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Original article

Antiinflamatuvar and Antioxidant Properties of *Spongosorites halichondriodes*, a Marine Sponge

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An array of biologically active compounds with unique, novel structures and pharmacological properties has been reported to occur in marine sponges. The present study provides information about extracts of *Spongosorites halichondriodes*, a marine sponge collected from western coast of India. The antioxidant and anti-inflammatory effects were investigated *in vitro* and *in vivo* experimental models. None of the extracts showed significant antioxidant activity. *N*-hexane, ethyl acetate and butanol extracts of *S. halichondriodes* were investigated for anti-inflammatory activity in carrageenan induced paw edema in Wistar rats, and compared to a positive control, diclofenac. The anti-inflammatory effect was investigated *in vitro* and experimental model in rats. The acute toxicity study of the methanolic extract on Wistar male rats exhibited an LD_{50} value of more than 5000 mg/kg with no behavioural changes. *n*-Hexane, ethyl acetate and butanol extract from *S. halichondriodes* (100, 200 and 500 mg/kg) were tested for anti-inflammatory activity. Ethyl acetate extract reduced hind paw swelling significantly with increasing dose at 200 and 500 mg/kg. No mortality was observed with increasing dose up to 500 mg/kg for intraperitoneal administration. The extract of the marine sponge *S. halichondriodes* possessed good anti-inflammatory activity. Therefore it can be further taken for studying the bioactive compounds responsible for the activity. As new marine bioactives are recently gaining importance, they could be helpful in combating chronic inflammatory degenerative conditions.

Key words: Marine sponge, Acute toxicity study, Carrageenan induced paw edema, DPPH assay.

Deniz Süngeri *Spongosorites halichondriodes*'in Antiinflamatuvar ve Antioksidan Özellikleri

Deniz süngerlerinde özgün, yeni yapılar ve farmakolojik özelliklere sahip biyolojik olarak aktif bir dizi bileşiğin varlığı bildirilmiştir. Bu çalışma, Hindistan'ın batı kıyılarından toplanan bir deniz süngeri olan *Spongosorites halichondriodes*'in ekstreleri hakkında bilgi vermektedir. Antioksidan ve anti-inflamatuvar etkileri *in vitro* ve *in vivo* deneysel modellerle araştırılmıştır. Ekstrelerin hiçbirisinde belirgin bir antioksidan aktivite gözlenmemiştir. *S. halichondriodes*'in *n*-hekzan, etil asetat ve butanol ekstreleri anti-inflamatuvar aktiviteleri için Wistar ratlarda karragenle indüklenmiş pençe ödemi ile incelenmiştir, ve pozitif kontrol olarak diklofenak ile karşılaştırılmıştır. Anti-inflamatuvar etki *in vitro* koşullarda ve ratlarda geliştirilen deneysel modelde incelenmiştir. Metanollü ekstrenin Wistar erkek ratlar üzerinde yapılan akut toksisite çalışmasında davranışsal değişiklikler olmaksızın 5000 mg/kg'dan daha büyük bir LD_{50} değeri gözlenmiştir. *S. halichondriodes*'den hazırlanan *n*-hekzan, etil asetat ve butanol ekstreleri (100, 200 and 500 mg/kg) anti-inflamatuvar aktivite için test edilmiştir. Etil asetat ekstresi 200 ve 500 mg/kg dozlarda arka ayaktaki şişkinliği önemli düzeyde azaltmıştır. Doz intraperitoneal olarak 500 mg/kg'a çıkarıldığında hiçbir deney hayvanında ölüm gözlenmemiştir. Deniz süngeri *S. halichondriodes*'in ekstresi iyi anti-inflamatuvar aktiviteye sahiptir. Bundan dolayı aktiviteden sorumlu biyoaktif bileşikleri ile ilgili ileri çalışmalar yapılabilir. Son zamanlarda yeni biyoaktif marin bileşikler önem kazanmaktadır ve kronik inflamatuvar dejeneratif hastalıklara karşı mücadelede faydalı olabilmektedir.

