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Immunomodulatory Potential of a Marine Sponge *Spongosorites halichondrioides* (Dendy, 1905)

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Abstract

Marine sponges are animals belonging to the Phylum Porifera. They are well known for its medicinal value. However, to prove its efficiency for the clinical utilization, more experimental data will be beneficial. The present study involved the investigation of immunomodulatory activities of methanolic extract of marine sponge *Spongosorites halichondriodes* from western coast of India. The extract was studied for acute toxicity (OECD-425 guideline), Haemagglutinating antibody (HA) titre, delayed-type hypersensitivity (DTH) response and cyclophosphamide-induced myelosuppression for their immunomodulatory potential. The evaluation of immunomodulatory potential by oral administration of methanolic extract of marine sponge (200 mg/kg) evoked a significant decrease in total WBC count as compared to control, in antibody titre values, and also in delayed type hypersensitivity reaction induced by sheep red blood cells. Also it prevented myelosuppression in cyclophosphamide drug treated rats. The results obtained in the present study indicated that extract of marine sponge *Spongosorites halichondrioides* possesses immunosuppressant activity and can be studied further for isolation of the compounds which can be used for organ rejection purpose in future.

Keywords: *Spongosorites halichondrioides,* Haemagglutinating antibody (HA) titre, Delayed-type hypersensitivity (DTH) response, Cyclophosphamide-induced myelosuppression

Introduction

Marine sponges are a rich source of biologically active secondary metabolites with novel chemical structures. Most bioactive compounds from sponges can be classified as anti-inflammatory, antitumor, immunosuppressive or neurosuppressive, antiviral, antimalarial, antibiotic and antifouling agents. The chemical diversity of sponge products is remarkable. In addition to the unusual nucleosides, bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and amino acid derivatives (which are frequently halogenated) have been described from sponges (Joseph and Sujatha 2011; Sipkema *et al*. 2005).

Immunomodulation is explained as any change in the immune response and may involve induction, expression, amplification of any part or phase in the immune response. Modulation may be very specific and limited to a given antigen/agent or non-specific, with a great effect on immune response. Stimulation of the immune response is preferred for certain people such as immunocompromised patient, whereas, suppression of immune response is consideration for others such as transplant recipient or inflammatory diseases. Immunomodulatory biomolecules of animal as well as plant sources activates the immune response in an organism against any foreign invaders by mimicking the immune reactions. Immunosuppressant's are structurally and functionally heterogeneous group of drugs, which are often concomitantly administered in combination regimens to treat various types of organ transplant rejection and autoimmune diseases.

In one of the previous study, a series of mono and bisindole alkaloids were isolated from the marine sponge *Spongosorites* sp. Seven (**1**−**7**) monoindole derivatives were isolated from the MeOH extract of this marine sponge *Spongosorites* sp. by bioactivity-guided fractionation. The planar structures were established on the basis of NMR and MS spectroscopic analyses (Figure 1). These bisindole alkaloids have been studied for cytotoxicity properties, but there are no reports of any immunomodulatory properties (Bao *et al*. 2005, 2007a, 2007b).

However, there is no scientific report available in the literature on the immunomodulatory activity of

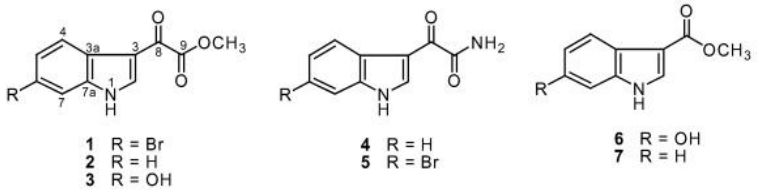

Figure 1. Seven (1–7) Monoindole derivatives isolated from the MeOH extract of a marine sponge *Spongosorites* sp., Mar Drugs.2007;5(2): 31–39.

Spongosorites halichondriodes sponge. Therefore, the present study was undertaken to assess the immunomodulating activities of the methanolic extract derived from the dried sponge *Spongosories halichondriodes* in relation with its reported medicinal properties.

