International Journal of Pharmaceutical Development & Technology

e ISSN - 2248 - 910X

www.ijpdt.com

Print ISSN - 2248 - 9096

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

John Peter Paul J.

Centre for Advanced Research in Plant Sciences (CARPS), Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.

ABSTRACT

Methanolic extract of *Gracilaria corticata* J.Ag. was subjected to paw edema anti-inflammatory test. The extract was injected into Wistar albino rats and the paw volume was measured in mm by Mercury Displacement method. Results revealed that the methanolic extract had good anti-inflammatory effects in the carageenan induced paw edema compared to those of control. Maximum inhibition of 0.35mm was found in the fourth hour after carageenen injection in *Gracilaria corticata* J.Ag. at 200mg/kg methanol extract. Similarly 0.4mm of inhibition was also noticed in 400mg/kg methanolic extract of *Gracilaria corticata* J.Ag. after the fourth hour. The anti-inflammatory activity had its peak at the dose of 400mg/kg as compared to 200mg/kg methanolic extract.

Keywords: Red Seaweed, Anti-inflammatory, *Gracilaria corticata*, Methanolic extract, Wistar rats.

INTRODUCTION

Seaweeds are also known as macroscopic algae which attached to the bottom in relatively shallow coastal waters. They form one of the important living resources categorized under three groups namely Chlorophyceae (green seaweeds), Phaeophyceae (brown seaweeds) and Rhodophyceae (red seaweeds). Seaweeds are considered as a potential source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities with valuable pharmaceutical potential [1, 2]. Several metabolized products especially oxylipins, resemble human eicosanoid hormones, which carry out a range of physiologically important functions. The anomalous production of these compounds underlies several diseases related to inflammation and thus eicosanoids and their derivatives have received wide attention in the search for anti-inflammatory drugs [3, 4]. Inflammation is the normal physiological and immune response to tissue injury. Increased blood supply, enhanced vascular permeability and migration of immune cells occur at damaged sites. The inflammatory process is a protective response that occurs in response to trauma, infection, tissue injury or noxious stimuli. It can be identified by tumor (swelling); Robor (redness), Calor (heat) and Dolar (pain) [5-7]. Seaweeds are the potential objects for the extraction of anti-inflammatory agents. The anti-inflammatory activity of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) *in vivo* and *in vitro* had been affirmed [8]. The anti-inflammatory substances with different nature have been separated from marine algae. Previous study explained that sterol glycoside from *Undaria pinnatifida* and *Enteromorpha linza* showed anti-inflammatory activity [9]. Nowadays, searches of natural medicinal herbs against inflammatory diseases, especially from marine organisms including seaweeds with some advantages are attracting the attention of many scientists and other countries in the world. With this background, an attempt has been made to evaluate the anti-inflammatory activity of methanolic extract of *Gracilaria corticata* J.Ag. (Red seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Plant Sample:

Gracilaria corticata J.Ag. (Figure 1) is red seaweed belonging to Rhodophyceae member showed much attention in the present study for muscle relaxant activity. *Gracilaria corticata* J.Ag. was collected from Hare Island, Thoothukudi, Tamil Nadu, India.

Corresponding Author :- **Dr. J. John Peter Paul,** **Email:-** johnarock2008@yahoo.com

The collected plant samples were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. The plants were brought to the laboratory. In the laboratory, the plants were once again washed in freshwater and stored in refrigerator for further analysis [10].

Figure 1. Natural Habit of *Gracilaria corticata* J.Ag.

Preparation of methanol extract

For the preparation of methanol extract of *Gracilaria corticata* J.Ag., the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered sample was packed in Soxhlet apparatus and extracted with methanol for 8h separately. The excess amount of methanol was evaporated and fine methanol crude powder was prepared and stored in the refrigerator for the analgesic activity [11].

