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LARVICIDAL AND BRINE SHRIMP LETHALITY ASSAY OF A LECTIN ISOLATED FROM MARINE SPONGE *AXINELLA DONNANI*

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Abstract
Lectins are glycoproteins of non – immune orgin which posses diverse biological and structural properties. In this study, a lectin isolated from marine sponge *Axinella donnani* (ADL) were evaluated for its cytotoxic and larvicidal activity. Bioassay of ADL was tested against brine shrimp; *Artemia* sp. ADL showed potent cytotoxic activity after 24 hours against the different concentrations of ADL (50 - 200 µg/mL) tested. Finally the larvicidal activity of ADL (100 -1000µg/mL) was checked against Bihar hairy caterpillar, *Spilosoma obliqua*. ADL effected mortality of *S.obliqua* in a dose dependent manner. Thus ADL could be a potent cytotoxic and larvicidal agent in clinical and pest management.

Key words: *A.donnani*, Lectin, *S. obliqua, Artemia* sp, Larvicidal, Cytotoxicity

Introduction

Marine sponges are oldest metazoans which produces a variety of biomolecules with insecticidal and cytotoxic activities (Rasjasa *etal.,* 2011; Burgess.,2012). For example; Maleimide -5-oxime and 18-bromooctadeca - (9E, 17E)-dien-7, 15-diynoic acid (2) insecticidal compounds isolated from marine sponge *X. testudinaria* have toxic effects on white fly *B. tabaci* (Genn.) and the Aphid *A. gossypii* (Glover) (Mostafa *etal*., 2019). Ethanolic extracts of marine sponges such as *C.longitoxa*, *C. diffusa*, *H. pigmentifera*, *S.carnosa*, and *D. nigra* exhibited insecticidal activity against fifth instar larvae of *C. quinquefasciatus* (Joseph *etal*., 2010). Crude extracts of marine sponges, N. *magnifica* and *C.siphonella* were tested for their antimicrobial, larvicidal, pupicidal, adulticidal effects against filarial vector *C.pipiens* were studied by Hasaballah *etal.,* (2017).

Pesticidal activities of lectins (carbohydrate binding proteins) are one of the interesting role of lectins in the host defense against pathogens and predators (Fitches *etal*., 2010). This property of lectins were utilized as naturally occurring pesticidal agents against, which restrain increased crop production (Hakim *etal*.,2010; Fitches *etal*., 2010; Kaur., 2009, 2006 a,b). However there are scanty reports available regarding the larvicidal activity of marine derived lectins. Artemia lethality bioassay have been successfully used for screening for cytotoxicity of bioactive compounds for their pharmacological activities including anticancer, antiviral, insecticides, pesticides and anti-HIV (Sorgeloos *etal*.,1978, Persoone;1980). Due to its sensitivity, easy availability and long stability *Artemia* sp. were suitable for biotechnological applications. There are many reports regarding the cytotoxic activity of lectins by Brine shrimp lethality test (Kawser *etal*., 2010; Khatun *etal*., 2011).

In the present work, the toxicity of a lectin isolated from the marine sponge, *A.donnani* were evaluated.

Materials and methods

Preparation of *A. donnani* lectin (ADL)

Lectin from the marine sponge *A.donnani* (ADL) were extracted with Phosphate buffered

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saline (PBS) buffer (pH - 7.4), fractionated by ammonium sulphate precipitation and purified by DEAE – Cellulose ion exchange and gel filtration chromatography. The purified lectin (ADL) was then lyophilized and kept under 4˚C until use.

Maintenance of test insects

Larvae of *S. obliqua* collected from the vegetable gardens of Sreekaryam, Trivandrum, Kerala, India .They were reared on *Clerodendrum infortunatum* leaves in the laboratory at 29 ± 3ºC and relative humidity 70 ± 10% in plastic trays (25 x 20 x 5 cm). Dead larvae were removed and the mother culture was cleaned every day. The second instar larvae were used for the experiments.

Insect bioassay

2nd instar larvae were bioassayed by treating ADL against sweet potato leaf based artificial diet (Armes *etal*., 1992). Leaves of were soaked in three concentrations of ADL (0.1, 0.25, 0.5 and 1 mg/ml as suggested in various papers). Control larvae were fed with distilled water treated leaves. The treated larvae were introduced into the plastic container (34 X 21mm) provided with moist cotton swab covered with tissue paper at the bottom of the container to provide humidity. The containers were covered with meshed lid to provide aeration to the larvae. The containers were incubated at room temperature 28 ± 0.5º C. Daily observation on larval mortality was recorded for a period of 10 days.

