LETHAL EFFECT OF COLCHICINE AND PACLITAXOL ON TROCHOPHORE LARVAL STAGE OF LYMNAEA STAGNALIS

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ABSTRACT

Lymnaea stagnalis is an oviparous snail. It is the most common serious pests of aquatic vegetation. In the present investigation, Effect of sublethal exposure to colchicine and paclitaxol on mortality, survival and reproductive performance of Lymnaea stagnalis was studied. The observed values of sublethal concentration 0.02% for colchicine and 0.01% for paclitaxol. The present investigation has also been taken to study the effect of colchicines and paclitaxol on deletion of proteins that is responsible for different developmental stages of Lymnaea stagnalis. So, to control the population density of Lymnaea stagnalis the treatment with colchicine and paclitaxol drugs would be significant for pest snail. Detection of negatively charged protein fractions in trochophore larval stage of control and treated snails by SDS-PAGE of Lymnaea stagnalis was assessed in the present investigation.

INTRODUCTION

Snails are distributed worldwide. The snail act as the intermediate hosts of trematode parasite, the causative agent of helminthes diseases. The common pond snail is a freshwater species widely used in embryological studied [1]. Snails are the pest of paddy crop, aquatic garden vegetation, coffee, tea, money and ornamental plants. Family Lymnaeidae is abundant in our lakes and ponds [2].

Colchicine is a toxic natural product and secondary metabolite and inhibits microtubule polymerization by binding to tubulin, tubulin one of the main constituents of microtubules. Availability of tubulin is essential to mitosis and therefore colchicine effectively functions as a “mitotic poison” or spindle poison. Paclitaxel is a mitotic inhibitor used in cancer chemotherapy. It was discovered in a U.S. National Cancer Institute program at the Research Triangle Institute in 1967 when Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific yew tree, Taxus brevifolia and named it taxol. The antitubulin drugs of analytic grade of Colchicine and paclitaxol procured from CDH and Sigma companies respectively.

Galactogen and protein form the main constituents of the eggs of Lymnaea stagnalis. The amount of galactogen per egg is fairly constant, irrespective of the size of the egg mass or the age of the snail [3]. In the experimental groups of Lymnaea spp that toxified with the vinorelbine observed the depletion, destruction and degeneration of protein metabolites in the trochophore larval stage, which correlated with the depletion of negatively charged protein fractions detected by SDS-PAGE.

Proteins play a very important role for overall growth, development and production of animals. The depletion, destruction and degeneration of protein metabolites in the various stages of experimental groups of Lymnaea spp., correlated with the depletion of negatively charged protein fractions were detected by SDS-PAGE and an important aspect of the present investigation. Acute toxicity studies are among the first steps in determining the water quality required for the sustenance of snails. These studies reveal the toxicant concentrations (viz. LC50) that cause snail mortality even at short-term exposure [4].

MATERIAL AND METHOD

Fresh water healthy and sexually mature snails of Lymnaea stagnalis belonging to family Lymnaeidae were selected for the present study. The selected snails were acclimatized under laboratory conditions. The young ones hatched from the freshly laid egg masses of Lymnaea were used for the experimental purpose. The egg masses laid by these snails were introduced to different concentration of antitubulin drugs in petri dishes in triplicate to study their toxic effects on protein in larval stage and calculated the lethal concentration values for colchicine.
(0.12%-LC100, LC50-0.06%, LC0-0.03% and 0.02% sublethal concentration) and for paclitaxel (0.08%-LC100, LC50-0.04%, LC0-0.02% and 0.01% sublethal concentration) by Probit analysis [5] and data was summarized in Table No.1 & 2.

**Detection of Protein in Trochophore Larval Stage**

For quantification of extract protein, egg masses of trochophore larval stage were collected from snails. The separated protein placed in individual eppendorfs that were stored at -20°C. 80-100 µl of distilled water was added to the eppendorfs containing egg masses of various stages of development. These materials were homogenized in Bloer’s mixture and the vials containing the egg masses of various stages of snails were centrifuged at 10,000 RPM for 10min at 5°C. Aliquots of the supernatants of the centrifuged extracts were used for protein content. In order to investigate the proteins from homogenized egg masses of various developmental stages of *Lymnaea* spp., 7 % SDS-PAGE was performed. In this 50 µl of pure egg masses of various developmental stages (approx 220 mg of protein) derived from control and treated snails were used. 50 µl of sample was added to 50 µl of sample buffer (Tris buffer pH 6.8 1.66 ml, glycerol 2 ml, 10 % SDS 4 ml, β-mercaptoethanol 200 µl, bromophenol blue 0.02 gm, distilled water 2.14 ml).

Protein samples of trochorephore larval stages were loaded on a prepared SDS-PAGE gel (7 % separating gel, 4 % stacking gel) in different lanes. The number and intensity of protein fractions were detected out in trochophore larval stages of *Lymnaea* spp.

**SDS-PAGE was carried out by the method adapted [6].**

**RESULTS**

In the present investigation, Figure 1: Showed that the number and intensity of negatively charged protein fractions were increased in cleavage stage of control groups of *Lymnaea stagnalis* while depletion in number and intensity of protein fractions was observed in trochophore larval stage treated experimental groups due to intoxication of colchicine and paclitaxol.

