Research Article

A Preliminary Study on the Anti-Inflammatory Activity of Marine Green Macro Algae Using Wistar Rats

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ABSTRACT

Studies on the bioactivities of marine algae have revealed numerous therapeutic effects. Sulfated polysaccharides present in algae were reported to affect multiple targets in inflammation cascade. The present study deals with screening of aqueous extract of green seaweed to evaluate anti-inflammatory activity using the paragon carageenan in wistar rats. As compared with the control, the aqueous extract showed a significant reduction in paw volume in biphasic carageenan induced edema. Also a remarkable anti-inflammatory activity with 200mg/kg and 400mg/kg doses was observed at second phase (i.e. 4th and 5th hrs) when compared to diclofenac sodium, the standard drug. Thus the results suggest that the extract has potentiality in combating both acute and chronic inflammation. The phytochemical screening revealed the presence of tannins, carbohydrates, gums and mucilages, which exemplifies the role of polysaccharides in inflammation. The study advocates characterizing the bioactive molecules from this seaweed for future therapeutic efficacy.

Keywords: Green macro algae, anti-inflammatory, ultrasonication, sulfated polysaccharides, marine seaweed.

INTRODUCTION

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents.[1] During the last four decades, numerous novel compound have been isolated from marine organism and many of these substances have been demonstrated to possess interesting biological activities.[2] India has long coastline extending to 8085 Kms. Approximately 841 species of marine algae found in both inter-tidal and deep water region of Indian coast.[3] Suryalanka beach in coastal Andhra overlooks the crystal waters of bay of Bengal supports luxuriant growth of macro algae. The macro algae (seaweed) occupy the littoral zone, which included green algae, brown algae, and red algae. The macro algae are generally grown in aquatic environment and have ability to withstand fluctuations in salinity around them, strong tidal current, variation in light intensity and constant fluctuations in temperature.[4] Seaweeds produce a variety of compounds such as carotenoids, terpenoids, xanthophylls, chlorophylls, vitamins, saturated and poly unsaturated fatty acids, amino acids, acetogenins, antioxidants such as polyphenols, alkaloids, halogenated compounds and polysaccharides such as agar, carageenan, proteoglycans, alginates, rhamnan sulfate, galactosyl glycerol and fucoidans.[5]

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells (or) irritants. it is linked with pathogenesis of many diseases like cancer, atherosclerosis, Rheumatoid arthritis, hay fever, asthma. Inflammation can be classified as acute and chronic. Acute inflammation is a short term process and occurs due to mediators from mast cells. It is characterized by 5 cardinal signs-calor, rubor, dolar, tumor and functiolesae by Rudolph Virchow. Sometimes it may extend as chronic inflammation if not controlled promptly.

Chronic inflammation is a long term process, it is characterized by infiltration of mononuclear immune cells and there mediators(macrophages, lymphocytes, monocytes and plasma cells), tissue destruction and attempts at healing which include angiogenesis and fibrosis.

Presently NSAIDS are used mainly to alleviate the symptoms of the disease and also recorded with several side effects. Also earlier according to the pyramid therapy they were the first choice in treatment of RA, later DMARD'S occupied their position, but DMARD's are also inevitable of side effects and adverse effects. Thus, safe biological sources are now been considered to explore new bioactivities in order to combat most panic inflammatory diseases.

Sulfated polysaccharides of macro algae origin act as promising ingredients for functional, nutraceutical, pharmaceutical and cosmaceutical applicataion due to their potential anti-inflammatory, antioxidant, anti-lipidemic and high nutritional value.[6&7] According to Vitor, green algae also possess sulfated galactans.[8] Thus the aim of the study is to carry out preliminary phytochemical screening and anti-inflammatory potential of collected green Seaweed from suryalanka beach.

Therefore, an effort has been made to corroborate and add scientific evidence for its anti-inflammatory bioactive molecules.

MATERIALS AND METHODS

Collection of algae[9&10]

The green seaweed (macro algae) were collected from suryalanka beach (province with coordinates of 15^0 53′20" N in latitude and 80^0 28′12"E in longitude) in Bapatla during in july 2015.

Cleaning and identification

It is washed thoroughly with sea water followed by fresh water to remove the soils, epiphytic forms, salts and other suspended materials. The cleaned algae were air dried in shade for 4-5 days and powered with electrical blender.