Anahtar kelimeler: Deniz süngeri, Akut toksisite çalışması, Karragenan pençe ödemi, DPPH testi.

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INTRODUCTION

Marine sponges have been considered as an important source during the past 50 years, with respect to the unique and diverse structure of their secondary metabolites. They have the potential to provide future drugs against important diseases like cancer, viral diseases, malaria, and inflammations. Although the molecular mode of action of most metabolites is still unclear, for a substantial number of compounds the mechanisms by which they interfere with the pathogenesis of a wide range of diseases have been reported. These are rich source of pharmacologically active compounds that can potentially be used as medicines to cure human diseases, and the isolation of these bioactive compounds from sponge has been already reviewed extensively (1).

They produce a wide array of secondary metabolites ranging from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols (2). These most bioactive compounds from sponges can be classified belonging to groups of anti-inflammatory, antitumor, immuno suppressive or neurosuppressive, antiviral, antimalarial, antibiotic, or antifouling class (1, 2). Pharmaceutical interest in sponges was aroused in the early 1950s by the discovery of nucleosides spongothymidine and spongouridine in the marine sponge *Cryptotethia crypta* (de Laubenfels, 1949). These nucleosides were the basis for the synthesis of Ara-C, the first marine-derived anticancer agent, and the antiviral drug Ara-A (1,3). Eighty four anti-inflammatory compounds have been isolated from marine sponges (2). A range of bioactive metabolites has been found in about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce powerful anti-cancer, anti-inflammatory agents, but their cultivation has not been studied.

Marine sponges are known for their anti-inflammatory activities, some examples are the compound cavernolide which was isolated from *Fasciospongia cavernosa* (Schmidt, 1862), contignasterol isolated from *Petrosia contignata* (Thiele, 1899) and the compound cyclolinteinone from *Cacospongia linteiformis*. The mechanism of action of these compounds were explained by the inhibition of enzymatic activities, inhibition of inducible nitric oxide synthase (iNOS), cyclooxygenase- 2 (COX-2) gene expression, plasma exudation *in vivo* in response to ovalbumin and prostaglandin E2 (3). Potent phosphate inhibitors have been isolated from sponges like, okadaic acid from *Halichondria okadai* (Kadota, 1922), motuporin from *Theonellas swinhoei* (Gray, 1868) and calyculin-A from *Discodermia calyx* (Doderlein, 1884) (4,5). Inhibitors of phospholipase such as manoalide and scalaradial have proved to be useful tools to study the role of this enzyme in the release of arachidonic acid, which is a key molecule, involved in the biochemical processes leading to inflammation. A number of receptor antagonists with potential as biochemical tools or structural leads to the development of therapeutics have been isolated from sponges. Examples include xestobergsterol (isolated from *Xestospongia berguista*), which inhibits immunoglobulin E mediated histamine release from mast cells and is 5000 times more potent than the antiallergic drug disodium cromoglycate. Leucettamine A isolated from *Leucettamicroraphis*, is a potent and selective antagonist for the receptor for leukotrine, a non-peptide metabolite of arachidonic acid produced mainly in inflammatory cell Batzelladine A & B, novel polycyclic guanidine alkaloids from the Caribbean sponge *Batzella* spp., exhibit potent inhibition to the binding of HIV glycoprotein, on CD4 receptors of T cells (6). Monoindole alkaloids such as hamacanthins and topsentins display potent biological cytotoxic activities in human solid tumor cell lines (7). However, there are no reports on the study of the anti-inflammatory and antioxidant effects from the marine sponge *Spongosorites halichondriodes* (Dendy, 1905).

The purpose of the present study was to investigate the anti-inflammatory activity of extracts of *Spongosorites halichondriodes* (Dendy, 1905) using the carrageenan -induced rat paw edema models and compare with the

commonly used non-steroidal anti-inflammatory drug (NSAID) diclofenac as a standard. We have also undertaken an evaluation of antioxidant properties of the extracts by antiradical activity assay using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) against ascorbic acid as a standard.

