Impact of Endosulfan on Protease and Lipase activity of Land Slug *Laevicaulis alte.*

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Abstract

Gastropods exposed to toxicants for even a short span of time may produce considerable destruction of the internal organs especially their enzymatic architecture. Majority of enzymes are functional in various metabolic pathways and changed pattern of enzyme activity induced by pesticide, is the surest indicator of functional disorder, enzyme assays and estimation of metabolites have been proposed as valid biochemical means of monitoring toxicity. Hence the present investigation was therefore, undertaken to study the alterations produced in the digestive enzymes protease and lipase metabolic enzyme in the gastropod, *Laevicaulis alte* after treatment of endosulfan.

Keywords: Metabolites, Biochemical, Enzyme, Toxicant.

INTRODUCTION

To satisfy the expanding food needs, a few kinds of pesticides are utilized for controlling different sorts of farming vermin. As such numerous valuable non-target living beings additionally have the poisonous impact of pesticide. Pesticides are known to be widespread environmental pollutants due to their bioaccumulation and persistence in the ecosystems. Residues of these compounds have been detected in different biological media of test organisms (Bolognesi and Merlo, 2011). A large portion of the pesticides meddle with the compound activity and produce numerous physiological and biochemical changes in the collections of non-target life forms. Gastropods presented to poisons for even a limited ability to focus time may create extensive annihilation of the interior organs particularly their enzymatic design. Larger part of catalysts are practical in different metabolic pathways and changed example of protein movement actuated by pesticide, is the surest marker of useful issue, chemical examines and assessment of metabolites have been proposed as substantial biochemical methods for checking poisonousness. Toxins act at the biochemical level, at various destinations yet a creature might have the option to adjust by typical homeostatic systems, with the goal that chemical hindrance may decrease the overall wellness of a living being. Compound bio-examines remain, notwithstanding, helpful procedures in searching for sublethal impacts of poisonous contamination. Pesticide-induced oxidative stress is well-known to cause genotoxicity (Franco et al., 2010).

Molluscs show a variety of digestion patterns as they have learnt to feed in different ways due to their ability to adapt themselves to life in so many different types of habitats. The comparative physiology of digestion in molluscs has been reviewed by Vanwheel (1961). The correlation between digestive enzymes and diet has been established but specific characterization of different enzymes of different animals presents many interesting and puzzling question (Prosser, 1973). Pesticides are known for their solid fascination in natural tissues. Pesticides which are invested in to body are fit for responding with an assortment of restricting destinations and afterward upsetting the ordinary physiology of a creature prompting harmfulness. Mackee and Wolf (1963) expressed that the significant harming of a compound framework relies upon its ability to respond with ligands. The living cells are the focuses of dominant part of chemical catalisation responses. At the point when a vitality unevenness happens in the cell because of introduction to infective operators or poisonous substances, chemicals spill through the layer into flowing liquids.

This makes their liquid level be increased over the typical level. It has been likewise contended that a portion of the cytoplasmic chemicals are spilled out of the tissues because of harmed cells. They are regularly delivered in liquids bringing about the diminished chemical exercises in tissues and comparing increment in the liquids. Several workers have reported the effect of pesticides and heavy metals on enzyme activity in molluscs, which was shown by either depletion of enzymes (Jackim et al., 1970; Hinton and Koehing, 1975) or elevation of enzymes (Banerjee et al., 1978; Verma and Prasad, 1972). Albeit a few specialists have announced the impact of various toxins on compound action, little data is accessible with respect with the impact of pesticides on stomach related and metabolic chemicals of the gastropods. The activities of antioxidant enzymes, in turn, can be affected by pesticides. Superoxide dismutase (SOD) plays a pivotal antioxidant role as evidenced by its presence in virtually all aerobic organisms examined to date (Lushchak, 2011)The current examination was hence, embraced to consider the changes delivered in the stomach related proteins protease and lipase in gastropod, L. alte after treatment of endosulfan.

MATERIAL AND METHODS:

Medium sized terrestrial snail *Laevicaulis alte* (8 to 10 cm in length and 2 to 3 cm in width) used in the present study were collected from Kalwan Taluka area. They were acclimatized to the laboratory conditions for four to five days. The air temperature was 31.25° +2.2173°. Since the animals are micro feeders, no special food was supplied during the experiment.

To study the effect of pesticides endosulfan on the enzyme activity of gastropods, L. alte were exposed to lethal concentration (LC50 ppm of 96 hrs) for acute treatment. The active and acclimatized medium sized animals were divided into five groups, one group was maintained as control and from the remaining four, and each one was separately exposed for acute treatment of pesticides up to 96 hours during pre-reproductive, reproductive and post-reproductive period. After an interval of 24 hours enzyme activity of treated and control animals was determined up to 96 hours during pre-reproductive, reproductive and post-reproductive periods. For digestive enzymes such as protease and Lipase the animals were dissected and the digestive gland was taken out, cleaned and homogenized in ice cold distilled water.

