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Research Article

INVESTIGATION ON *IN VIVO* ANALGESIC ACTIVITY OF METHANOL EXTRACT OF MARINE BROWN ALGA *SPATOGLOSSUM ASPERUM* J. AGARTH

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ABSTRACT

Objective: In the present study, evaluation of the methanol extract of *Spatoglossum asperum,* brown marine algae on the analgesic activity using animal model was focussed.

Methods: Acetic acid-induced writhing test on Wistar albino rats at a dosage of 10 mg/kg body weight of algal extract have been carried out. It showed significant analgesic activity by reducing the number of acetic acid-induced writhing.

Results: The animals at a dosage of 10 mg/kg body weight exhibited, 70.52% of the animals were protected using *S. asperum* extract, on the other hand, the standard, diclofenac protected 84.21% of the animals. The results are statistically significant at $p<0.001$, and the investigation revealed dose-dependent significant activity in comparison with standard and control.

Conclusion: Hence, it can be concluded that the methanol extracts of the brown alga *S. asperum* have potent analgesic activity at moderate doses.

Keywords: Acetic acid writhing, Analgesic, Methanol extract, *Spatoglossum asperum.*

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INTRODUCTION

An analgesic or painkiller is any member of the group of drugs used to achieve (analgesia) relief from pain [1]. For neuropathic pain, traditional analgesics are less effective, and the drugs based on tricyclic antidepressants and anticonvulsants could be often showed benefit as analgesics [2]. Analgesia is associated with several pathological conditions.

The drug with lesser side effects would be a viable option when selecting a drug for the treatment of any ailments. Several ongoing studies around the globe have been involved to identify the healing agents based on natural sources. Consequently, there are many natural products that exhibit analgesic properties with relatively low incidences of side effects have been reported [3,4]. An increasing number of studies on the drugs of marine origin are demonstrating that many compounds produced by marine life have useful pharmacological activities. Among these organisms, the macroalgae are considered to be a rich source of bioactive substances suitable for therapeutic medical applications in analgesics [5-9].

Among the algae, brown algae are one of the most interesting phyla with respect to pharmacologically active compounds, were investigated widely in the past decade [10]. Nevertheless, there are many natural products that exhibit anti-inflammatory and analgesic properties and have relatively low incidences of side effects. An increasing number of studies on marine flora and fauna are demonstrating that many compounds produced by marine life have useful pharmacological activities. Among these organisms, the macroalgae are considered to be a rich source of bioactive substances suitable for therapeutic medical applications including use as an antiprotozoal [8], antibacterial [11-16], antifungal [17-22], antiviral [23], antioxidant [24-26], antidiabetic [27-29], anti-inflammatory [23], and analgesics [5,7,9].

METHODS

Sample collection and preparation

The samples of experimental marine brown alga, *Spatoglossum asperum* was collected from the Mandapam coast (Lat. 09° 17.417'N; Long. 079° 08.558'E) of the Gulf of Mannar. The alga was authenticated by the monograph of Phaeophyceae [30]. Then, the alga was washed thoroughly with sterilized seawater to remove extraneous materials. The sample was shade dried to constant weight obtained and ground. The powdered samples were stored in an airtight container for future use.

Preparation of algal extracts

About 50 g of dried seaweed powder was soaked in the methanol solvent (1:3 w/v) and kept in a hot air oven overnight at 323 K, and the extracts were collected and concentrated. The extract was then filtered through a Buchner funnel with Whatman No.1 filter paper. The filtrate was evaporated to dryness under pressure using a rotary vacuum evaporator at 323 K, and the crude extracts were weighed. The yield of the powdered extract obtained was 5 g (yield: 10%) in methanol solvent. These crude extracts were then tested for their analgesic activity.

Selection of animals

Wistar albino rats, weighing 180–220 g of their body weight were selected for writhing analgesic method by Eddy's hot plate method and carrageenan-induced rat paw edema antiinflammatory effects. They were housed in polypropylene cages and maintained in standard laboratory conditions.

Acetic acid induced writhing test

The methods of Koster *et al*. (1959); Williamson *et al*. (1996) and García *et al*. (2004) were used [31-33]. Mice were used in three animals per group per dose of methanolic extract of *S. asperum.* The animals were kept individually in transparent perspex cages (25 cm × 15 × 15 cm) for 30 min to acclimatize to their new environment before the commencement of the experiment. Control mice were pre-treated with normal saline in a volume of 1 mL/100 g of body weight, and after 15 min each mouse was injected with 0.2 mL of 3% acetic acid. 5 min after the administration of acetic acid, the writhes were counted for 20 min. Other groups of animals were pre-treated with algal extract,

Table 1: Analgesic activities of methanolic extract of *S. asperum* on acetic acid-induced writhing reflux in rats

Groups	Dose (mg/kg)	No. of writhing	% reduction in reaction time
Normal control (Group I)	Inject 1% v/v acetic acid 1 mL/100 g of body weight	38.0±3.5	-
Positive control diclofenac sodium (Group II)	10 mg/kg/body weight I. P. diclofenac sodium	6.0±0.8	84.21%**
Treatment control (methanolic extract of S. asperum (Group III)	10 mg/kg/body weight administered through orally	11.2±2.0	70.52%**

Values are expressed as mean±standard error of the mean. Values were found out by using one-way ANOVA followed by Newman's cause multiple range tests. **Values were considered significant at P<0.001. *S. asperum: Spatoglossum asperum*

15 min before injecting them intraperitoneally with 0.2 mL of 3% acetic acid. All experiments were performed between 08:00 and 16:00 h in a quiet laboratory with an ambient temperature of 298±2 K. The ability of the algal extract was significantly reduced the number of acetic acid-induced writhes was taken as an analgesic activity. Animal experiments were performed in accordance with the CPCSEA norms and are approved by the Institute of Animal Ethical Committee (IAEC) No: IAEC/KMCP/153/FT/9599/2013-2014.

