Evaluation of marine brown algae *Sargassum ilicifolium* extract for analgesic and anti-inflammatory activity

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ABSTRACT

Background: The methanolic extract of *Sargassum ilicifolium* (Pheophyceae) was used to evaluate its analgesic and anti-inflammatory activity in the present study. **Materials and Methods:** Analgesic activity was tested using Acetic acid writhing method and Eddy hot plate method in Male albino mice and Wister rats respectively at a dose level of 1, 10, 50, 100mg/kg p.o. At the same dose, its anti-inflammatory activity was also tested using Carrageenan induced rat paw edema method Result Acetic acid writhing test and Eddy's hot plate episodes were significantly and dose dependently reduced. Carrageenan (a standard inflammatory agent) induced paw edema in rats was significantly reduced after intraperitonal administration of methanolic extract. **Results:** showed dose dependant significant activity in comparison with standard and control. **Conclusion:** Methanolic extracts of the brown seaweeds *Sargassum ilicifolium* have potent analgesic and anti-inflammatory activity at moderate doses.

Key words: Acetic acid writhing, analgesic, anti-inflammatory, carrageenan, granuloma, hot plate, paw edema, *Sargassum ilicifolium*

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INTRODUCTION

Brown alga is one of the most interesting phyla with respect to pharmacological active compounds, were investigated widely in the last decade.[1] The ethno-medicinal uses of *Sargassum* sp. have been reported form Vietnam for the preparation of tea and source of iodine, whereas in China and Korea it is used for chronic gastric ulcer, lump, dropsy, swollen and painful scrotum, and urination problems. In India *Sargassum sp.* is used for the treatment of goiter by natives. A number of pharmacological activities reported for *Sargassum* sp. viz antitumor, cytotoxic antioxidant, anthelmintic, anticoagulant, antibacterial antifungal, hepatoprotective effects and inhibits DNA polymerase and xanthine oxidase.[2] Based on these ethnobotinacal clues, the present study is to evaluate the bioactivity screening of marine brown algae *S.ilicifolium* methanolic extract for its analgesic and anti-inflammatory activity in laboratory animals.

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MATERIALS AND METHODS

Algal material

S. ilicifolium was collected manually at the Devgad Island, near Karwar (14 49'12"N 74 7'12"E) of the Arabian Sea during May 2004. After carefully removing the associated algae, the material was washed with fresh water, dried in shade and weighed (1.9kg). The taxanomy was performed through the courtesy of Dr. B. B. Chaugule, Professor, Department of Botany, Poona University, Pune. The herbarium specimen were carefully prepared and deposited in Department of Pharmacognosy, BLDEA College of Pharmacy under the code No. BLDE-2004-10.

Preparation of extract

Algal material was washed with sea water and later repeatedly by tap water to remove epiphytic growth, after drying on blotting paper; wet weight was obtained and then dried under shade for about four to five days. The powdered sample of alga was subjected to methanol extraction (maceration) for three days, concentrated and dried under reduced pressure.

Animals

Male albino mice (18-30g) were selected for writhing analgesic method and Wistar rats (120-180gm) of either sex were used

for assaying analgesic activity by Eddy's hot plate method and carrageenan induced rat paw edema anti-inflammatory effects. They were housed in polypropylene cages and maintained in standard laboratory conditions. Animal experiments were performed in accordance with the CPCSEA norms and obtained from BLDEA Medical College Bijapur Animals House. (REF: 1076/c/07/CPCSEA)