Protease:

The protease activity was determined by following Sorenson's formaldehyde titration method as modified by Prosser and Vanwheel (1958). The reaction mixture contained 3.0 ml gelatin (3%), 1.0 ml phosphate buffer (pH 7.5) and 1.0 ml tissue homogenate (10% w/v). The reaction mixture was incubated for 60 minutes at 37°C. The enzyme activity was terminated by keeping it in boiling water bath for 5 minutes, then equal amount of neutral formaldehyde was added and titrated against 0.1 N KOH solution by using alcoholic phenol phthalein (0.5%) as an indicator. The difference between boiled and unboiled tissue homogenate showed protease activity. The amount of amino acid liberated in terms of ml of KOH (0.1 N) solution was taken as an index of enzyme activity.

Lipase:

The lipase activity was determined by the method of Sinha (1976) based on titrimetric estimation of liberated fatty acids from substrate during enzyme actions. The reaction mixture consists of 1.0 ml olive oil, 1.0 ml phosphate buffer (pH 8) and 1.0 ml tissue homogenate (10% w/v). The reaction mixture was incubated for one hour at 37°C with frequent shaking. The enzyme activity was terminated by boiling reaction mixture in water bath. The lipolytic activity was determined by titrating the reaction mixture with NaOH (0.1 N) solution after adding 3.0 ml of alcohol (95%) using alcoholic phenolphthalein as an indicator. The difference

between the volume of NaOH (0.1 N) solution utilized in unboiled and boiled homogenate containing reaction mixtures indicated the lipase activity.

RESULTS:

The compound exercises were concentrated in the control and pesticide treated slugs *Laevicaulis alte*. The outcomes are summed up in figures 1 to 6. The exercises of various stomach related catalysts treated with various pesticides were discovered to be subject to the substance idea of pesticide and time of presentation alongside conceptive status of the gastropods.

Protease (Figs. 1to 3):

The increase in protease activity in the digestive gland of the slug was different in different pesticidal stresses. The fluctuation in the elevation of activity was observed, but overall increase in the activity was noted. The percent increase in protease activity from endosulfan varied from 24 to 96 h. The increase in pre-reproductive period varied from 5.0 % to 44.90 ($P < 0.01$), 17.3633 % to 26.1782 % in reproductive period and 11.440 to 20.5430 % ($P < 0.001$) in post-reproductive period.

Fig.1: Changes in the Protease activity of *Laevicaulis alte.* after acute pesticidal stress during Pre-reproductive period.

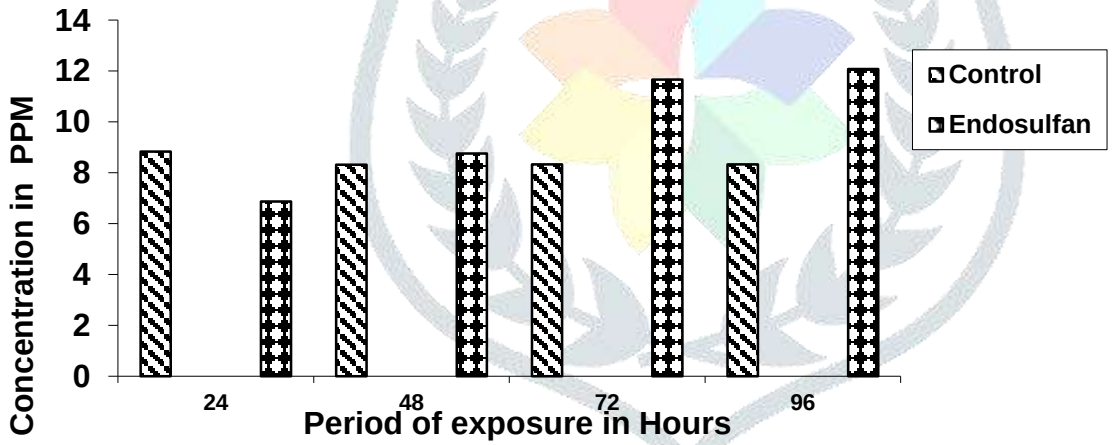


Fig. 2: Changes in the protease activity of *Laevicaulis alte* after acute pesticidal stress during reproductive period.

