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EFFECT OF DENATURING AGENTS ON A LECTIN ISOLATED FROM MARINE SPONGE *AXINELLA DONNANI* AND ITS POTENTIAL CYTOTOXIC ACTIVITY

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Abstract

Marine sponges are oldest metazoans which were found to have structural and biologically diverse lectins. A lectin isolated from marine sponge *Axinella donnani* (ADL) were evaluated for its stability against denaturing agents such as Urea, Thiourea and Guanidine hydrochloride. Urea and Thiourea decreases the stability of ADL in higher concentration (5 mM) while Guanidine hydrochloride does not show any activity. Finally, cytotoxicity of ADL at different concentrations (0.25-500 mg/mL) was tested against MCF-7, A431 and HeLa cell lines by MTT assay. ADL exhibited potential cytotoxicity against these cell lines after 24 hours in a dose dependent manner. Thus, ADL could be a potent cytotoxic agent in clinical applications.

Keywords : *Axinella donnani*, Lectin, MCF-7, A431, HeLa, MTT assay.

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1. INTRODUCTION

Marine sponges are promising sources of new biologically-active molecules, many of which are still remain unknown and unexplored biotechnological resources. The sponges which belong to genus *Axinella* (class Demospongiae, order Halichondridae, family Axinellidae) contains almost 20 species distributed world-wide is known source of metabolites such as

bromo compounds, cyclopeptides, polyethers, sterols, and terpenes [1-3]. Among them *Axinella donnani* is a dark, thick, cup shaped marine sponge with dark brownish colour [4,5]. Antibacterial, cytotoxic, larvicidal, antifouling and ichthyotoxic activity were reported from these sponges [6,7].

Lectins are carbohydrate binding proteins which are widespread in nature including sponges [8]. Lectins can be utilized for structural studies, molecular and cell biology, immunology, pharmacology, medicine and cell biology. Lectins and other proteins from marine sponges have proved as potent candidates for new drugs, due to their wide range of biological activities, such as antibacterial, pro-inflammatory and antitumoral [9]. According to literature sponge lectins possess many unique structural, physical and chemical properties [9, 10]. Many research works were undergoing to elucidate lectins from marine sponges.

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

2. MATERIALS AND METHODS

2.1. Reagents

Trypsin was purchased from Gibco (USA). Fetal bovine serum (FBS), Dulbecco´s modified eagles medium (DMEM), 3-(4, 5-dimethylthiazol-2-yl) -2, 5 diphenyltetrazolium bromide (MTT), Penicillin-Streptomycin were obtained from Himedia laboratories, Mumbai, India. All of the reagents used in the study were of analytical grade.

2.2. Preparation of *Axinella donnani* lectin (ADL)

Lectin from marine sponge *Axinella donnani* were extracted with Phosphate buffered saline (PBS) buffer (pH - 7.4), fractionated by ammonium sulphate precipitation and purified by DEAE-Cellulose ion exchange followed by gel filtration chromatography. ADL was found to be a molecular mass of 30 kDa approximately after SDS-PAGE. The purified lectin (ADL) was then lyophilized and kept under 4˚C until use.

2.3. Cell lines

Human breast **(**MCF-7), Skin (A431) and cervical (HeLa) cancer cell lines were purchased from NCCS, Pune, India. The cells were maintained in DMEM, supplemented with 10% fetal

bovine serum (FBS) and 100 U/l penicillin. Cells were cultured at 37ºC in a humified atmosphere of 5% CO_2 and 95% air. The cells were sub cultured at regular intervals.

