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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF MARINE SPONGE

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ABSTRACT

A variety of constituents with wide range of bioactivities have been reported with different sponge species. But pharmacological study of sponges was still scanty because of lack of standard screening procedures. This study progress in the analysis of analgesic and anti-inflammatory activity of marine sponge. Five selected sponges were extracted with methanol for analyzing the analgesic and anti-inflammatory efficacy. The analgesic activity determined through hot plate and tail compression method at various doses (5, 50, 300, 2000 mg/kg body weight) using Swiss albino mice. On the other hand, anti-inflammatory assay was performed by carrageenan induced paw edema of methanol extract of sponges at 100 and 200 mg doses in animal models (Swiss albino mice). Morphine hydrochloride and diclofenac were employed as a standard for analgesic and anti-inflammatory studies, respectively. Our present study demonstrated that H.exigua extract bears significant analgesic and anti-inflammatory activities in those animal models

KEY WORDS

Analgesic, Anti-inflammatory, Sponge

INTRODUCTION

Plant based drug have formed the basis of traditional medicines. But the scarcity of the sources and need for large scale production of drug due to the emergence of infectious diseases, research for drug development gradually shifted from terrestrial sources to marine sources. Since the few last decades, marine environment has been recognized to be rich source of bioactive metabolites with varied biological and pharmacological activities (10,7). By the invention of new technologies, it is possible to access the marine resources to control various diseases. Marine organisms have a shorter history of utilization in the treatment and prevention of human disease. Among marine sources, sponges ranked very high in the priority of natural product research for drug development. Sponges, a group of marine invertebrates, multicellular and immobile are a point of interest for marine natural product researchers as a source of novel bioactive compounds (3). Marine sponges have been deliberated as a very fertile field for the finding of bioactive natural chemical substances with regards to the variety of their primary and secondary chemical components and metabolites. By the chemical diversity of metabolites, marine sponges provide remarkable pharmacological efficiency such as ceramide from *Negombata corticata* (2) which displayed anticonvulscent activity, manoalide, a sesterpenoids compound, from *Luffariella variabilis* which displayed anti-inflammatory, analgesic and antibacterial activities (9,5). The objective of present study was to assess pharmacological potential of selected sponge extracts and its bioactive molecules.

MATERIALS AND METHODS

Preparation of sponge extract for pharmacological evaluation

Five different sponge extracts were prepared by using methanol then these extracts were filtered using muslin cloth and the filtrate was evaporated. This extract were

transferred to a container and kept at refrigerated temperature. The extract was diluted with double distilled water under sterile condition and used for experimental evaluation.

Selection of experimented animals

The albino mice (26 g – 28 g) obtained from Kings Institute, Guindy, Chennai, Tamilnadu was used throughout the study. Animals were fed with standard diet and allowed free access to drinking water. Prior to experiment, the animals were acclimatized to laboratory condition. Housing condition and in vivo experiments were approved according to the guidelines established by Institutional Animal Ethical Committee (IAEC) of School of Pharmaceutical sciences, S 'O' A University, Bhubaneswar, Orissa.

Determination of lethal dose (LD_{50})

LD_{50} was carried out according to OCED guide lines. The method used defined doses (5, 50, 300, 2000 mg/kg body weight). The starting dose of the methanolic extract of the sponge was 50 mg/kg body weight p.o. The different doses administered to the mice, which were fasted over night with water *ad libitum* and observed for signs of toxicity. The dose was repeated with another three rats and were observed for 72 h for the development of change in skin color, salivation, diarrhea, sleep, convulsions, and also respiratory-, autonomic- and CNS defects.

Evaluation of analgesic activity

Analgesic activity was determined by chemical and mechanical methods. Chemical methods was suitable produce for analyzing analgesics extract activity of selected sponge extract. In this study morphine hydrochloride used as control for the evaluation of analgesic activity of sponge extract. Hot plate method and tail immersion test in albino mice were conducted for evaluation of analgesic activity of sponge extracts.

