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Changes in Negatively Charged Protein Fraction after Treatment with Antitubulin Drug on Trochophore Larval Stage of *Lymnaea Stagnalis*

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Abstract: *Lymnaea stagnalis a pest of aquatic vegetation, prolific breeders and the intermediate host of helminthes diseases. So it is essential to control the fertility. The objective of these study larval stages of Lymnaea stagnalis was treated with different concentration of paclitaxol and colchicine. The values of LC_{100}, LC_{50}, LC_0 and sub lethal concentration were detected out and data was summarised in the table. Mortality during larval stages was high in treated group in snail which was more pronounced in trochophore larval stage. Detection of negatively charged protein fraction in the larval stage of control and treated snails by SDS-PAGE of Lymnaea stagnalis was assessed in the present investigation.*

Keywords: *Lymnaea stagnalis, Taxol, Colcincine, Mortality, Toxicity, SDS-PAGE*

1. INTRODUCTION

Lymnaeids are distributed worldwide [1]. Snails are the pest of aquatic vegetation and ornamental plants. They can be easily procured from any fresh water body. Snail is a hermaphrodite and also prolific breeders, capable of cross-fertilization. As a result snail population increases. Snails are harmful pests of various valuable crops and directly decline the crop productivity which affects the economy of the country. Severe damage caused to standing crops resulted into scarcity of raw material and create serious problem of food scarcity in that particular area. To save our valuable crops from the disaster of these pestiferous snails it is essential to control their fecundity and viability.

A lot of research work has been done on the neurons of *Lymnaea stagnails* by using antitubulin drug but nobody has paid any attention on the development of pond snail *Lymnaea stagnalis* with the effect of antitubulin drug. The present investigation was undertaken to study the lethal toxicity on trochophore larval stage of *Lymnaea stagnalis* with the effect of antitubulin drugs and for the study of negatively charged protein fraction by SDS-PAGE on the trochophore larva of fresh water snails treated with alkaloid is very scanty and some research work has been done in some gastropods e.g. in *Pacificoyster Crassostrea gigas* [2], in *Lymnaea stagnalis* after thiourea treatment [3], in Gyraulus convexiusculus after treatment of some pesticides and in *Lymnaea stagnalis* after treatment with colchicines but no literature is available on the toxicity of alkaloids on the trochophore larval stage of *Lymnaea stagnalis*.

2. MATERIALS AND METHOD

2.1. Selection of Pestiferous Snails

Common pond snails of *Lymnaea stagnalis* belonging to family Lymnaeidae were selected for the present investigation

2.2. Procurement of Rearing of Snails

Sexually mature specimens of *Lymnaea stagnalis* were collected from botanical garden pond of Dr. H. S. Gour University, Sagar and Sagar Lake by fishing nets or picking by hands and were kept in glass containers or troughs (2-5 liter water capacity). The collected snails were acclimatized for 7 days under laboratory conditions [6].The water was replaced with fresh water 3 times in a week. They were fed regularly with aquatic vegetation e.g. Hydrilla to avoid the stress of starvation.

2.3. Test Animal

The young ones hatched from the freshly laid egg masses of *Lymnaea stagnalis* were used for the experimental purpose. The egg masses laid by these snails were introduced to different concentration of antitubulin drugs separately in glass Petri dishes (50 ml capacity) in triplicate, used in the present investigation.

2.4. Antitubulin Drugs

The antitubulin drugs of analytic grade are Paclitaxel or Taxol and Colchicine procured from Sigma and CDH companies respectively.

2.5. Experiments with Different Dosages of Antitubulin Drugs

Fresh egg masses with subsequent developmental stages of *Lymnaea stagnalis* of F_0 generation were introduced via media to different concentrations of antitubulin drugs and the data was collected in triplicate and calculated the values of LC_{100}, LC_{50}, LC_0 and sub-lethal concentration were detected out for each group separately and data was summarized in table no.1 & 2 Probit analysis method [7]. Each egg masses contain about 30 egg capsules.