2.4. MTT assay

Cytotoxicity of ADL on viability of cell lines was determined by MTT assay on MCF-7, A431, HeLa cells [11]. The assay relies on the reduction of the MTT, Primarily by the mitochondrial dehydrogenases, to purple coloured formazan crystals [12]. The formazan product is analyzed spectrophotometrically at 570nm. The spectra of ADL-treated and untreated cells giving an estimate extent of cytotoxicity. Viability of cells were calculated using the following equation (1),

$$\% \ viability = \frac{Absorbance \ of \ treated \ cells}{Absorbance \ of \ untreated \ cells} \times 100 \qquad (1)$$

2.5. Effect of denaturing agents

The effect of denaturing agents on lectin activity was analyzed by using Guanidine hydrochloride, Urea and Thiourea at a concentration ranging from 0.5-5M. Then 50 µL of each solution was incubated with 50µL of lectin solution (1mg/ml) in a microtitre plate at 37°C for 1 h and the haemagglutination activity of the lectin was checked in treated and untreated samples [13].

2.6. Statistical analysis

All the experiments were performed in triplicate (n = 3). The data were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The reducing agent Guanidine hydrochloride does not exhibit any activity against ADL. While Urea and Thiourea decreases the stability of ADL in higher concentration (5 Mm) (Fig-1). There was scanty literature available with respect to the activity of denaturing agents on sponge lectins. The activity of denaturing agents on marine sponge lectin *Fasciospongia cavernosa* was studied by Sadanandan and Rauf., [14]. The high concentrations of denaturing agents are presumed to allow water molecules to interrupt the hydrophobic interactions in the internal lectin structure thereby maintaining its native structure [15]. Nevertheless, there are reports available with the inhibitory activity of Urea, Thiourea and Guanidine hydrochloride

in plant lectins [13, 16].

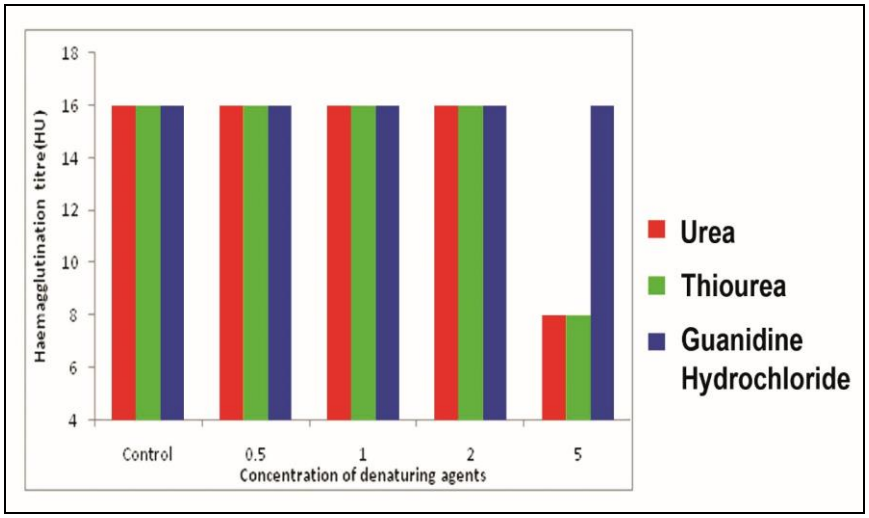

Fig.1. Effect of denaturing agents on ADL

The cytotoxic activity of ADL to HeLa, MCF-7 and A431 after an incubation period of 24h 24 using the calorimetric MTT assay. These cell lines showed an inhibition of cell growth in a in a dose dependent manner., with LC_{50} of 500 mg/mL after (Fig- 2 a, b, c). Cytotoxic activity of methanolic extract of *A. donnani* against leukemia were studied by Yalçın *etal.*, [17]. Toxicity of lectins from sponges such as *Axinella corrugata* [18], *Haliclona cratera* [18], *Cinachyrella apion* [20], *Cliona varians* [21], *Haliclona caerulea* [22] against different cancer cell lines were reported earlier. Anyway, further studies were required are required to determine the mechanism of action of ADL on the antiproliferative activity of cancer cell lines studied.