Hot plate method in albino mice

Hot plate consists of electrically heated surface with temperature controller for 55 ± 2 °C. The animals were placed on the hot plate and the time until either licking or jumping occurs was recorded by a stop watch. Swiss albino mice weighing between 26 – 28 g were used for evaluation of analgesic activity. Mice were grouped into six and each group consists of six animals. Food was withdrawn 12 h before the drug administration till completion of experiment. The animals were weighed and numbered with marker. The test (methanol extract of selected sponge extract) and standard drug (morphine hydrochloride) were given orally. After 60 min, the animals were placed on the hot plate and the observations were recorded and the time intervals of 30, 60 and 90 min. In this experiment a cut off period of 15 seconds was used to prevent paw damage to the animals.

Tail compression method

This experiment is on the basis capability of extending the reaction time of the typical tail withdrawal reflex in albino mice by immersing the end of tail in warm water of 55 °C, after administration of morphine and methanol extract of selected sponge extract. The difference in reaction time was observed and recorded. For this experiment, mice of either sex were divided into six groups. Each group consists of six animals. The animals were weighed between 26 g - 28 g and numbered with marker. Before starting experiment, lower portion of tail was marked and immersed into the water bath for about 55 °C. The sample and standard were given orally. Within few seconds the mice react by withdrawing the tail. The reaction time was recorded in 0.5 s units by stop watch. After each evaluation the tail was carefully dried. The reaction time was analyzed before and after oral administration of test and standard substance.

Anti-inflammatory activity

The anti-inflammatory activity of the extracts was assessed by carrageenan-induced right paw edema in mice. Acute inflammation was induced by sub-plantar injection of 0.05 ml 1% carrageenan (sigma-type I) in normal saline for 30 min after treatment. The diclofenac was used as reference drug comparing the anti-inflammatory potential of sponge extract. The change in paw diameter was measured using a digital vernier caliper 30 min before injection of carrageenan and at 1, 2, 3 and 4 h after induction of inflammation (4). The percentage of inhibition in inflammation was calculated using the formula as described previously (13).

$$\text{Inhibition (\%)} = \frac{C_t - T_t}{C_t} \times 100 \text{ Where,}$$

C_t=Paw diameter at 1 h after carrageenan administration

T_t=Paw diameter after Treatment

Statistical analysis

The results obtained with various experiments were subjected to statistical analysis by using one-way analysis of variance (ANOVA) to assess the significant difference if any among the groups. *P* values >0.05 were

regarded as non-significant and *P* values <0.001 was considered as very significant

RESULTS

Analgesic activity by Eddy's hot plate method

The results of analgesic activity of sponge extracts by Eddy's hot plate method were mainly on the basis of retention time of mice after administrating sponge extract. It was revealed that *H. exigua* extracts injected mice showed high retention time of 19 sec in 90 min during experiment. Higher retention time of 24 sec in 90 min produced by Morphine hydrochloride administered mice group. The sponge *D. nigra* exhibited average retention time of 8 sec in 90 min of experimental period. But the other sponge extracts administered group produced least retention time in Eddy's hot plate experiments. The results were displayed in Table-1.

Table 1: Analgesic activity Eddy's hot plate method

Sponge extracts	Average body weight (g)	Reaction time (sec.)			
		0 min	30 min	60 min	90 min
D. nigra	26	5.00±1.00	6.00±2.00	6.00±1.00	8.00±1.00
H. exigua	27	8.00±1.00	13.00±2.00	16.00±2.00	19.00±1.00
A. donnani	26	4.00±1.00	6.00±1.00	7.00±1.00	9.00±1.00
Callyspongia sp.	28	5.00±1.00	6.00±1.00	7.00±1.00	9.00±1.00
Sigmodiosia sp.	26	5.00±2.00	7.00±1.00	8.00±1.00	9.00±1.00
Morphine hydrochloride	27	6.00±1.00	15.00±1.00	18.00±1.00	24.00±1.00

Table 2: Tail compression method

Sponge extracts	Average body weight (g)	Reaction time (sec.)			
		0 min	30 min	60 min	90 min
D. nigra	26	2.00±1.00	4.00±1.00	6.00±1.00	4.00±1.00
H. exigua	27	4.00±1.00	8.00±1.00	9.00±1.00	5.00±1.00
A. donnani	26	3.00±1.00	4.00±1.00	6.00±1.00	4.00±1.00
Callyspongia sp.	28	2.00±1.00	5.00±1.00	7.00±1.00	4.00±1.00
Sigmodiosia sp.	26	4.00±2.00	7.00±1.00	7.00±1.00	4.00±1.00
Morphine hydrochloride	27	2.00±1.00	9.00±1.00	10.00±1.00	7.00±1.00