***Artemia* Nauplii Hatching**

The *Artemia* cysts were hatched in artificial seawater at 30°C with constant lighting and strong aeration. The cysts were incubated in a plastic container with 1 g cysts per liter of artificial seawater. After a period of 24 h, the nauplii are then collected and used for bioassay.

Artemia Lethality Test

The cytotoxic activity of ADL was evaluated against *A.salina* according to Mayer *etal*., (1982). 48 h cultured nauplii (n=10) were dispensed to a petridish (90×15 cm). ADL (50 - 200 μg/mL) were tested in triplicate and the system was incubated in a dark place for 48 h. As a negative control synthetic saline was used. After incubation, the number of surviving nauplii was counted under a microscope.

Statistical analysis

All the experiments were performed in triplicate (n = 3). One way Anova was performed to determine the LC_{50} value.

Results and Discussion

ADL have shown high lethality against brine shrimp, *A.salina*. LC_{50} of ADL was found to be 50 µg/mL (Table-1). Similarly ACL-1, a lectin isolated from marine sponge from *Axinellidae* family ie., *A.corrugata* have shown higher toxicity at 0.951µg/mL against Brine shrimp (Dresch *etal.,* 2012). Cytotoxic study of Moura *etal*., (2015) from CVL-2, a galactose specific trimeric lectin from marine sponge *C. varians* have shown lower lethality (LC_{50} - 850 µg/mL). On the other hand lectins from (H-1 and H-2) from marine sponge *H.caerulea* (LC_{50} - 6.4 & 142.1µg/mL respectively) showed higher toxicity (Carneiro *etal*., 2013). The exact mechanism by which lectins play its toxicity on *Artemia* is still unclear; however, fluorescence studied by Arruda *etal*., (2013) showed the presence of lectins in the digestive tract of *Artemia* nauplii, suggesting that the surface of the digestive tract is extensively glycosylated

Table 1. *Artemia* lethality bioassay

Lectin Concentration (µg/mL)	Mortality (%)
Control	6.67 ± 5.78
50	26.67 ± 5.78^{a}
100	53.33 ± 5.78^{a,b}
150	83.33 ± 5.78^{a,b,c}
200	100 ± 0^{a,b,c,d}

ADL exhibited larvicidal activity against *S. obliqua* in a dose-dependent manner. LC_{50} of

ADL was found to be 500 µg/mL, against this larva (Table-2). There was not any literature available on the effect of marine sponge lectins on any larvae. Sadanandan and Rauf (2021) reported insecticidal activity of marine sponge lectin *F.cavernosa* against cowpea aphid, *Aphis craccivora*. However there are reports of pesticidal activity of plant lectins against *A.sordens* and *P. nubilalis* (Keburia *etal*.,2010) , *H. armigera* (Arora *etal.,*2005; Shukla *etal*.,2005 .,Ohizumi *etal*., 2009) ,*Spodoptera litura* (Sadeghi *etal*., 2007., Namasivayam.,2014), *A. kuehniella* (Macedo *etal.,*2007). This larvicidal property of lectins may be due to orchestration of enzymatic activity of larvae. After treatment with lectins, alteration of the enzymes such as esterases, acid phosphatase and alkaline phosphatase occurs in larvae and thus affecting the mortality (Hamid *etal*., 2013). However this is the first report of a marine sponge lectin on *S. obliqua* to our knowledge. Further studies required for the possible utilization of lectin as an effective biopesticidal agent and study its mechanism of action.

Table 2. Mortality of *S.obliqua* treated with ADL

Lectin Concentration (µg/mL)	Mortality (%)
Control	0^{a}
100	0^{a}
250	20.5 ± 0.7^{b}
500	51.25 ± 1.9^{c}
1000	87.6 ± 2.56^{d}

Conclusion

A galactose specific lectin isolated from marine sponge *A.donnani* (ADL) exhibited cytotoxic and larvicidal activity in a dose dependent manner. These results indicate that the lectin may be utilized in therapeutic and pesticidal applications.

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