Material and Methods

General

Hematological readings were recorded on Sysmex; poch-100i. Rota Evaporator used for preparation of crude extract was from Buchi. Thermo scientific, Forma orbital shaker 420 was used for extraction.

Spongosorites halichondriodes Sponge Material

The sponge was collected from Khardanda beach in western costal region Khar, Mumbai, in the month of April, 2011. It was sent for identification to Zoological Survey of India, Chennai and is registered in Marine Biology Regional Centre, ZSI.

Preparation of Extract

10 g of air dried sponge sample was soaked in 200ml of methanol for 1 day. The solvent was removed after squeezing the sponge and filtered through Whatman filter paper no.1. The solvent was evaporated at low pressure using rotary evaporator at 40˚C. The dried extract mass was stored in refrigerator at 4˚C for further use.

Drugs

Accurately weighed quantities of the ethanol extract were suspended in 0.5 % sodium carboxy methylcellulose (SCMC) to prepare suitable forms of the dosages. Cyclophosphamide was used as a standard immunosuppressant drug. It was obtained from Nanavati hospital located at Vile Parle, Mumbai. Endoxan-N vials containing 1 gm of cyclophosphamide were brought and reconstituted using sterile water.

Antigen

Fresh Sheep blood was collected in Alsver's solution (Dextrose 20.5 g, Sodium citrate 8.0 g, and Sodium Chloride 4.2, Citric Acid 0.55 g dissolved in 1000 ml of deionized water) in 1:1 ratio from local slaughter house. This blood was centrifuged at 8000 rpm for 10 mins. The pellet of Sheep RBC's was resuspended in Phosphate Buffer Saline and centrifuged at 8000 rpm for 10 mins. This process was repeated 2-3 times. The Sheep RBC's pellet was finally resuspended in PBS. The dilution was made upto concentration of 0.5×10^9 cells/ml for immunisation and challenge.

Acute Toxicity Assay

An acute oral toxicity study of extract was carried out as per the OECD guidelines-423 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India. Male swiss albino mice weighing 25-30 gm were used for toxicity studies. The animals were obtained from Haffkine Institute, Mumbai. The animals were housed under standard conditions of temperature (25±10˚C) and relative humidity (60±10%), 12/12 h light/dark cycle, and fed with standard pellet diet and tap water. Animals were fasted prior to dosing and the test substance was administered in a single dose by oral route. Three animals were used for each step. Dried test extract administered orally to different groups of mice in dosages ranging from 100 to 1000 mg/kg for the LD_{50} study using the method of Joshi C. S *et al*., 2007 with some modifications.

Immunomodulatory Studies

The immunomodulatory study was approved by the Institute's Animal Ethical Committee and conformed to national guidelines on the care and use of laboratory animals. Female Wistar rats weighing 120- 150 g were obtained from Haffkine Institute for *in vivo* immunomodulatory studies. The animals were housed under standard conditions of temperature (25±10˚C) and relative humidity (60±10%), 12/12 h light/dark cycle, and fed with standard pellet diet and water.

Cyclophosphamide-Induced Myelosuppression

Ziauddin *et al*. (1996) method was employed for cyclophosphamide-induced myelosuppression. Albino rats were divided into three groups each comprising

six animals. The group I served as control, fed with distilled water, the group II served as a negative control, which was given cyclophosphamide at the dose of 30mg/kg, i.p. while group III rats received varied concentrations of methanolic sponge extract of *Spongosorites halichondriodes* (200–500mg/kg, p.o.) for 10 days. On day 11, blood samples were collected from the retro-orbital plexus of individual animals and analyzed for hematological and serological parameters.

Haemagglutinating Antibody (HA) Titre

Puri *et al*. (1993) described the method for haemagglutinating antibody titre. The animals were immunized by injecting 0.1ml of SRBCs suspension containing 0.5×10^9 cells intraperitoneally on day 0. Blood samples were collected in micro-centrifuge tubes from individual animal by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the hemagglutination technique. Equal volumes of individual serum samples of each group were pooled. Twofold serial dilutions of pooled serum samples made in 25µl volume of normal saline in microtitration plates was added to 25µl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37 °C for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum agglutination was taken as the antibody titre.

