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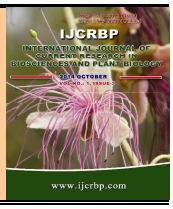

Original Research Article

In Vitro Anticancer Activity and DNA Fragmentation Capacity of a Marine Sponge, *Spongia tosta*

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Abstract

More than 60% of currently used anticancer agents have been derived from natural sources including plants, marine and microorganism. Many natural products form marine sources are endowed with promising immunomodulating activities, thus representing invaluable leads in the drug discovery. Sponges (Phylum: Porifera) are sessile marine invertebrates and are known to be the richest source of pharmacologically-active compounds. This work was taken to investigate the antibacterial, antifungal activity and cytotoxicity form marine sponge. The study was aimed to evaluate the anticancer activity of the marine sponge, *Spongia tosta* on the EAC cell line. The methanolic extract of *S. tosta* can be used as anticancer activity. More over in DNA fragmentation assay induction of apoptosis was confirmed by electrophoretic pattern of separated DNA fragments in HeLa cell line, which could be considered as one of the cytotoxic mechanisms of the compounds as potential anticancer agents.

Keywords

Cytotoxicity
DNA fragmentation
EAC cell line
HeLa cells
Marine sponge

Introduction

Cancer is a serious clinical problem that possesses significant social and economic challenges to the healthcare system. Cancer is the major public difficulty and one of the top causes of death in the prosperous countries. Despite improved imaging and molecular diagnostic techniques. Cancer continues to affect millions of people globally. In many countries, cancer is the second leading cause of death after heart diseases. Overall survival rate has only improved slightly despite advances in surgery, radiotherapy and chemotherapy. Most of the anticancer drugs to normal cells and cause immunotoxicity which affects not only tumour development, but also aggravates patient's recovery. The discovery and identification of new antitumor drugs with low side effects on immune system has become an essential goal in many studies of immunopharmacology (Subhadradevi et al., 2010).

Marine sponges have been considered as a gold mine during the past 50 years, with respect to the diversity of their secondary metabolites. The biological effects of new metabolites from sponges have been reported in hundreds of scientific papers, and they are reviewed here sponges have the potential to provide future drugs against important diseases, malaria, and inflammations. Although the molecular mode of action of most metabolites is still unclear, for a substantial number of compounds the mechanism by which they interfere with the pathogenesis of a wide range of diseases have been reported. This knowledge is one of the key factors necessary to transform bioactive compounds into medicines. Sponges produce a plethora of chemical compounds with widely varying carbon skeletons, which have been found to interfere with pathogenesis at many different points. The fact that a particular disease can be fought at different points increases the chance of developing selective drugs for specific targets (Munro et al., 1999)

Toxicity testing can reveal some of the risks that may be associated with their usage. This intern will facilitate in the identification of toxicants at an early stage of drug discovery and development from sponges sources. Hence the present study was taken up with intent to understand the cytotoxic property and DNA fragments assay of extracts form *S. tosta* which will help in dosage fixation for further exploration of their therapeutic efficacy.

Materials and methods

Collection of Sample

The sponge sample (Fig. 1) was collected as entangled specimens from a bottom trawl fish net operated off Manoli and Hare Islands of Mandapam group of Islands, Gulf of Mannar at Rameswaram. It was collected by bicatching method. The samples were placed inside sterile ethyl polythene bags under water and transferred to the lab aseptically in ice boxes.

Reagents

Trypanblue, Fetal Bovine Serum [FBS], Phosphate Buffered Saline [PBS], Dulbecco's modified Eagle's Medium [DMEM] and Trypsin were obtained from Sigma Aldrich Co, St. Louis, USA. EDTA, Glucose and antibiotics from [Hi-Media Laboratories Ltd, Mumbai]. Dimethyl Sulfoxide [DMSO] and Propanol [E.Merck Ltd, Mumbai, India].

Cells and Culture Medium

EAC [Ehrlich Ascites Carcinoma] cells were propagated in the peritoneal cavity of mice by transplanting one million DLA [Dalton's Lymphoma Ascities] cells per ml of Phosphate Buffered Saline [PBS], for experimental purposes, the tumour cells were aspirated from tumour bearing mice ascetically.

Preparation of Test Solutions

For cytotoxicity studies, each weighed *S. tosta* was separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Preparation of Sponge Extracts

Prior to the extraction, samples were washed with water, cleaned, air dried, lyophilized and powdered. They were stored for further use. For the extraction of crude bioactives, 100 g of powdered material was exhaustively extracted with 200 ml of methanol using Soxhelt apparatus and concentrated in a rotary evaporated at reduced pressure (Wang et al., 2009).