Statistical analysis

All animal experiments were performed with at least 7 mice per group, and the highest and lowest values were discarded. Data are presented as means± standard error. The significance of the results was calculated using one-way ANOVA using SPSS 17.0, and the differences were deemed statistically significant at p<0.01.

RESULTS

The methanol extract of *S. asperum* (10 mg/kg body weight) showed significant analgesic activity by reducing the number of acetic acid-induced writhes (Table 1). The analgesic activity of *S. asperum* at a dose of 10 mg/kg body weight on acetic acid-induced writhing reflex could be clearly observed and nearly, 70.52% of the animals were protected as against *S. asperum*, whereas 84.21% by diclofenac (10 mg/kg body weight), a standard peripherally acting analgesic drug. The observed results are statistically significant at p<0.001. Many studies have found interesting biological activities in polar fractions from marine algae [34], and similar results were also obtained in our study.

The results of the present study showed a presence of triterpenes and steroids. Some of these compounds such as phenols, terpenes, polysaccharides, and steroids have been reported to possess anti-edematous effects [23,35-41]. In agreement with these reports, it is possible that such compounds are present in the methanol extract of *S. asperum* and are able to inhibit the synthesis, release of inflammatory mediators involved in inflammation.

DISCUSSION

The present study demonstrated that the extracts, as well as structurally diverse compounds obtained from marine brown and green seaweeds, have been shown to inhibit inflammation [42]. The compounds in the seaweed extracts may play as competitive inhibitors of cyclooxygenase or lipoxygenase in an inflammation reaction, resulting in decreased production of prostaglandins and leukotrienes [43,44].

The analgesic test used in the present study was chosen to test the different nociceptors stimuli, namely cutaneous thermic and chemical visceral stimuli [45]. In acetic acid-induced abdominal writhing causes analgesia by liberating endogenous substances and many others excite pain to the never-ending. Based on the percentage inhibition on the number of writhes obtained with different doses of methanol extract of *S. asperum*, it was found that the intensity of the analgesic effect was similar to that of the diclofenac. Diclofenac and related drugs can inhibit cyclooxygenase in peripheral tissues, thus interfering with mechanical transduction in primary afferent nociceptors. The ostagladina amplify the pain mechanism and enhance vascular permeability while the leukotrienes contract smooth muscle of blood vessels. Prostaglandins also enhance the vascular permeability and mediate pro-inflammatory and allergic responses [46,47]. The results of the present study showed that all the doses of the methanol extract of *S. asperum* produce a significant analgesic effect which may be due to the release of endogenous substances that stimulate pain in nerve endings similar to diclofenac. The methanol extract of *S. asperum* antagonized the pain produced by the acetic acid analgesic test method; it is possible that the seaweed produces its analgesic activity both peripherally and centrally.

The treatment of animals with the methanol extracts of *S. asperum* has shown significant inhibition of the writhing induced by 1.0% acetic acid solution. The maximum reduction in the number of writhing was 70.52%, although the dose required to be higher than the acetic acid dose required for a similar effect. Nevertheless, all doses given were found to be more potent. The doses of positive control showed the percentage of reductions in the number of writhing of 84.21%. The search for new metabolites from marine organisms has resulted in the isolation of some compounds such as terpenes, peptides, and sulfated carbohydrates that exhibit analgesic effects [7,48,49]. The analgesic activity observed may be associated with the presence of such compounds and other secondary metabolites in the methanolic extract of *S. asperum*, which are able to inhibit the release of endogenous mediators in response to acetic acid.

In the present investigation, confirmed that the methanolic extract of *S. asperum* has potent anti-inflammatory activity at moderate doses. This result suggests that the constituents in the algal extract could inhibit chemical mediators responsible for inflammation and that the inhibitory role in the migration of leukocytes to the site of inflammation is a strong indication in this study. This could, therefore, support the anti-inflammatory property of the *S. asperum*. Therefore, the results possess significant analgesic activity as compared to the control group in the pain model *in vivo*, with no serious toxic effect at moderate doses. The present findings reinforce the claims of the health-care industry and indigenous medicine that *S. asperum* can be used as a remedy for inflammation-related symptoms. Further, chemical analysis of the methanolic extract of *S. asperum* is necessary to isolate and identify the bioactive compounds that may have potential applications in therapeutic fields as analgesics. It is also understood that the rich diversity of marine biota with its unique physiological adaptations to the harsh marine environment provides a fruitful source for the discovery of life-saving drugs.

CONCLUSION

The marine brown alga *S. asperum* antagonized the pain produced by acetic acid writhing test methods; it is possible that the seaweed produces its analgesic activity both peripherally and centrally. The present study confirmed that the methanol extracts of the brown alga *S. asperum* have potent analgesic activity at moderate doses. This result also suggests that the constituents in this extract could inhibit chemical mediators responsible for analgesic activity is a strong indication in this study.

AUTHORS' CONTRIBUTIONS

All authors have contributed in the completion of this research work.

CONFLICTS OF INTEREST

Authors have none to declare.

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