Delayed-Type Hypersensitivity (DTH) Response

The rats were challenged by injection of 0.5×109 cells SRBCs in right hind foot pad. Foot thickness was measured after +24 and +48 h of this challenge. The differences obtained for pre- and post challenge foot thicknesses were taken for the measurement of DTH and were expressed in mm. The extract was administered orally on day 0 and continued till day 7 of challenge (Shivaprasad *et al*., 2006).

Statistical Analysis

All experiments were repeated thrice and results are expressed as mean ± SD. Statistical analysis of data was done using student's test. Differences between the data were considered significant at $P<0.05$.

Results

Acute Toxicity Study

Administration of stepwise doses of extract according to body weight up to the dose 2000 mg/kg body weight caused no considerable signs of the toxicity in the tested animals upto 14 days. There was no lethality in any of the groups after 14 days of treatment. Hence, One tenth of this dose (200 mg/kg) was selected as the level for examination of immunomodulatory potential (OECD Guidelines 2000).

Immunomodulatory Studies:

The crude extract of *Spongosorites halichondriodes* effect was studied for Immunomodulatory effect, with hematological parameters (WBC & RBC COUNTS), Haemagglutinating Antibody titer and Foot Paw measurement.

Cyclophosphamide Induced Myelosuppression:

There was no change in total WBC count in control group as compared to standard group and test group. Cyclophosphamide at the dose of 30 mg/kg, i.p. caused a significant reduction in the total WBC count as compared to control group (group-I). Combined treatment of cyclophosphamide and methanolic extract of marine sponge (200 mg/kg, p.o.) resulted in a significant reduction in total WBC as compared to group treated with cyclophosphamide (group-II) alone. The methanolic extract of marine sponge decreased the levels of WBC at dose of 200 mg/kg as compared to the group treated with cyclophosphamide alone. Thus, the test drug shows immunosuppressant activity. The results of total WBC count on day 0 and day 11 are shown in Table 1.

Haemagglutinating Antibody (HA) Titre

According to the results obtained, it was observed that there was significant reduction in the HA titre values of group treated with cyclophosphamide alone (group-II) as compared to that of the control group (group-I). Also, there was reduction in HA titre values of group treated with methanolic extract of marine sponge (group-III) as

Table 1. Effect of methanol extract on WBC in rats treated with cyclophosphamide (CP)

Group	Treatment	Dose (mg/kg)	Total WBC count (per µl)			
			Day 0		Day 11	
			Mean ± S.E.M.	S.D.	Mean ± S.E.M.	S.D.
I	Control	-	6016.66 ± 454.17	1112.5	6533.33 ± 350.87	859.45
II	Standard (CP)	30	6050.00 ± 361.24	884.87	1700.00 ± 211.345 *	517.68
III	Test extract+CP	200	6066.66 ± 282.45	691.85	850.00 ± 42.81 **	104.88

Values are mean ± SEM (n=6) * P<0.001; ** P<0.005

compared to group treated with cyclophosphamide alone (group-II). Thus, the test extract showed immunosuppressant activity. The results of Haemagglutinating antibody (HA) titre are shown in Table 2.

The results of Haemagglutinating antibody (HA) titre revealed that there is decrease in HA titre values in group treated with cyclophosphamide alone (group-II) as compared to that of the control group (group-I). Also it was observed that there is decrease in HA titre values in test group treated with methanol extract of marine sponge (group-III) as compared to group treated with cyclophosphamide alone (group-II). Thus, the low values of HA titre obtained in case of methanol extract of marine sponge has indicated that immunosuppression was achieved in rats.

Delayed Type Hypersensitivity Reaction (DTH)/ Footpad Thickness Test in Rats

There was significant reduction in the DTH response in group treated with cyclophosphamide alone (group-II) as compared to that of the control group (group-I). Also, there was reduction in DTH response in group treated with methanolic extract of marine sponge (group-III) as compared to group treated with cyclophosphamide alone (group-II). Thus, the test drug showed immunosuppressant activity. The results of DTH studies are shown in Table 3.