MATERIALS AND METHODS

Spongosorites halichondriodes (Dendy, 1905) sponge material

The sponge was collected from Khardanda beach in western costal region Khar, Mumbai, in the month of December, 2012. They samples were washed and freed from all visible surface debris with sea water, cleaned, washed rapidly with freshwater, and immediately stored at -80°C. The frozen samples were also immersed in ethanol and maintained at) 20°C. The biological sample was sent for identification to Zoological Survey of India, Chennai and the voucher specimen no. S- 165 is registered in Marine Biology Regional Centre, ZSI.

Chemicals and standard drugs

Diclofenac (Merck) and DPPH (Sigma Aldrich) were obtained at the Nanavati hospital pharmacy, Vile Parle (w), Mumbai. Carrageenan sodium salt was purchased from SD Fine Chemicals and ascorbic acid from Qualigens, Mumbai. The extracts and drugs were dispersed or dissolved in saline solution (0.9%) for administration. Bovine Serum albumin was procured from Himedia Laboratories, Mumbai.

Extract preparation

The sponge samples were crushed to fine powder form using mortar and pestle. The weighed amount of powdered sponge (500 g) was soaked in specific amount (3.0 L) of methanol so that the sponge is completely immersed in the methanol overnight. After 24 hours, extract was filtered and wet sponge was reimmersed in methanol (2.5 L) overnight. Maceration was repeated for three cycles. All the extracts collected throughout the process were pooled and concentrated in rotary vacuum evaporator to obtain extract (yield-10.26%). The extract was successively extracted using solvents with increasing polarity by partitioning with *n*-hexane, ethyl acetate and butanol. All the extracts were concentrated and evaporated to dryness and kept at 4°C until further use (8).

Extract characterization

A systematic and complete study of crude drugs includes a complete investigation of both primary and secondary metabolites derived from sponge metabolism. Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. The extracts obtained were subjected to chemical tests for identification of various phytoconstituents as per the methods given by Harborne, 1973 (9).

High performance thin layer chromatography (HPTLC)

Method was developed for wavelength of scanning the lanes through the densitometer. HPTLC method was also optimized for loading amount and loading concentration of the sample. Samples are tested in different various solvents and efficiency was checked in manner to signal to noise ratio. The band resolution for fingerprinting purpose was optimized. Method development was done through software DESAGA ProQuant specially designed for densitometer. Bands under 366 nm- long and 254 nm short UV light was observed. Chloroform: methanol = 9: 1 mobile phase was then selected as an optimized phase for TLC and HPTLC as well.

In vitro antioxidant assay

100 µg/mL solution stock was prepared for both standard ascorbic acid and test extracts. Working solutions of 20, 40, 60, 80 and 100 µg/mL were prepared. To 2 mL of each working solution 2 mL of DPPH working solution was added and incubated the mixture for 20 min in dark. Absorbance is measured at 517 nm using methanol as blank (10).

$$\% \, Scavenging = 100 * \left(1 - \frac{Mean\ absorbance\ of\ test}{Mean\ absorbance\ of\ control}\right)$$

In vitro anti-inflammatory assay
Inhibition of albumin denaturation

Anti-denaturation study was performed by using Bovine Serum Albumin (BSA). 2 mL of 1 mM BSA solution was added to 2 mL of the test solutions of both extracts and standard. In case of control sample 2 mL of phosphate buffer containing 2.5% DMF was added to 2 mL of 1 mM BSA solution. The mixtures were incubated at 27±1°C in BOD incubator for 15 minutes. After incubation the mixture was heated at 60±1°C, in water bath for 10 minutes for denaturation. The mixture was allowed to cool and turbidity thus produced after heating was measured spectrophotometrically at 660 nm. The reaction was performed in triplicates (11-14).

$$\% \, Inhibition\ of\ denaturation = 100 * \left(\frac{Mean\ absorbance\ of\ sample}{Mean\ absobance\ of\ control} - 1\right)$$