Fig. 1: The marine sponge, *Spongia tosta* used in the present study.

Cytotoxicity assay

Trypan Blue Dye Exclusion Technique

The cytotoxicity studies were done on EAC cells by Trypan blue exclusion method. Cells were aspirated from the peritoneal cavity of tumor bearing mice and washed in PBS twice and counted using a haemocytometer 2×10^5 cells / ml were taken for cell cytotoxicity studies. Although an aseptic technique is not essential in all stages of this procedure, it is good laboratory practice to maintain sterility throughout the procedure different concentration of the compound was added to the cells and then made up to 1 ml with PBS. Cells were incubated for 3 h at 37°C.

After incubation, the cell death was evaluated using Trypan blue exclusion method. With an equal volume of Trypan blue, 50 ml of cell suspension was added and mixed well using a pipette, transferred to a hemocytometer to count the live cell as clear form and dead cell as blue cells. After staining with Trypan blue solution counting was commenced < 5 min. The percentage growth inhibition was calculated and CTC_{50} value is generated from the close response curves for each cell line calculating percent viability by using formula:

$$\text{Growth Inhibition} = 100 - \left[\frac{\text{Total Cells} - \text{Dead Cells}}{\text{Total Cells}}\right] \text{x} 100$$

DNA Fragmentation Studies

Using apoptotic DNA ladder kit [G Bioscience], HeLa cells (3×10^6 cells / well) were seeded in two 6 well plates and allowed to adhere for 24 h then incubated for 48 h at 37°C in humidified atmosphere of 95% air and 5% carbondioxide after trypsinization, cells were washed with 200 µl of PBS. Apoptotic cells were incubated with 200 µl of lysis/ binding buffer in 15-25°C for 10 min.

After incubation, the based sample was mixed with 100 µl isopropanol and pipetted into a filter tube containing glass fleere. DNA which was bound to the filter tube was isolated from the by sate through centrifugation of the sample [1 min; 8000 rpm; twice] which was followed by a final high speed spin [13rpm; 1 min; then 10 sec in RT].

The flow through liquid containing unbound by sate components was then discarded after washing the bound DNA. The filter was inserted into 1.5 ml centrifugal tube. 200µl warmed (70°C) elution buffer was then added and the eluted DNA was collected by centrifugation [1 min; 8000 rpm; RT]. 20 µl of DNA eluted sample was mixed with 4µl of loading buffer, electrophoresed on 0.8% agarose gels at 90V for 1.5h and visualized using a UV transilluminator and then photographed (Azizi et al., 2009).

Results and discussion

In the present study the cytotoxic effect of extracts from *S. tosta* was determined. The extract was tested against a panel of normal and cancer cell line at range of 62.5 to 1000 µg / ml using Trypan blue exclusion method. The CTC_{50} values were shown separately for normal and cancer cell lines (Table 1). The CTC_{50} values for short term study are depicted in Fig. 2.

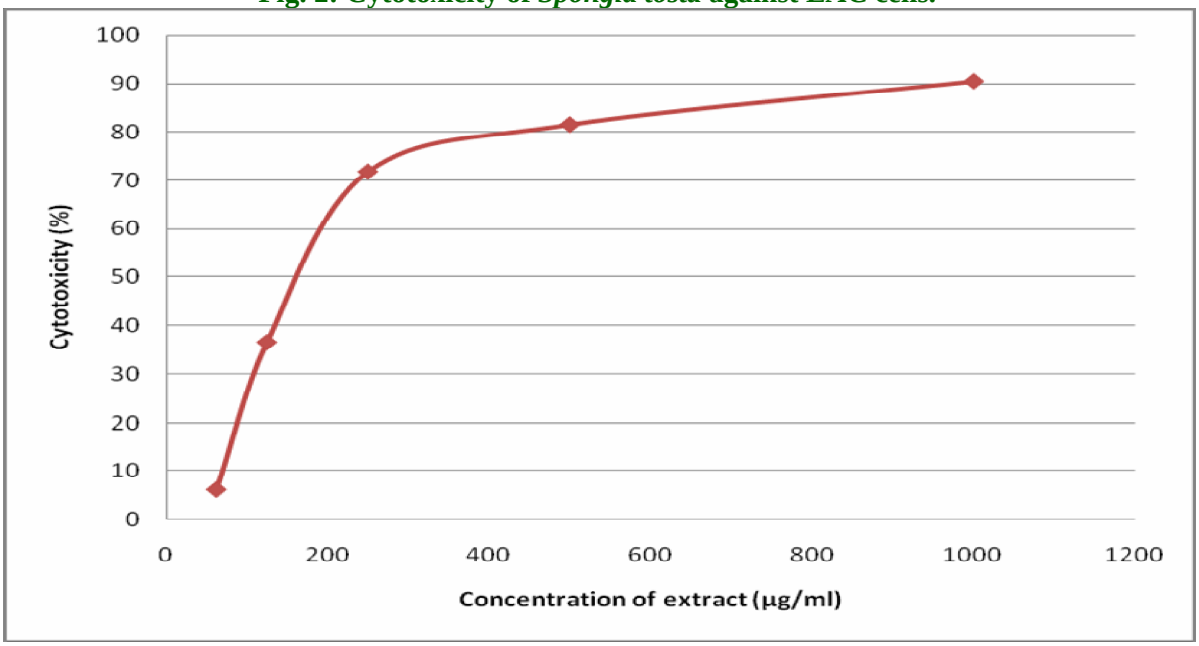
In the present study, only methanolic extracts from *S. tosta* was evaluated against EAC cells and was found to possess cytotoxicity. The cytotoxicity was found to be 90.38, 81.48, 71.72, 36.55 and 6.30% (Fig. 2). Moreover in DNA fragmentation assay induction of apoptosis was confirmed by electrophoresis pattern of separated DNA fragments in Hela Cell Lines (Fig. 3). The results of other studies also revealed that induction of apoptosis and DNA fragmentation are the most reported mechanisms.

