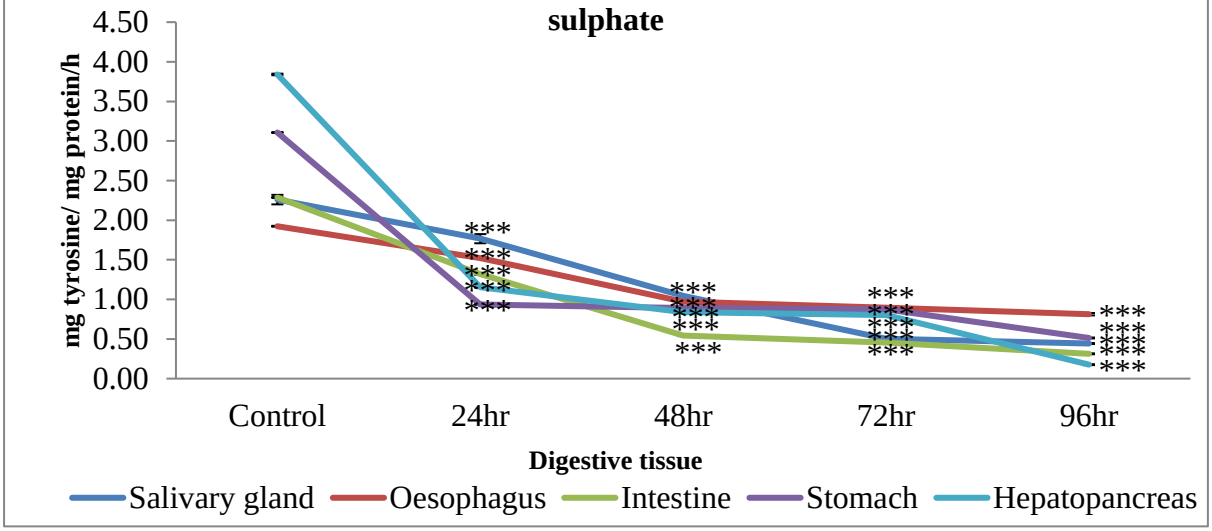

Figure 2: Alterations in the Protease activity after exposure to *Acacia sinuata*

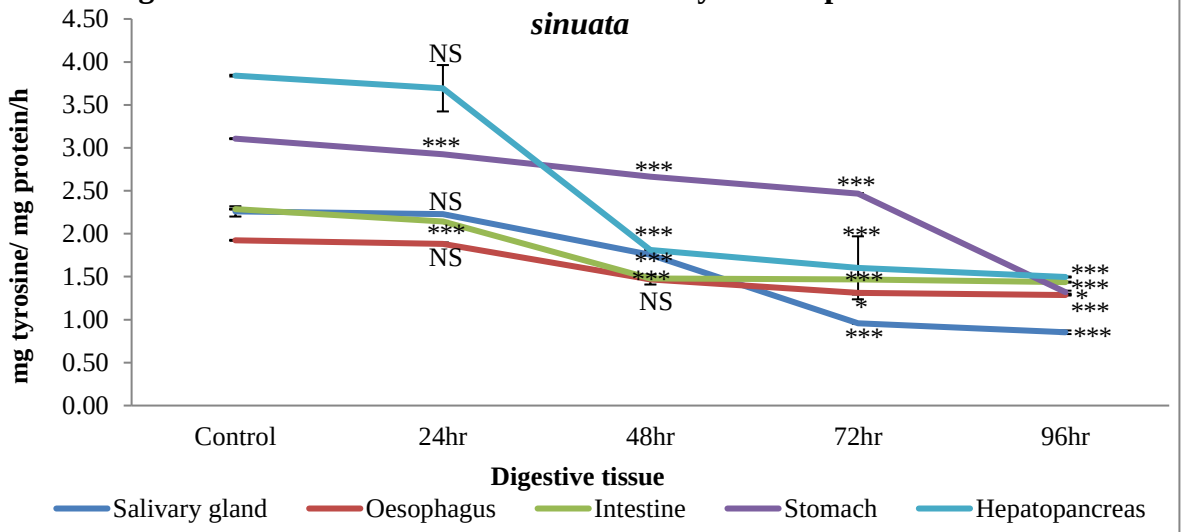

Figure 3: Comparative account of Protease activity in digestive organs after 96 hrs intoxication of $CuSo_4$ and *A. sinuata*