In vivo anti-inflammatory assay
Animals

According to standard protocols, male or female rats can be used for this study (15). Female Wistar albino white rats (150 - 200g) were used in this study which were nulliporous and nonpregnant. The animals were purchased from the Animal House of Haffkinn Institute, Parel, Mumbai and were housed in animal house with controlled temperature (20-22°C), under 12-h: 12-h light/dark cycles. Standard rat diet and tap water was provided *ad libitum* allowing the animal to self-regulate intake according to its biological needs. The experiments were performed in accordance with the OECD Guidelines 420 (fixed dose procedure) (16, 17), and to the ethical guidelines with permission no: CPCSEA/IAEC/SPTM/P-01/2012.

Carrageenan-induced hind paw edema model

The animals were starved overnight. Animals were randomly divided into 5 groups of 6 animals each. Group 1 served as negative control and was given 0.1% w/v carboxy methyl cellulose (CMC) in distilled water. Group 2 served as positive control and was given 20 mg/kg diclofenac. Group 3, 4 and 5 were given 100 mg/kg of *n*-hexane, ethyl acetate and butanol extract respectively. 0.1% CMC, diclofenac and the extracts were administered in single dose by gavage using stomach tube or intubation canula. Thirty minutes later rats were challenged with the sub-cutaneous injection of 0.05 mL 1% solution of carrageenan into the planter side of left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection, 1, 3 and 5 hr, and eventually 24 hr after challenge. The same procedure was implemented later with increased dose of 200 mg/kg and 300 mg/kg for each extract. The percentage inhibition of the edema was calculated by the following equation:

$$\% \, Inhibition = 100\left(1 - \frac{Vt}{Vc}\right)$$

where, Vc = edema volume of control
Vt = edema volume of test

Acute toxicity assay

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 rats in dosage ranging from 100 to 1000 mg/kg for the LD_{50} study using the method of Joshi C. S et al., 2007 (18) with some modifications.

Statistical analysis

The results are expressed as mean ± SD. All animal study results were statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad Prism 5 software, where p values <0.05 were considered significant.

RESULTS

Extract characterization by HPTLC

HPTLC results showed eleven bands which were not detectable in TLC. The bands were visible under UV light at 366 nm- and proper resolution was only observed in chloroform: methanol (9: 1) mobile phase. Mobile phase chloroform: methanol (9:1) was selected as an optimized phase for TLC and HPTLC as well. Bands in both the phases were scanned and chromatogram in Figure 1 shows the HPTLC resolution pattern.

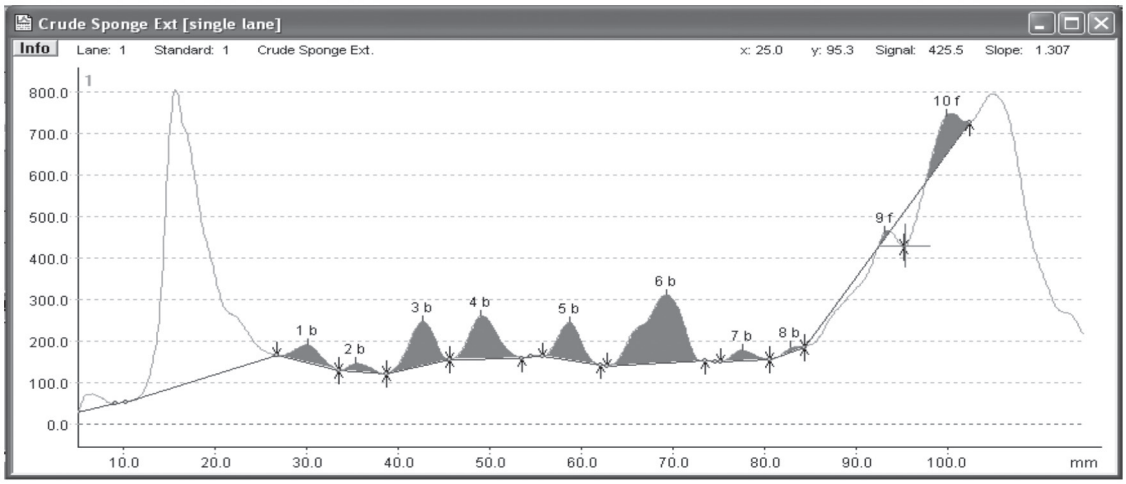

Figure 1. HPTLC chromatogram after optimization with mobile phase chloroform: methanol (9:1)