2.6. Preparation of Sample for the Detection of Protein Fractions in the Egg Masses Containing Trochophore Larval Stage of Control and Experimental Group of Lymnaea Stagnalis

Egg masses of *Lymnaea stagnalis* containing different developmental stages of control and experimental groups were cut into fine pieces and grind with Bloor's mixture into a homogenizer, then centrifuged at 3000 rpm for 20 minutes. The supernatant contained lipoprotein fractions was discarded while the residue collected was dried in air and further extracted with cold TCA (10%) and then centrifuged at 3000 rpm for 15 minutes. The supernatant contains nucleic acid was discarded, while pallet or residue was dissolved in 0.1 N NaoH (1 ml) (Boiled at 90^{o}C) and stored as protein sample for the detection of protein fractions.

Table1. *Data on Toxicity of Paclitaxel on the Trochophoral larval stage of Lymnaea stagnalis*

S.No	Name of the antitubulin drug	Concentration of the antitubulin drug	Duration (hrs.)	Mortality (%)	Lethal conc. Value
1.	Paclitaxel	0.12%	72	100%	LC_{100}
2.		0.06%	72	50%	LC_{50}
3.		0.03%	72	Nil	LC_0
4.		0.02%	72	Nil	Sublethal concentration

Result: *0.02% Concentration of paclitaxel was considered as sublethal concentration value*

Table2. *Data on Toxicity of Colchicine on the Trochophoral larval stage of Lymnaea stagnalis*

S.No	Name of the antitubulin drug	Concentrationof the antitubulin drug	Duration (hrs.)	Mortality (%)	Lethal conc. Value
1.	Colchicine	0.14%	72	100%	LC_{100}
2.		0.07%	72	50%	LC_{50}
3.		0.04%	72	Nil	LC_0
4.		0.03%	72	Nil	Sublethal concentration

Result: *0.03% Concentration of Colchicine was considered as sublethal concentration value*

2.7. Sample Preparation and Detection of Protein

For protein quantification trochophore larval stage were collected from snails, separated and individually 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 trochophore larval stage. These materials were homogenized in Bloor's mixture and the vials containing the larval stage of snails were centrifuged at 10,000 rpm for 10 min at 5˚C. Aliquots of the supernatants of the centrifuged extracts were used for protein content. In order to investigate the proteins from homogenized larval stage of *Lymnaea*

stagnalis 7 % SDS-PAGE was performed. In this 50 µl of pure stages (approx. 200 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). Samples containing 20 – 40 µl of proteins diluted 1: 1 in the buffer, were boiled in a water bath for 5 min, and after they had been cooled on ice, they were applied onto the polyarcylamide gel. The molecular mobility of proteins was determined by interpolation from mobility of commercial prestained standards (GeNei PMWM) by computer analysis and their profiles were analyzed through SDS-PAGE [8] and the larval stage was processed for the extraction of protein samples [9].

3. RESULTS

3.1. Behavior in the Control Groups

In control groups after immersion in water the snails *Lymnaea stagnalis* retracted body inside the shell.

3.2. Behaviour in Experimental Groups

Marked behavioural changes were observed when adult snails were introduced to the higher concentration of the antitubulin drugs as follows.

i. After immersion the gastropods retracted the body in the shell.

ii. The snails slightly protruded foot.

iii. The foot got adhered to the substratum with extended proboscis, peripodial lobe and tentacles.

iv. The snails when died, retracted bodies inside the shell to maximum extend.

The treated snails showed dullness throughout the experimental duration. They were apathetic during the experimental period. Although sample of aquatic vegetation was available but they did not show any attraction towards feed upto 30±6 hrs and 52±4 hrs in colchicine and paclitaxel treatment in *Lymnaea stagnalis*. Their feeding rate was also slow throughout the experimental period while the control snails fed at regular rate. If these snails were placed in fresh water under normal laboratory conditions their normal behaviour retained within three weeks. Detection of protein fractions in trochophore larval stages of *Lymnaea stagnalis* by SDS-PAGE is an important aspect of the present investigation because the biochemical metabolites like proteins play a very important role in overall growth, development and reproduction of the animals. In this investigation which evident the continuous increase (in control group) or regular decline (in experimental groups) in the number and intensities of protein metabolites in the detected fractions by SDS-PAGE and on the basis of these observations one can correlate this increase (in control group) or decrease (in experimental groups as the case may be) in molecular level.