Table 3: Anti-inflammatory activity of marine sponge extracts on albino mice

Treatment	Mean body weight (g)	Mean paw volume (ml)	% Oedema inhibition
Control	25	2.00 ± 1.00	-
Diclofenac sodium	25	0.28 ± 0.011	64
Callyspongia	26	0.66 ± 0.10	12
Sigmadiocia	26	0.61 ± 0.10	18.66
D.nigra	24	0.60 ± 0.02	20
A.donnani	24	0.56 ± 0.03	25.33
H.exigua	25	0.39 ± 0.03	48

Values are expressed as Mean ±SD (n=6). Difference in groups were analyzed by one-way analysis of variance (ANOVA)

Statistical analysis

In accordance with statistical study, all groups were more significant ($p<0.01$) as compared to standard drug even in different time intervals. As per ANOVA study, it was showed that there is no significant difference among groups.

Tail compression method

Analgesic activity of methanol extract of sponges was evaluated by tail compression method displayed in Table -2. In this experiments, sub lethal dose of sponge extracts were used and for comparing analgesic activity, morphine hydrochloride as standard reference drug. The results indicated that *H. exigua* administered mice group produced high retention time. *D. nigra* and *A. donnani* produced moderate retention time compared to standard drug received mice group. But *Callyspongia* and *Sigmodiosia* produced very low retention time. Higher retention time response perceived in drug

administered mice group. Values are expressed as Mean ±SD (n=6). Difference in groups were analyzed by one-way analysis of variance (ANOVA).

Statistical analysis

By statistical evaluation, it was displayed that all groups were significant ($p<0.01$) when compared to standard drug activity. According to ANOVA, there is significant difference among groups.

Anti-inflammatory activity

The results of anti-inflammatory activity of selected sponge extracts were shown in Table-3. It was significantly displayed that standard drug Diclofenac sodium produced high rate of edema inhibition. At the same time *H. exigua* produced inhibition of 48% edema. But other extracts were did not showed anti-inflammatory activity. Values are expressed as Mean ± SD (n=6). Difference in groups were analyzed by one-way analysis of variance (ANOVA).

Statistical analysis

In this study all groups were significant ($p<0.01$) except *A. donnani* group.

DISCUSSION

The major class of analgesics used in the management of moderate to severe pain is NSAIDs and Opioids. They are associated with many side effects and provide only symptomatic relief. Analgesic study was conducted by using Eddy's hot plate method and tail compression method. Tail compression method is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level **(**12). In present study, methanolic extracts of *H.exigua* has peripheral analgesic activity probably by inhibiting the inflammatory process compared to other sponge extracts. The high retention time exhibited by reference drug, morphine administered mice group, which was almost similar to retention time of *H.exigua* administered mice group. Hot plate method has been used by many investigators and has found to be suitable for centrally acting analgesic. The validity of the test has been even in the presence of substantial impairment of motor performance. In present study, *H. exigua* administered group exhibited high retention time than other sponge extract administered group. Dellai *et al.* (1) reported that analgesic and aniconvulscent potential of crude extracts of Mediterranean sponge, *Spongia officinalis.* Still very few sponges were studied pharmacological analysis successfully. The present study postulated that *H. exigua* showed analgesic activity due to the presence of novel metabolites.

Anti-inflammatory properties have already been reported in various marine sponges, including cavernolide from *Fasciospongia cavernosa* (12) and cyclolinteinone from *Cacospongia linteiformis* (6). These effects can be explained by the inhibition of enzymatic activities such as inhibition of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) gene expression, and plasma exudation *in vivo* in response to ovalbumin and prostaglandin E2. In present study, *H. exigua* showed anti-inflammatory activity as per mice edema assay. This activity seemed to be contributed by a specific metabolite evolved from *H. exigua.* Studies are currently underway to chemically characterize the compounds produced by *H.exigua* are profound effects on cancer cell lines for future drug development. Extension of this approach may help elucidate the function of individual molecular species and prioritize them as drug targets.

CONCLUSION

In this study, we meant to disclose our findings on analgesic and anti-inflammatory activity of selected five sponge extracts. The methanolic extracts of *H.exigua* exhibited higher activity compared to specific standard drug. These results indicated that the promising analgesic and anti-inflammatory potential of selected sponge extracts.

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