Measurement of antinociceptive activity

a. **Acetic acid writhing test:** The methods of Koster *et al,*[3] Williamson *et al.*[4] and García *et al.*[5] were used. Mice were used in groups of six per dose of methanolic extract or drug. The animals were kept singly in transparent perspex cages (25 × 15 × 15cm) for 30 minutes to acclimatize to their new environment before the commencement of the experiment. Control mice were pre-treated with normal saline in a volume of 1ml/100g of body weight and after 15 minutes each mouse was injected with 0.2 l of 3% acetic acid. 5 minutes after the administration of acetic acid, the writhes were counted for 20 minutes. Other groups of animals were pre-treated with algal extract or paracetamol 15 minutes prior to injecting them intraperitoneally with 0.2 l of 3% acetic acid. All experiments were performed between 08:00 and 16:00hrs in a quite laboratory with an ambient temperature of 22 ± 1°C. The ability of the plant extract to significantly reduce the number of acetic acid-induced writhes was taken as an analgesic activity.
b. **Hot plate test:** The hyper analgesic response on the hot plate is considered to result from a combination of central and peripheral mechanism. Hot plate method was assessed using Woolfe and MacDonald modified method.[6] In this rats were individually placed on Eddy's hot plate with the temperature adjusted to 55±1°C. The latency to the first sign of paw licking or jumping response, to avoid the heat, was taken as an index of the pain threshold. The cutoff time was 15 sec in order to avoid damage to the paw. The response was recorded after administration of consecutive seven days of methanolic extract (1, 10 50 and 100g/kg) by i.p route which is compared with standard drug Asprin (20g/kg) and control saline 10l/kg p.o route. Thermal stimulation was measured at 0, 15, 30, 45 and 60 minutes.

Assessment of anti-inflammatory activity

The anti-inflammatory activity was examined in rats according to the method of Winter *et al,* with slight modifications.[7] The rats were divided into group of six each and were treated with vehicle, methalonic extract of (1, 10 50 and 100mg/kg) by i.p route, one hour prior to carrageenin injection. 0.1ml of 1% carrageenin was injected into the subplantar region of left hind paw of each rat animals. Swelling of carrageenin induced foot was measured at 0, 1, 2, 3, 4 and 24hrs by using plethysmometer. The right paw was injected with 0.1ml of vehicle. Reduction in left paw volume is recorded for 24hrs and it was compared with Indomethacin (10mg/kg p.o.) and control. Inflammation or paw volume in rat was calculate by taking the difference between final (Vt) i.e. after carreageenan and initial volume (Vo) i.e. before carreageenan administration average volume (Vt-Vo) for each group at different time intervals. Result reported as anti-inflammatory effect were calculated as percentage inhibition in edema, obtained at each time interval for each group, using the formula % inhibition of edema = [{(Vt-Vo) control- (Vt-Vo) treated}/(Vt-Vo) control] × 100.

Statistical analysis

All animal experiments were performed with at least six rats for each group. Data are reported as means ± S.E.M. The significance of the results was calculated using Student's *t*-test and was deemed statistically significant at $P < 0.01$.

The present study the methanolic extract of *S. ilicifolium* (1-100mg/kg), shows significant analgesic activity, in a dose dependent manner, by reducing significantly the number of acetic acid-induced writhes. According to Tables 1, 1mg/kg extract of *S. ilicifolium* protected 45% of animals against acetic acid-induced writhes. Whereas 10, 50 and 100g/kg extracts of *S. ilicifolium* protected 51, 65 and 78% of the mice against 0.2ml of 3% acetic acid-induced writhes respectively. Similarly, paracetamol (300mg/kg), a standard peripherally acting analgesic drug an tagonized the acetic acid-induced writhing elicited by 0.2 l of 3% acetic acid. 94% of the animals were protected against the writhes by paracetamol.

In the hot-plate test model, *S. ilicifolium* (1-100g/kg) significantly prolonged the reaction time of 36.5 minutes to thermal stimulation over the period of 30 minutes observation when compared with control and standard [Table 2].

Table 1: Analgesic activities of methanolic extract of Sargassum ilicifolium (MESA) on acetic acid induced withers. Values represent the Means + SEM of 6 mice. Significant-t-test level *P < 0.05, ** P < 0.01 and *** P < 0.001 as compared with control

Treatment group	Dose (mg/kg)	No of writhes in 20 in Mean ± SEM	Protection (%)
Control Saline	10 ml/kg	28.11 ± 0.88	—
MESA	1	13.62 ± 0.71*	45
	10	9.30 ± 0.32*	51
	50	8.05 ± 0.15*	65
	100	6.75 ± 0.25*	78
Paracetamol	300	2.15 ± 0.15**	94

Table 2: Analgesic activities of methanolic extract of *Sargassum ilicifolium* (MESA) on Eddy hot plate test. Values represent the Means + SEM of 6 rats. Significant-t-test level * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with control