The algae was identified as *cladophora indica Martens* belonging to cladophorales, chlorophyta.[10] The herbarium specimen with the accession number 20150724001 has been deposited at National Facility for Marine Algae Herbarium, Marine Algal Research Station (CSIR), Mandapam camp.

Preparation of extract[11]

Dried Seaweed sample (200mg) was extracted with 6ml of distilled water in an ultrasonic bath for 20 mins. The extract was centrifuged at 1500 rpm for 10 mins at 4°c in cooling centrifuge and filtered through Whatmann Grade 1 filter paper.

Phytochemical screening[12]

The extract was subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the algae using the standard qualitative tests for screening of phytochemical constituents.

Pharmacological screening of anti-inflammatory activity

Anti-inflammatory activity of aqueous extract of algae on carrageenan induced inflammation in wistar rats was evaluated by adopting the well recognized method of Winter.[13]

Materials used

Carageenan was used to induce inflammation. Diclofenac sodium was used as standard, aqueous extract of macroalgae was used as a test drug.

Experimental animals

Adult male wistar rats, weighing between 180-200 gms were procured from animal house of Bapatla college of Pharmacy. The animals were starved overnight and deprived of water only during the experiment. The experimental protocol was duly approved by IAEC of Bapatla College of Pharmacy. A total of 24 animals were randomly selected and divided equally into 4 groups namely.

Group1: Normal control (untreated)

Group 2*:* Standard Diclofenac sodium (i.p, 10mg/kg)

Group 3: Aqueous extract of *Cladophora indica* (200mg /kg administered orally)

Group 4: Aqueous extract of *Cladophora indica* (400mg/kg administered orally)

The volumes of standard and test extracts administered are variable as they depend on the body weights of animals.

Procedure

All the groups were treated with test and standard drugs 1 hour prior to the carrageenan injection. The measurement of paw volume was accomplished by displacement technique using the mercury plethysmometer before the carrageenan injection and at 30 mins, 1, 2, 3, 4, 5 hours after carrageenan injection.[14] Edema was expressed as the increment in paw volume due to carrageenan administration. The percentage inhibition of each group was calculated by the following equation.[15]

$$\%\ inhibition\ of\ paw\ edema = \frac{(v_t - v_o)_{control} - (v_t - v_o)_{treated}}{(v_t - v_o)_{control}} X\ 100$$

Where

v_t = *paw volume after carageenan administration*

v_o = *paw volume before carageenan administration*

The percentage of inhibition was carried out for different time interval.

Statistical analysis

The data presented in Table 1 were expressed as Mean± Standard Error of Mean (SEM) (n=6). Significant difference among the mean were calculated at the level of $p<0.001$ and analyzed by one-way analysis of variance by Dunnett's t-test, a value of $p<0.05$ was defined as significant.

RESULTS AND DISCUSSION

Extensive literature survey of this algae reveals no past work on anti-inflammatory activity. Hence, in this study an attempt was made to screen for Anti-inflammatory activity.

The Anti-inflammatory activity of aqueous extract of *Cladophora indica* was assessed by carrageenan induced rat hind paw edema. Carrageenan, a phlogistic agent induced edema is commonly used as an experimental animal model of inflammation and is believed to be biphasic.

The first phase is due to release of histamine and serotonin from mast cells. The second phase is caused by release of Bradykinin, Protease, Prostaglandins and Lysosome.

It has been reported that second phase of oedema is sensitive to most clinically effective anti-inflammatory agents.

Table 1: Effect of aqueous extract of green macroalgae i.e., *Cladophoraindica* in carrageenan induced rat hind paw edema.

Groups	Treatment	Dose (mg/Kg)	Mean Paw Volume (ml) ±*sem*					
			½ hr	1 hr	2 hr	3 hr	4 hr	5 hr
1	Control	-	0.29±0.001	0.4±0.002	0.65±0.003	0.8±0.004	0.95±0.005	1.1±0.006
2	Standard	10	0.27±0.001 a** (6.89)	0.37±0.002 a*** (7.50)	0.52±0.003 a*** (20.00)	0.65±0.003 a*** (18.75)	0.73±0.004 a*** (23.15)	0.63±0.003 a*** (42.72)
3	Test drug	200	0.25±0.001 a**b^{ns} (13.69)	0.35±0.002 a***b*** (12.50)	0.50±0.002 a***b* (23.07)	0.77±0.004 a***b*** (3.75)	0.70±0.004 a***b*** (26.31)	0.62±0.003 a***b*** (43.63)
4	Test drug	400	0.27±0.001 a**b^{ns} (6.89)	0.32±0.001 a***b*** (20.00)	0.45±0.002 a***b* (30.76)	0.60±0.003 a***b*** (25.00)	0.57±0.003 a***b*** (40.00)	0.52±0.003 a***b*** (52.72)