Table 1. Cytotoxic properties of a marine sponge *Spongia tosta* on EAC cells.

Test Sample	Concentration (µg/ml)	Dead cells	Viable cells	Total cells	Cytotoxicity (%)
Methanol extracts of *S. tosta*	1000	141	15	156	90.38
	500	110	25	135	81.48
	250	104	41	145	71.72
	125	53	92	145	36.55
	62.5	8	119	127	6.30
IC_{50}					**174.50**

Cancer can be described as the major non communicable disease that India is going to face in the coming decades with projected cases of over 10 lakhs by 2015 (Ali et al., 2011). The increased socio economic burden arising out of the treatment regimens and adverse side effect is supposed to affect a larger population which raises concerns.In this aspect *S. tosta* was studied for its anticancer potential owing to the fact that apart from screening studies no scientific evidence is available till date. The anticancer potential of *S. tosta* and the mechanism of cytotoxicity has been many Marine Algae have been used as food among people in some part of the world and certain algae have long been used in traditional Chinese herbal medicine in the treatment of cancer (Yamamotoi et al., 1984).

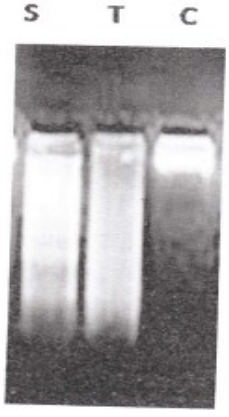
Fig. 2: Cytotoxicity of *Spongia tosta* against EAC cells.



There is an increasing number of studies which have shown the bioactive potential of compounds produced by marine algae (Albano et al., 1990). It is long time that human beings have known marine algae as a rich source of pharmacologically active metabolites with antineoplastic, antimicrobial and antiviral effects (Faulkner et al., 2000). The anticancer activity is one of the most important activities in marine algae and many algae have shown cytotoxic and anticancer activities (Mayer et al., 2003). The anticancer activities can play an important role in leading to new pharmaceutical compounds for anticancer drugs (Yoo et al., 2002). Several species of algae have been found to be sources of metabolites with antitumor and immune stimulant activities (Konig et al., 1995). But the studies on biological and pharmacological activities of marine sponges are limited.

Marine sponges are also having many pharmacological activities. Some of the drugs which are isolated from marine organisms including sponges have already been available in the pharmaceutical market. For example, Prialt® (ziconotide; potent analgesic) and Yondelis® (trabectedin or ET-743; antitumor). Many such drugs are under clinical trials (alpidin and kahalalide F) (Ebada et al., 2010). In *S. tosta*, biological activity studies are at preliminary level.

Fig. 3: DNA fragmentation by *Spongia tosta* extract against HeLa cells.



S: Standard- Doxorubicin (5µg/ml)

T: Test extract- 300 µg/ml

C: Untreated HeLa cells

Conclusion

This study confirms that methanolic extract of the marine sponge *S. tosta* was found to possess anticancer properties. More over in DNA fragmentation assay induction of apoptosis was confirmed by electrophoretic pattern of separated DNA fragments in HeLa cell line. Further indepth studies on *S. tosta* may yield a new marine resource for anticancer medicine.

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