Discussion

The present investigation suggests that methanolic extract derived from Spongosorites halichondriodes may suppress both cellular and humoral immune responses. The extract not only suppresses nonspecific immune response, but also decreased humoral as well as cell-mediated immunity effectively. A high degree of cell proliferation renders the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone marrow is the organ most affected during any immunosuppression therapy with this class of drugs. Loss of stem cells and inability of the bone marrow to regenerate new blood cells results in thrombocytopenia and leucopenia. Administration of the extract of marine sponge was found to decrease the total WBC count as compared to cyclophosphamide, a cytotoxic drug, indicating that the methanolic extract of marine sponge suppresses the bone marrow activity.

Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses. IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins, etc. Antibody production to T-dependent antigen SRBC requires co-operation of T- and B-lymphocytes and macrophages. Cyclophosphamide has a particularly intense effect on short-lived lymphocytes known to include a great proportion of B-cells. The results of Delayed type hypersensitivity (DTH) studies showed that there was decrease in DTH response in group treated with cyclophosphamide alone (group-II) as compared to that of control group (group-I) as cyclophosphamide damaged the short lived suppressor T-cells in immune regulatory systems. Also, decrease in DTH response was observed in group treated with methanolic extract of marine sponge (group-III) as compared to that of group treated with cyclophosphamide alone (group-II). Thus, decrease in the DTH reaction in rats in response to T-cell dependent antigen revealed immunosuppressant activity of methanolic extract of marine sponge. Thus, finally it can be concluded that the immunomodulatory studies on methanolic extract of marine sponge using three *in vivo* models like cyclophosphamide induced myelosuppression, Haemagglutinating antibody (HA) titer and Delayed type hypersensitivity (DTH) reaction, revealed that the test extract shows immunosuppression activity. However, future scope suggests purification and characterization of the biomolecules from the extract

Table 2. Effect of methanolic extract of marine sponge on HA titre using SRBCs in rats

Group	Treatment	Dose (mg/kg)	HA titre	
			Mean ± S.E.M.	S.D.
I	Control	-	48.0 ± 9.238	18.47
II	Standard (CP)	30	14.0 ± 2.0 *	4.00
III	Test extract + CP	200	3.0 ± 0.632 **	1.41

Values are mean ± SEM (n=6) ***** P<0.01; ** P<0.001

Table 3. Effect of methanolic extract of marine sponge on DTH response using SRBCs in rats

Group	Treatment	Dose (mg/kg)	DTH response (mm)			
			After +24h of challenge		After +48h of challenge	
			Mean ± S.E.M.	S.D.	Mean ± S.E.M.	S.D.
I	Control	-	0.69 ± 0.00187	0.037	0.615 ± 0.017	0.034
II	Standard (CP)	30	0.477 ± 0.0075*	0.015	0.44 ± 0.01 ***	0.021
III	Test extract + CP	200	0.41 ± 0.0132**	0.029	0.348 ± 0.008 ****	0.019

Values are mean ± SEM (n=6) ***** p<0.05; ** p<0.001; *** P< 0.001; ****P<0.001.

which possess the immunosuppressive activity in animal models. The extensive work can be planned *in- vitro* to confirm the bioactivity in animal models.

There are reports on Glycolipids, membrane constituents of animals and plants playing a major role as cell surface- associated antigens and recognition factors. Glycosphingolipids with immunomodulating activity are synthesized by sponges belonging to the genera *Agelas* and *Plakortis* while atypical glycolipids, formed with a sugar head glycosidically linked to a long alkyl chain, have been isolated from species belonging to the genera *Erylus* and *Plakortis*. From *Erylus placenta*, Fusetani's group isolated the erylusamines (Fusetani *et al.*, 1993; Sata *et al.*, 1994) while simplexides (Costantino *et al.*, 1997) have been isolated from *Plakortis simplex.* It is noteworthy that both eylusamines and simplexides possess potent immunosuppressive activity with a non-cytotoxic mechanism (Costantino *et al.*, 2004). In the current study, the nature of the compounds causing immunosuppressive activity is not known. However the presences of alkaloids, steroids, terpenoids and flavonoids have been detected by preliminary pharmacognosy chemical tests. The immunosuppressive activity can be attributed to any of these chemical constituents. The compounds can be claimed after isolation and characterization of the bioactive compounds from the crude methanolic extract.

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