Values are mean ± sem of 6 animals (n=6); a-group 2, 3, 4 vs group 1; b-group 3, 4, vs group 2

* = p < 0.05, ** = p<0.01, ***= p<0.001, ns= non-significant as compared to control and standard, as per one way analysis of variance (ANOVA) followed by Dunnett's multiple comparision test.; Values in parenthesis indicate percentage inhibitions at different time intervals.

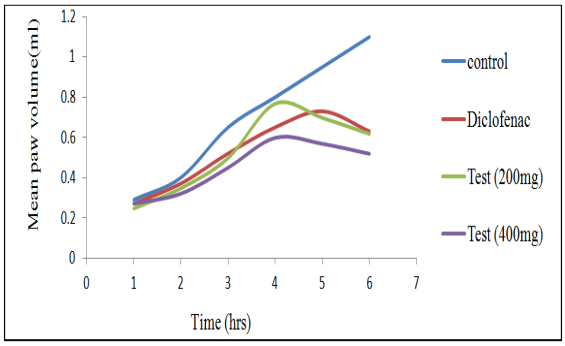

Figure 1: Effect of aqueous extract *of Cladophoraindica* in carrageenan induced rat hind paw edema

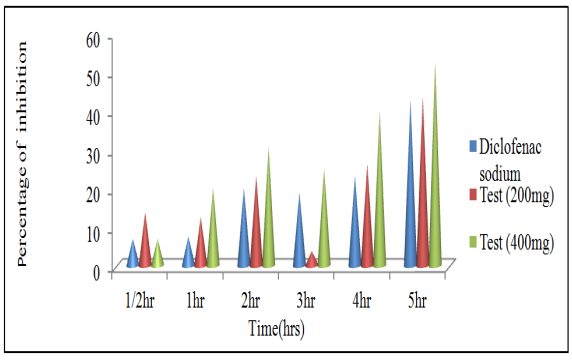

Figure 2: Percentage inhibition of standard drug and aqueous extract of *Cladophoraindica* in rat hind paw edema.

Carrageenan induced rat paw oedema is suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-oedematous effect in natural product. When compared with the control, aqueous extract of *Cladophoraindica* and Diclofenac sodium treated group showed significant reduction (P≤0.001) in paw oedema volume for 1 to 5 hrs. The result showed significant reduction (P<0.001) anti-inflammatory activity when compared to Diclofenac sodium (standard drug – group 3) at second phase i.e. (4th and 5th hours) of inflammation (Table I, Fig. 1) with aqueous extract of test drug.

The Percentage inhibition of paw oedema was significant when compared to Diclofenac sodium at both 200mg/kg and 400mg/kg dose of green algae at (4th and 5th hours) (Table I, Fig. 2). It appeared from the study that the anti-inflammatory activity might be due to inhibition of Bradykinin, Protease, Prostaglandins and Lysosome.

The result obtained in the present study indicate that the aqueous extract of green algae is capable of imparting protection against carrageenan induced inflammation and as it showed remarkable control over second phase when compared to diclofenac 400mg/kg, it indicates the extract is as potential as other anti-inflammatory agents in effecting the sensitive phase of edema.

This suggests that the extract is containing certain components which exert inhibitory effect on the induction of inflammation. The present investigation was confined only to the experiment with aqueous extract of green algae and not with the individual components.

Hence, it is not possible to attribute the anti-inflammatory activity to any particular component. However, this is worthwhile to mention that some of the components like Carbohydrates, Tannins, Gums and Mucilage in the extract of green algae may possibly be playing significant role.

CONCLUSION

It can be concluded that green algae are hidden treasure of naturally obtained pharmacologically active substances. In this study the selected green algae extract which was later identified as *Cladophora indica* showed better anti-inflammatory activity to that of standard drug Diclofenac sodium by inhibiting second phase response of carrageenan induced inflammation. This study also

suggests for further investigation of other pharmacological effects and also to isolate bioactives (belonging to either tannins, carbohydrates, gums and mucilages) responsible for those properties.

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