Fig 1.Showed the –vely charged protein fractions were increased in number and intensity in trochophore larval 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 paclitaxel.The molecular mass of trochophore larval stage of control of *Lymnaea stagnalis* ranged from 2.5 to 40.1 kDa, while trochophore larval stage of *Lymnaea stagnalis* treated with colchicine ranged from 12.0 to 30.0 kDa and trochophore larval stage of *Lymnaea stagnalis* treated with paclitaxel ranged 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, 30.1 and 40.1 kDa were observed and of low intensity. Two bands of 2.5 and 5.8 kDa were observed and of very low intensity. Five bands in lane 2 of Fig. 1 were observed in colchicine treated trochophore larval stage of *Lymnaea stagnalis*. The bands were of 12.0, 15.1,

18.4, 22.2 and 30.1 kDa. One band of 15.1 kDa was observed and of very high intensity. Two bands of 18.4 and 22.2 kDa were observed and of high intensity. Two bands of 12.0 and 30.1 kDa were observed and of 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 and of high intensity.

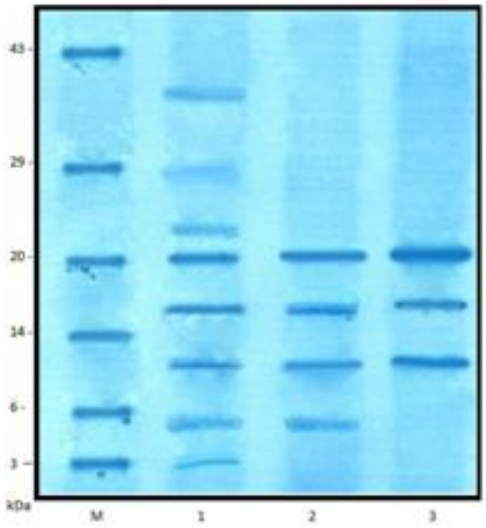

Fig1. *Showing Samples of Trochophore Larval Stage of Lymnaea stagnalis after Treatment with Colchicine and Paclitaxel. 1, 2 and 3 were Loaded on a Prepared SDS-PAGE Gel (7 % separating gel, 4 % stacking gel)*

- **Lane 1:** Trochophore larval stage of control *Lymnaea stagnalis*.
- **Lane 2:** Trochophore larval stage of *Lymnaea stagnalis* treated with Colchicine**.**
- **Lane 3:** Trochophore larval stage of *Lymnaea stagnalis* treated with Paclitaxel.
- The gel was stained with Coomassie Brilliant blue R-250.

4. DISCUSSION

Detection of negatively charged protein fractions by electrophoresis is the integrated part of the present investigation. In control the successive stages of development showed the gradual increase in the protein fractions indicated the progressive development of corresponding snails [10] but due to intoxication of the antitubulin drugs most of the developing stages showed the gradual decline not only in the number of protein fractions but also showed gradual decline in the intensities of some protein fractions in *Gyraulus convexiusculus* after treatment with some pesticites [4], in *Lymnaea Stagnalis* after treatment with some pesticides [11], in *Gyraulus convexiusculus* after treatment with Baygon studied. In *Lymnaea* spp. and *Gyraulus* spp. reported alternation in the number of protein fractions in larval stages observed [13,14].

The depletion in the number of protein fractions were due to partial or total arrest in the transcription of mRNA and ultimately affecting the translation and that is why specific fractions were missing in the corresponding developing stages as observed in trochophore larval stages and prior to hatching in *Lymnaea* spp. and proved the larvicidal nature of the alkaloid in the present investigation. The kinetic profiles of vitelline degradation by protease activity in embryos and larvae of the pacific Oyster, *Crassastrea gigas* have been investigated during the period from the unfertilized egg through the 48 hr straight hinge larva [2]. 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 [2]. It is observed that paclitaxel was more toxic than colchicine in gastrula stage observed [15].

In the present investigation it is observed that paclitaxel was more toxic than colchicine as evident by the depletion in the number of protein bands in comparison to colchicine treatment. So to control the population density of *Lymnaea stagnalis* the treatment with antitubuline drugs would be more significant.

5. CONCLUSION

It could be the suggested that different antitubulin drugs act as antifertility agents for decreased the fecundity of the snails. The strategy may be adopted to control the population of snail pest after a detailed research.

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