Antioxidant activity

DPPH staining allows for semi-quantitative visualization and rapid screening of antioxidant activity. Each concentrated sample extract was applied as a dot on the TLC plate and then stained with DPPH solution. White spot was seen for ethyl acetate extract. The extract was further analyzed quantitatively by DPPH, while the rest two extracts were not taken into consideration further because no spot was seen on the plate. It was thus concluded that there no or less antioxidant constituents in n-hexane and butanol extracts. The antioxidant activity of the ethyl acetate extract is shown in Table 1. The ethyl acetate extract showed negligible antioxidant activity.

Table 1. % scavenging of ascorbic acid and ethyl acetate extract of *S. halichondriodes.*

Conc. (µg/mL)	% Scavenging	
Control	---	
	Ascorbic acid	**Ethyl acetate extract**
10	9.83	4.38
20	11.13	4.68
40	38.67	5.92
60	59.08	7.22
80	84.05	10.87
100	95.99	12.25

Statistically represented as mean±SD, n=3

Anti-inflammatory activity

In vitro assay (inhibition of albumin denaturation)

Denaturation of proteins is well documented cause of inflammation. The anti-inflammatory drugs have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied and % inhibition of protein denaturation is calculated and shown in Table 2. When Bovine serum albumin is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus. Thus the assay applied for the discovery of those drugs which can stabilize the protein from denaturation process. The anti-inflammatory activity was greater in ethyl acetate extract. This may be due to the presence of bioactive compounds in ethyl acetate extract than n-hexane and butanol extract. The percentage inhibition of the standard, diclofenac used shows the potency of the test for anti-inflammatory activity. The test was further confirmed with *in vivo* assay.

Table 2. % inhibition of denaturation of BSA of diclofenac and extracts of *S. halichondriodes.*

Conc. µg/ml	% Inhibition of denaturation of BSA			
	Diclofenac	***n*-Hexane extract**	**Ethyl acetate extract**	**Butanol extract**
10	20.00	3.78	9.71	6.67
20	30.61	12.94	28.00	16.36
40	38.13	16.36	32.41	23.87
60	53.60	23.87	29.73	32.41
80	61.34	28.86	56.10	47.69
100	92.00	36.17	76.15	47.69

BSA: Bovine serum albumin

In vivo assay by carrageenan induced paw edema

Carrageenan induced paw edema is the most frequently used technique to screen the anti-inflammatory activity. Edema developed after the injection of carrageenan serves as an index of acute inflammatory changes and can be determined from the differences in the paw volume measured immediately after carrageenan injection and then every hour or at an interval of 2 hrs up to 6 hrs and later after 24 hrs. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation and the inflammation is characterized by increased tissue water and plasma metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathway. Edema induced by carrageenan occurs in two phases. The first phase begins immediately after injection and diminishes within 1 hr. In this phase serotonin and histamine release occurs which is responsible for early hyperaemia. The second phase begins after 1 hr. This delayed phase involves release of prostaglandin, cyclooxygenase products. Table 3 shows the % inhibition of edema by various extracts conc. at 100, 200 and 500 mg/kg body weight in rats. To evaluate the anti-inflammatory property of the extracts on carrageenan induced paw edema, diclofenac at 20 mg/kg body weight was suspended in 1.0 % CMC and suspension was given as a single oral administration 1 hr before the carrageenan injection. The control group received only 1.0 % CMC suspension.

Table 3. % inhibition of edema by diclofenac and various extracts (100, 200 and 500 mg/kg) body weight) of *S. halichondriodes* at different time intervals.