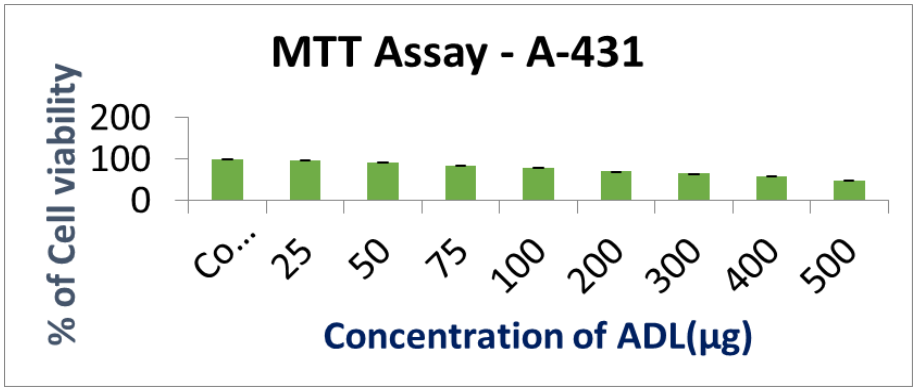

Fig.2a. Effect of ADL on A431Cellline

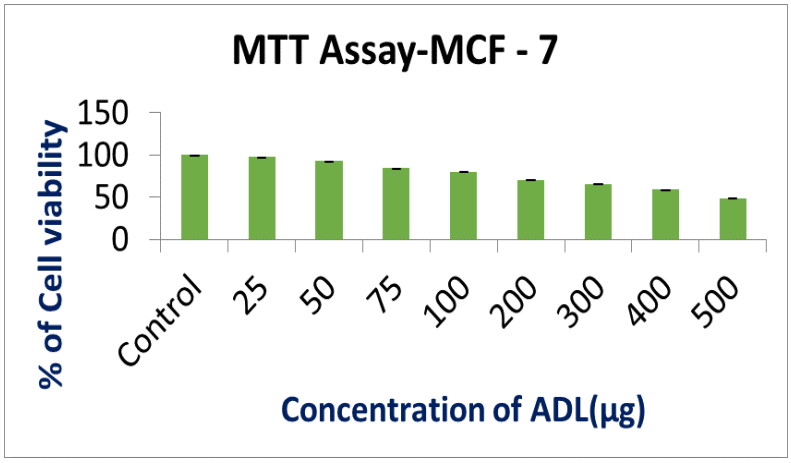

Fig.2b. Effect of ADL on MCF-7 Cellline

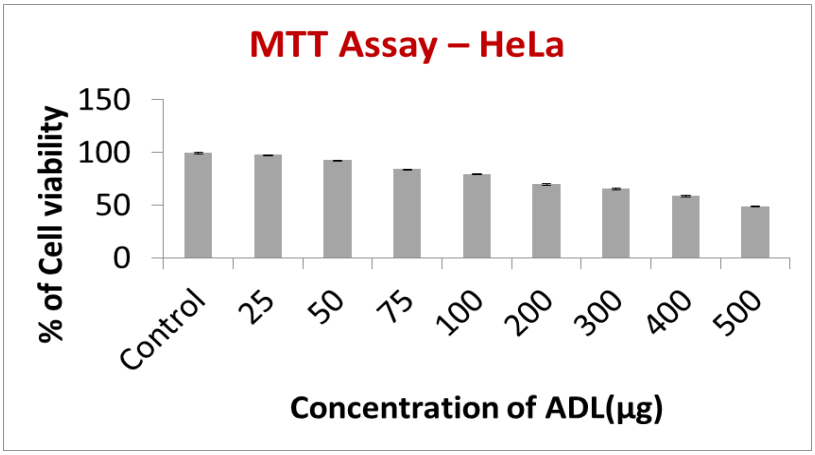

Fig. 3c. Effect of ADL on HeLa Cellline

4. CONCLUSION

A lactose specific lectin isolated from marine sponge *Axinella donnani* (ADL) exhibited cytotoxic activity against cancer cell lines tested in a dose dependent manner. The stability of ADL was decreased by the denaturing agents urea and Thiourea in increasing concentrations. These results indicate that the lectin may be utilized in therapeutic applications.

5. ACKNOWLEDGEMENT

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