Treatment	Dose, *p.o* (mg/kg)	Mean reaction in seconds				
		0 min	15 min	30 min	45 min	60 min
Control saline	10 l/kg	4.13 ± 0.04	10.25 ± 0.04	11.75 ± 0.05*	7.13 ± 0.11	5.13 ± 0.13
MESA	1	6.63 ± 0.08	7.78 ± 0.04	12.38 ± 0.02*	6.88 ± 0.01	6.25 ± 0.04
	10	6.63 ± 0.09	7.78 ± 0.04	12.38 ± 0.02*	6.88 ± 0.01	6.25 ± 0.04
	50	5.25 ± 0.04	19.13 ± 0.02*	26.63 ± 0.02**	22.63 ± 0.02**	24.5 ± 0.01**
	100	5.3 ± 0.04	26.63 ± 0.01**	36.5 ± 0.05***	22.88 ± 0.06**	16.63 ± 0.05*
Aspirin	20	5.7 ± 0.01	26.5 ± 0.02**	23.0 ± 0.01**	20.2 ± 0.01**	18.1 ± 0.01*

Table 3: Antiinflammatory activities of methanolic extract of *Sargassum ilicifolium* (MESA) on carrageenan induced rat paw edema. Values represent the Means + SEM of 6 rats. Significant-t-test level * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ s compared with control

Treatment	Dose, *p.o* (mg/kg)	Measurement of paw volume in ml/hr (% inhibition)				
		1 hr	2 hr	3 hr	4 hr	24 hr
Control saline	10 ml/kg	0.08 ± 0.07	0.09 ± 0.014	0.08 ± 0.012	0.08 ± 0.01	0.08 ± 0.011
MESA	1	0.1 ± 0.019 (25.0)	0.13 ± 0.062 (44.4)	0.15 ± 0.028*** (87.5)	0.11 ± 0.058 (37.5)	0.09 ± 0.014 (12.5)
	10	0.09 ± 0.024 (12.5)	0.12 ± 0.046 (33.3)	0.13 ± 0.057* (62.5)	0.11 ± 0.087 (37.5)	0.1 ± 0.014 (25.0)
	50	0.11 ± 0.027 (37.5)	0.14 ± 0.068 (55.6)	0.15 ± 0.047*** (87.5)	0.15 ± 0.087*** (87.5)	0.13 0.048* (62.5)
	100	0.09 ± 0.089 (12.5)	0.14 ± 0.078 (55.6)	0.14 0.012** (75.0)	0.12 ± 0.045 (50.0)	0.1 ± 0.036 (25.0)
Indomethacin	10	0.12 ± 0.025 (50.0)	0.16 ± 0.048** (77.8)	0.16 ± 0.037*** (100.0)	0.15 ± 0.057*** (87.5)	0.12 ± 0.072 (50.0)

According to Table 3, the control group progressively produced swelling of rat paw edema that reached a maximum (0.09 ± 0.014) in two hour and gradually declined over four hours. The dose of the methanolic extract (50-100mg/kg) produced a dose dependent response i.e., % inhibition of edema, the effect increases over a 3hrs period. The greatest effect was observed at a dose of 50mg/kg at 3hrs of 87.5% inhibitory effect elicited 3hrs after injection of carrageenan 0.1 l (1%) compared to standard indomethacin (10g/kg) which produced 100% inhibition of edema.

Therefore, results obtained in this study suggest that the *S.ilicifolium* possess significant analgesic activity and anti-inflammatory activity as compared to the control group in the pain model and carrageenan induced model. Form the result we may propose that methanolic extract of *S.ilicifolium* inhibits the inflammatory meadiators like bradykinine and substance P which are involved in stimulation of pain sensory neurons and inflammations.

CONCLUSION

Since *Sargassum ilicifolium* antagonized the pain produced by both acetic acid and hot-plate analgesic test methods, it is possible that the sea weed produces its analgesic activity both peripheraly and centrally. In conclusion, the present investigation confirmed that the methanolic extracts of the brown seaweeds *Sargassum ilicifolium* have potent anti-inflammatory activity at moderate doses. This result suggests that the constituents in this extract could inhibit chemical mediators responsible for inflammation and that the inhibitory role in the migration of leucocytes to the site of inflammation is a strong indication in this study. This could therefore support the anti-inflammatory property of this plant.

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