Groups	**Doses (mg/kg)**	**0 hr.**	**1 hr**	**2 hr**	**3 hr**	**6 hr**
Group I (control)	-	4.95 ±1.01	12.04 ±0.94	13.18 ±1.62	16.13 ±1.67	17.75 ±1.74
Group II (Standard)	20	3.69 ±0.53	21.20 ±1.23**	42.10 ±1.53***	49.80 ±1.80***	53.79 ±1.71***
Group III (n-Hexane Extract)	100	5.00 ±1.07	9.17 ±1.80	8.42 ±0.95	8.49 ±2.38	8.95 ±1.96
	200	4.86 ±1.62	12.24 ±0.31	15.72 ±0.50	17.41 ±1.69	19.31 ±1.11
	500	3.95 ±0.54	12.24 ±0.31	21.13 ±0.88	32.31 ±1.48**	37.80 ±2.29***
Group IIV (Ethyl acetate Extract)	100	9.00 ±0.69	14.80 ±2.02	15.86 ±2.34	15.79 ±1.57	15.67 ±1.98
	200	4.15 ±1.16	16.00 ±1.05	16.14 ±1.61	17.12 ±1.25	20.16 ±0.64
	500	3.84 ±0.15	18.58 ±2.41	30.68 ±1.79**	40.90 ±0.55***	49.61 ±1.92***
Group IIV (Butanol Extract)	100	4.90 ±1.42	13.08 ±1.23	11.70 ±1.66	11.48 ±1.87	13.84 ±2.12
	200	5.42 ±1.61	15.12 ±0.73	17.04 ±2.44	16.55 ±1.38	19.28 ±1.84
	500	3.64 ±0.21	13.96 ±2.80	23.98 ±1.00	32.93 ±1.08**	42.21 ±1.03***

Data represented as mean±SD (n=6), by one way ANOVA followed by Dunett'stest,*p<0.05, **p<0.01, ***p<0.001 as compared with diseased control group.

Diclofenac significantly ($p<0.01$) reduced rat paw edema by 21.20±1.23 % in the 1 hr, 42.10±1.53% and 49.80±1.80 ($p<0.001$) respectively during the 2nd and 3rd hour. *n*-Hexane, ethyl acetate and butanol extracts showed insignificant inhibition at 100 mg/kg body weight and 200 mg/kg body weight, while inhibition was significantly ($p<0.001$) reduced in ethyl acetate extract and ($p<0.01$) in butanol and n-hexane at dose of 500 mg/kg body weight. The anti-inflammatory activity of ethyl acetate extract was higher than the other two extracts and comparable to that of standard, diclofenac. The effect may be due to high concentration of the anti-inflammatory constituents in ethyl acetate extract as compared to n-hexane extract and butanol extract.

DISCUSSION

The sea is a rich source of useful compounds with new chemical structures and pharmacological effects like significant immunomodulation (against allergy), anti-inflammatory (and as a consequence, antitumor and analgesic), antibacterial and antiviral activities. New marine bioactives, such as COX inhibitors (Pacifenol, Epitaondiol), marine steroids (Contignasterol, Xestobergesterol), molecules interfering with NF-κB (Cycloprodigiosine, Hymenialdisine and Cycloamphilectenes), macrolides, peptides Cyclomarins, Salinamides and Halipeptins), other metabolites (Scytonemin and Petrocortyne) and many antioxidant agents (phenols and marine carotenoids, such as astaxanthin, fucoxanthin) have been recently discovered and characterized, in order to assess their potential role in contrasting inflammatory diseases (19). Sponges have proved to be an interesting source of anti-inflammatory compounds. Their anti-inflammatory action is based on the irreversible inhibition of the release of arachidonic acid from membrane phospholipids by preventing the enzyme phospholipase A2 from binding to the membranes. Phospholipase A2 inhibition has been recorded for many sesterterpenes from sponges of the order Dictyoceratida, and also for bis-indole alkaloids such as topsentin (20). Other potent PLA2 inhibitors, petrosaspongiolides were obtained from the New Caledonian sponge *Petrosaspongia nigra*, the most active being petrosiaspongiolide M, a potential antiinflammatory compound (21). Seven known and seven new bisindole alkaloids from the topsentin and hamacanthin classes were have been earlier isolated from the methanol extract of a *Spongosorites* sp. by bioactivity-guided fractionation (22). Anti-inflammatory metabolites from sponges are dominated by sesterterpenes. Manoalide and related compounds are probably the most well known of all antiinflammatory sponge metabolites. Cacospongionolide B is a tetracyclic sesterterpene originally obtained from *Fasciospongia cavernosa* have shown anti-inflammatory activity on TPA induced mouse ear edema after oral doses of 5, 10 or 20 mg/kg and also inhibits secretory PLA2 *in vitro* (23). Few anti-inflammatory steroids have also been reported from marine sponges like contignasterol, isolated from the sponge *Petrosia contignata.* This compound inhibited the anti-immunoglobulin E stimulated release of histamine from sensitized rat mast cells in a dose dependent manner (23).