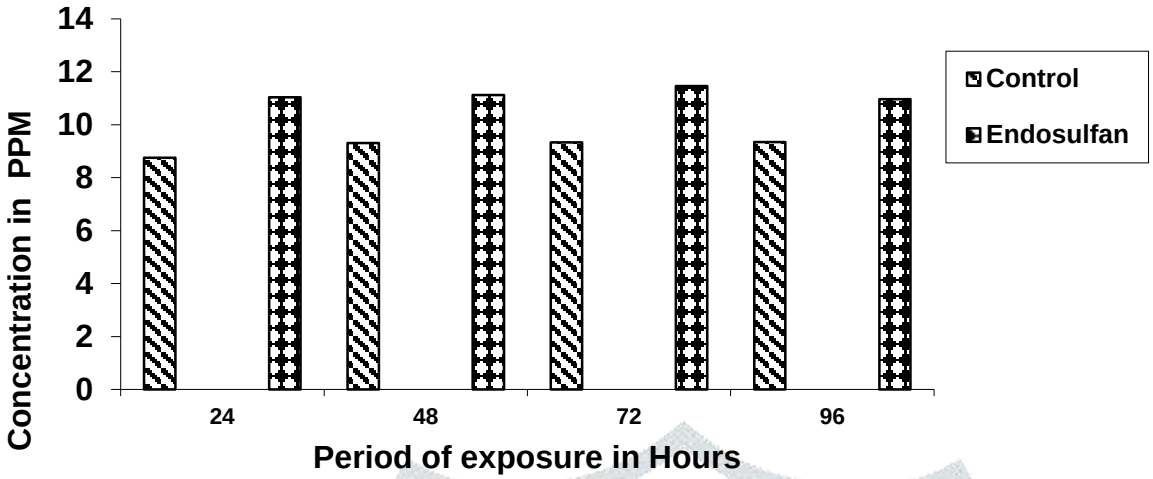
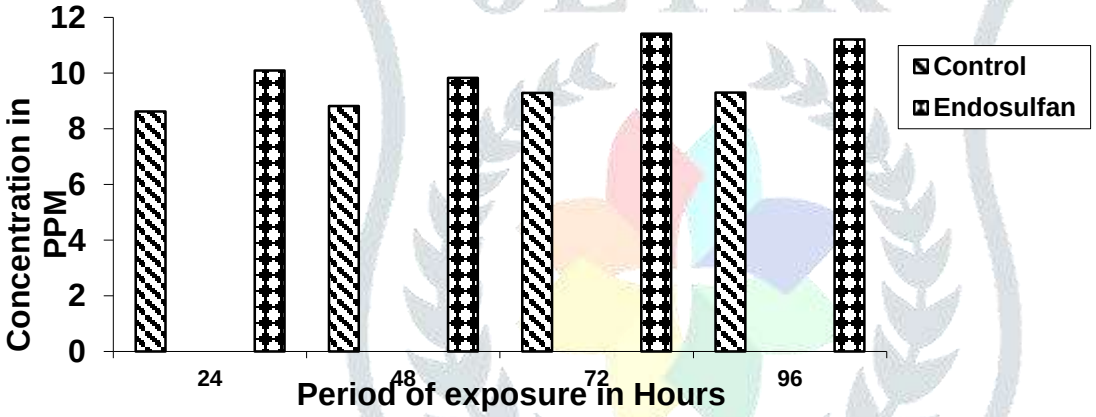


Fig. 3: Changes in the protease activity of *Laevicaulis alte* after cute pesticidal stress during Post-reproductive period.



Lipase (Figs. 4 to 6):

Lipase activity was decreased in the digestive gland of slugs after pesticidal stress.In endosulfan this decreased varies from 4.34 % to 32.72 % ($P < 0.001$) in pre-reproductive, 50.4528 % ($P < 0.001$) to 61.3523 ($P < 0.001$) in reproductive and from 49.3738 % ($P < 0.001$) to 74.1202 % ($P < 0.001$) in post-reproductive period.

Fig.4 : Changes in the lipage activity of Laevicaulis alte after cute pesticidal stress during Pre-reproductive period.