The molecular weight of the trochophore larval stage of *Lymnaea stagnalis* in control ranged from 2.5 to 40.1 kDa, while trochophore larval stage of *Lymnaea stagnalis* treated with with colchicine ranged from 12.0 to 30.0 kDa and trochophore larval stage of *Lymnaea stagnalis* treated with paclitaxel ranged from 10.4 to 14.8 kDa as exhibited in Fig 1. Eight bands in lane 1 of Fig. 1 were observed in the trochophore larval stage of control *Lymnaea stagnalis*. The bands were of 2.5, 5.8, 12.0, 15.1, 18.4, 22.2, 30.1 and 40.1 kDa molecular weight. One band of 15.1 kDa was observed of very high intensity. Two bands of 18.4 and 22.2 kDa were observed of high intensity. Two bands of 12.0 and 30.1 kDa were observed of low intensity. Two bands in lane 2 of Fig. 1 were observed in colchicine treated trochophore larval stage of *Lymnaea stagnalis*. Two bands of 10.4 and 14.8 kDa were observed of low intensity. Two bands of 18.4 and 22.2 kDa were observed of very high intensity. Two bands of 10.4 and 14.8 kDa were observed of very low intensity. Two bands in lane 3 of Fig. 1 were observed in paclitaxel treated trochophore larval stage of *Lymnaea stagnalis*. Two bands of 10.4 and 14.8 kDa were observed of low intensity.

**Table 1 Data on Toxicity of Colchicine on Egg Masses of *Lymnaea stagnalis***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the antitubulin drug</th>
<th>Concentration of the antitubulin drug</th>
<th>Duration (hrs.)</th>
<th>Mortality (%)</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colchicine</td>
<td>0.06%</td>
<td>72</td>
<td>100%</td>
<td>LC100</td>
</tr>
<tr>
<td>2.</td>
<td>Colchicine</td>
<td>0.03%</td>
<td>72</td>
<td>Nil</td>
<td>LC50</td>
</tr>
<tr>
<td>3.</td>
<td>Colchicine</td>
<td>0.02%</td>
<td>72</td>
<td>Nil</td>
<td>LC5</td>
</tr>
<tr>
<td>4.</td>
<td>Colchicine</td>
<td>0.01%</td>
<td>72</td>
<td>Nil</td>
<td>Sublethal conc.</td>
</tr>
</tbody>
</table>

**Result:** 0.02% concentration of colchicine was considered as sublethal concentration value.

**Table 2 Data on Toxicity of Paclitaxel on Egg Masses of *Lymnaea stagnalis***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the antitubulin drug</th>
<th>Concentration of the antitubulin drug</th>
<th>Duration (hrs.)</th>
<th>Mortality (%)</th>
<th>Lethal conc. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Paclitaxel</td>
<td>0.08%</td>
<td>72</td>
<td>00%</td>
<td>LC100</td>
</tr>
<tr>
<td>2.</td>
<td>Paclitaxel</td>
<td>0.04%</td>
<td>72</td>
<td>50%</td>
<td>LC50</td>
</tr>
<tr>
<td>3.</td>
<td>Paclitaxel</td>
<td>0.02%</td>
<td>72</td>
<td>Nil</td>
<td>LC5</td>
</tr>
<tr>
<td>4.</td>
<td>Paclitaxel</td>
<td>0.01%</td>
<td>72</td>
<td>Nil</td>
<td>Sublethal conc.</td>
</tr>
</tbody>
</table>

**Result:** 0.01% concentration of paclitaxel was considered as sublethal concentration value.

**DISCUSSION**

Detection of negatively charged protein fractions by electrophoresis is the integrated part of the present investigation. In control the successive development stages showed the gradual increase in the protein fractions indicated the progressive development of corresponding snails [7] but due to the intoxication of the pesticides most of the developmental stages showed the gradual decline not only in...
the number of protein fractions but also showed gradual decline in the intensities of some of the protein fractions as reported [8,9] in Lymnaea stagnalis after nuvan treatment.

The decline in the number of protein fractions could be correlated with the increase in enzymatic activity of protease during the corresponding stage e.g. trochophore but increase in free amino acids have not been investigated. Increase in number of protein fractions could be correlated with the synthesis of new types of proteins by the combination of different types of free amino acids as observed in the pacific oyster Crassastrea gigas observed [4]. It is observed that paclitaxel was more toxic than colchicine in gastrula stage observed [2] and the number and intensity of protein fractions were detected out in cleavage stages of Lymnaea stagnalis [11] after the treatment with docetaxel and vinorelbine.

CONCLUSION

The present investigation, concluded that vinorelbine was more toxic than docetaxel as evident by the depletion in the number of protein bands in comparison to docetaxel treatment. So to control the population density of Lymnaea stagnalis the treatment with vinorelbine antitubuline drugs would be more significant. Toxic effect of antitubuline drugs on snails were detected by the depletion of proteins on SDS page.

References


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