This study is the first report on anti-inflammatory and antioxidant activity induced by extracts of *S. halichondriodes* of family Halichondriidae in rats and *in vitro* conditions. Marine natural compounds have recently shown presence of antioxidant activity, steroids and several novel molecular entities potentially were able to target COX-1, COX-2 and the NF-κB pathway (24). To attempt the antioxidant potential, all the three extracts were subjected to biochemical DPPH assay. But none of the three extracts showed a notable free radical scavenging effect in DPPH. It was thus concluded that the antioxidant constituents are absent in *Spongosorites halichondriodes*. The animals chosen in toxicity studies had no symptoms of toxicity and signs of behavioural changes or mortality, concluding that the extracts were non-toxic. In present study crude

n-hexane, ethyl acetate and butanol extracts were subjected to anti-inflammatory (*in vitro* inhibition of albumin denaturation and *in vivo* inhibition of rat left hind paw edema induced by carrageenan) assays. The results indicated that the ethyl acetate extract of *S. halichondriodes* possessed anti-inflammatory activity greater than n-hexane and butanol extract of the same and is comparable to that with the standard anti-inflammatory drug, diclofenac at dose of 500 mg/kg body weight. However this dose has to be reconsidered as its too high, which can be improved by lowering with further studies by isolating and purifying the bioactive compounds responsible for anti- inflammatory activity.

In summary, the results of the present study demonstrate that the marine sponge *S. halichondriodes* exhibits anti-inflammatory effects against classical models of inflammation in rats, and does not possess antioxidant activity. Moreover the non-toxicity of the extract suggests that the sponge is valuable candidate to investigate and isolate active constituents responsible for the anti-inflammatory effect to counter various disorders of inflammation and further authenticate its mechanism of action. Phytochemical studies and LC-MS-MS analysis indicated presence of steroids, flavonoids, and terpenoids as the major component in the ethyl acetate extract which has been characterized in detail (25). Thus the steroids and terpenoids might be the active compounds responsible for anti-inflammatory activity in *S. halichondriodes* as suggested in literature reports with different classes of compounds like terpenoids, steroids, alkaloids, peptides and other novel secondary metabolites having potent anti-inflammatory activity.

CONCLUSION

In our study, the ethyl acetate extract of the sponge *S. halichondriodes* has shown strong anti-inflammatory and very weak antioxidant activity. The bioactive compounds responsible for the activity can be further confirmed by bioassay guided fractionation *in vitro* assay. The sterol esters have been known for their anti-inflammatory activity and might be the responsible compounds for showing activity in *S. halichondriodes*. The strong anti-inflammatory activity can be a combinatorial effect of the bioactive compounds such as steroids, flavonoids and terpenoids present in ethyl acetate extract. The results of the present work suggest that the anti-inflammatory activities of *S. halichondriodes* could be explained, by multiple effects mainly corticoids like effects and very weak free radical scavenging activity. However, a more extensive pharmacological investigation on the extract of *S. halichondriodes* is needed to understand the mechanism of action on targets like enzymes and other mediators responsible for inflammation.

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