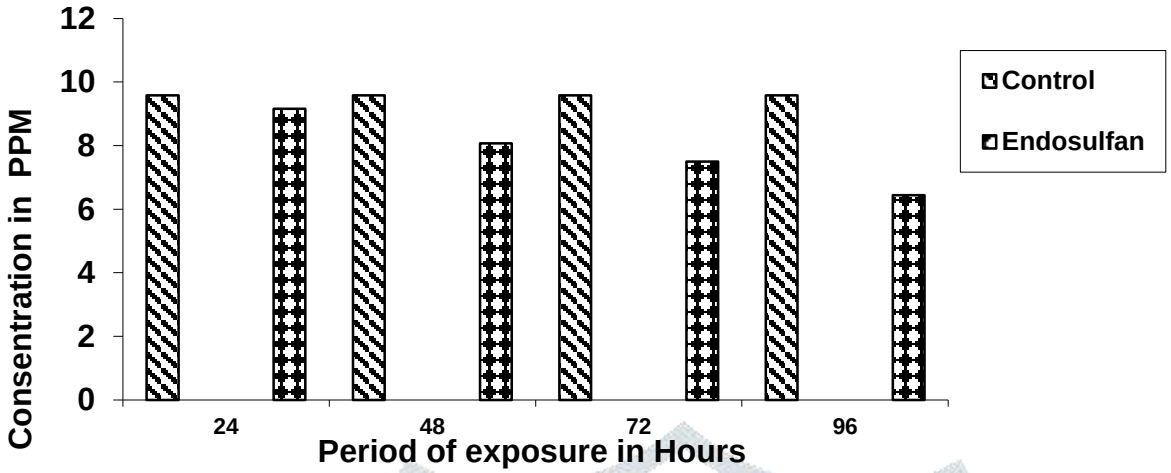


Fig.5 : Changes in the lipage activity of Laevicaulis alte after cute pesticidal stress during reproductive period.

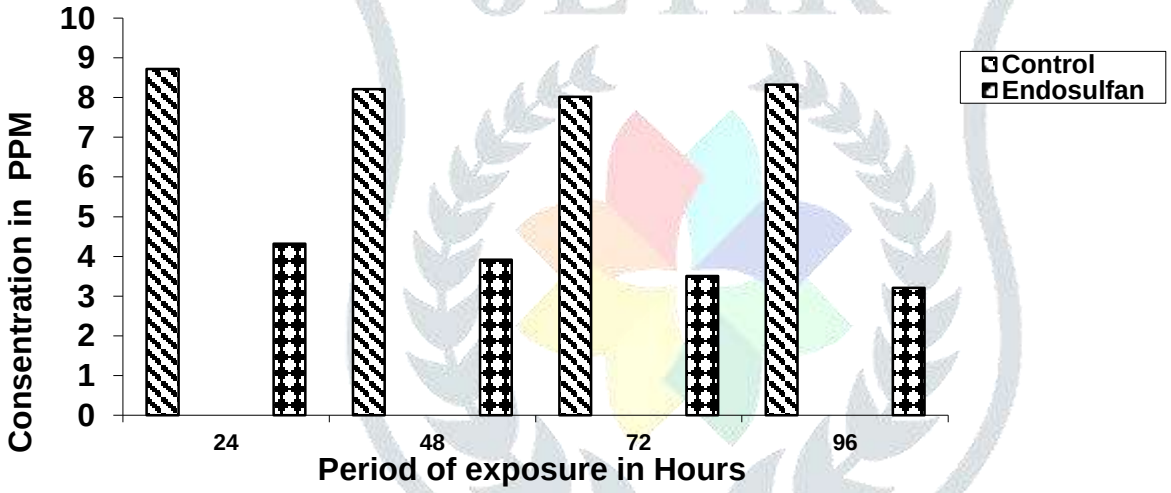
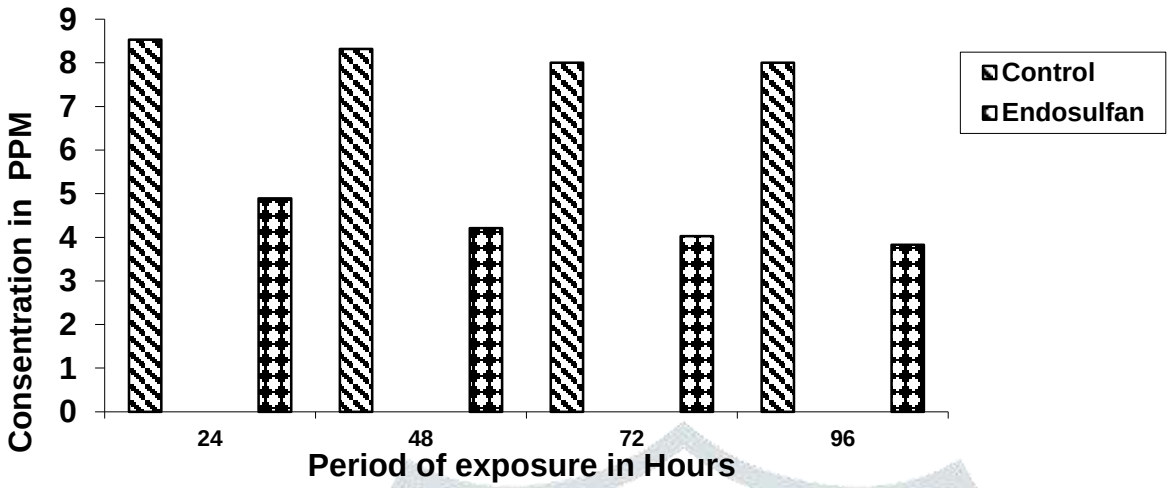


Fig. 6 : Changes in the lipase activity of Laevicaulis alte after cute pesticidal stress during Post-reproductive period.



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