

Original Research Article

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TOXIC EFFECT OF DOCETAXEL AND VINOURELBINE ON PROTEIN FRACTION OF *LYMNAEA STAGNALIS* DURING CLEAVAGE STAGE

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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 docetaxel and Vinorelbine on cleavage stages of *Lymnaea stagnalis* was studied. The observed values of sublethal concentration were 0.1 for docetaxel and 0.09 for Vinorelbine. The present investigation has been taken to study the effect of both drugs on depletion and deletion of proteins that is responsible for cleavage stages of *Lymnaea stagnalis*. So to control the population density of *Lymnaea stagnalis* the treatment with vinorelbine antitubuline drugs would be more significant for pest snail.

KEYWORDS: *Lymnaea stagnalis*, docetaxel, vinorelbine, sublethal concentration, SDS-PAGE.

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1. INTRODUCTION

Snails are distributed worldwide [1]. The snail act as the intermediate hosts of trematode parasite, the causative agent of helminthes diseases [2]. The common pond snail is a freshwater species widely used in embryological studied [3]. Snails are the pest of paddy crop, aquatic garden vegetation, coffee, tea, money and ornamental plants. Family Lymnaeidae is abundant in our fresh water lakes and ponds [4]. Docetaxel has been one of the most important chemotherapeutic drugs for cancer treatment and a number of clinical studies have been conducted to extend its clinical applications. a more potent semisynthetic derivative of paclitaxel, derived from extracts of the leaves of the European yew tree (*Taxus baccata*), was discovered in the 1980s [5]. Docetaxel is

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now generally recognized as one of the most active agents, and possibly the most active drug, currently available for the treatment of metastatic breast cancer [6]. Vinorelbine is a semi-synthetic vinca-alkaloid. Vinorelbine induces cytotoxicity by inhibiting the polymerization of tubulin dimers into microtubules, which in turn disrupts mitotic spindle formation and prevents cell division. This promotes apoptosis of cancer cells. Vinorelbine is selective for mitotic microtubules, with minimal activity on axonal or other microtubule classes[7]. 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 [8]. In the experimental groups of *Lymnaea* spp that toxified with the vinorelbine and docetaxel observed the depletion, destruction and degeneration of protein metabolites in the cleavage 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.

2. MATERIALS AND METHODS

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 for 7 days. 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 and data was collected in triplicate and calculated the value of sublethal concentration [9] and the sublethal values of docetaxel and vinorelbine was 0.1% and 0.09% respectively, to study their toxic effects on protein during various cleavage stage.

Detection of protein in cleavage stage

For quantification of extract protein, egg masses of cleavage stage were collected from snails egg masses. 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 Bloor'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 stagnalis*, 7 % SDS-PAGE was performed. In this 50 µl of pure egg masses of cleavage stage (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

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cleavage 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 cleavage stages of *Lymnaea stagnalis*.

3. RESULTS AND DISCUSSION

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 cleavage stage treated experimental groups due to intoxication of Docetaxel and Vinorelbine. The molecular weight of the cleavage stage of *Lymnaea stagnalis* in control ranged from 2.0 to 32.0 kDa, while cleavage stage of *Lymnaea stagnalis* treated with Docetaxel ranged from 5.1 to 12.2 kDa and cleavage stage of *Lymnaea stagnalis* treated with vinorelbine ranged from 11.8 to 17.8 kDa as exhibited in Figure 1. Eight bands in lane 1 of Figure 1 were observed in the cleavage stage of the control group. The bands were of 2.8, 4.7, 6.1, 12.2, 16.2, 18.2, 26.2 and 31.2 kDa molecular weight. One band of 16.2 kDa was found very high intensity. Two bands of 6.1 and 12.2 kDa were observed of high intensity. Two bands of 4.7 and 18.2 kDa were observed of low intensity. Three bands in lane 2 were observed in Docetaxel treated cleavage stage of *Lymnaea stagnalis*. The bands were of 5.1, 8.3 and 12.2 kDa. Two bands of 8.3 and 12.2 kDa were observed of high intensity. One band of 5.1 kDa were observed of very low intensity. Two bands in lane 3 were observed in Vinorelbine treated cleavage stage of *Lymnaea stagnalis*. The bands were of 11.8 and 17.8 kDa. Both two bands were observed of high intensity.

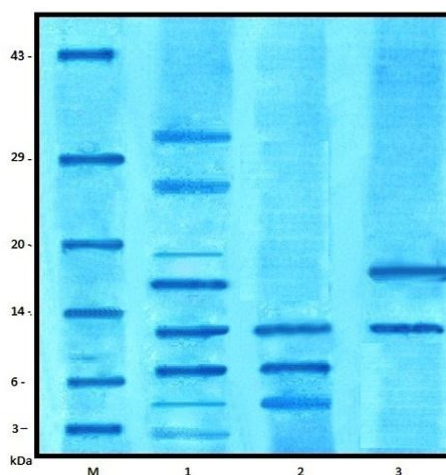


Figure 1: Depletion of protein in cleavage stage of *Lymnaea stagnalis* after treatment with vinorelbine and docetaxel.

Lane 1: control cleavage stage of *Lymnaea stagnalis*.

Lane 2: Cleavage stage of *Lymnaea stagnalis* treated with docetaxel.

Lane 3: Cleavage stage of *Lymnaea stagnalis* treated with vinorelbine.

Lane M: Protein Marker

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 [10] 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 by [11,12] 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 [13]. It is observed that paclitaxel was more toxic than colchicine in gastrula stage observed [14].

4. 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.

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CONFLICT OF INTEREST

There is no conflict of interest.

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