Experimental Animals

Wistar albino rats (160-200g) of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% *Arachis* oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain [12]. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines [13]. Albino mice (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5 mg/Kg body weight by gastric intubation and observed for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/Kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

Anti-Inflammatory activity

Carrageenan-induced paw oedema in rats

Wistar albino mice, weighing 150-200g maintained under standard husbandry conditions (temperature 23±2°C, relative humidity 55±10% and 12hr light: 12hr dark cycle) were used for the experiment. Animals were allowed to take standard laboratory feed and tap water. In the present study, anti-inflammatory activity was determined in albino mice according to the method described by Winter *et al*. [14]. Albino mice of either sex were divided into four groups of six each. Group I served as control and received normal saline at a dose of 1ml/kg. Group II served as standard and treated with Diclofenac (10mg/kg). Group III was treated with 200mg/kg methanolic extract of the selected seaweeds. Group IV was treated with 400mg/kg methanolic extract of *Gracilaria corticata* J.Ag. After 30 minutes 0.1ml of 1% (w/v) carageenan was injected in the plantar region of the left paw of all groups of animals. The right paw served as reference non-inflamed paw for comparison. The paw volume of both legs of all groups of animals at 1, 2, 3 and 4 hrs after carrageenan challenge was measured by pleythysmometer. Values were taken in mm for calculation. This procedure was done prior to irritant injection, and afterwards on 1st, 2nd and 3rd day.

RESULTS AND DISCUSSION

In the present study, for the analysis of anti-inflammatory activity, a mark was made on right the hind paws to ensure constant paw volume. The extract at the dose level of 200 and 400mg/kg body weight were administered orally to the treated group and Diclofenac sodium at the dose level of 20mg/kg was administered orally to the standard group. After 30 min, an inflammatory edema was induced in the left hind paw by injecting of carrageenan (0.1 mL) in saline (1% w/v), in the plaster tissue of all the animals.

The paw volume was measured at 0h, 1h, 2h, 3h and 4h after administration of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. The anti-inflammatory effect of methanolic extract of *Gracilaria corticata* J.Ag. was studied on carrageenan induced paw edema of Wistar rat (Table 1 and Figure 2). After the administration of

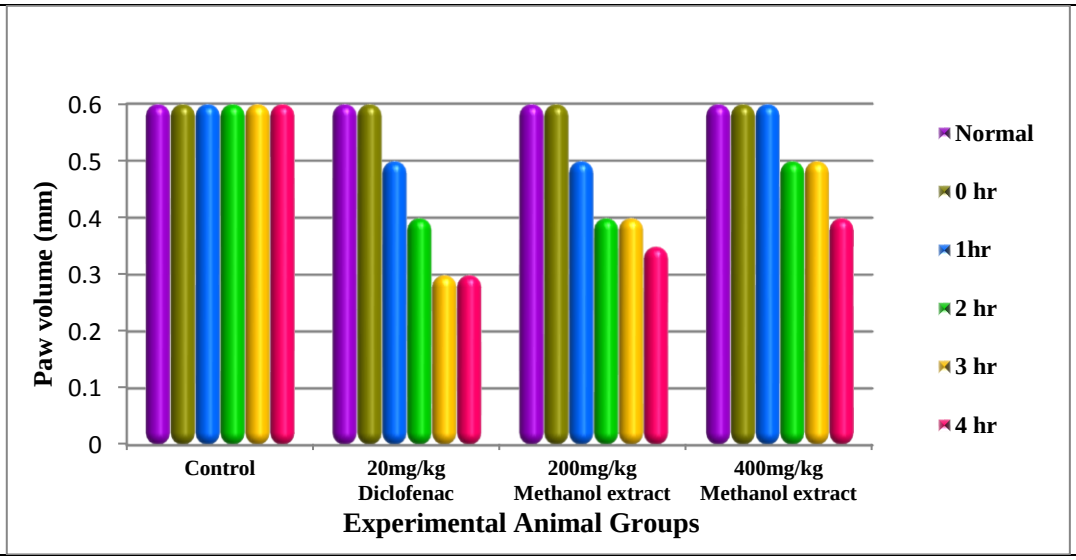
methanolic extract of *Gracilaria corticata* J.Ag. at 200mg/kg orally, the paw volume was reduced by 0.5mm, 0.4mm, 0.4mm and 0.35mm within 1h, 2h, 3h and 4h respectively. Whereas after the administration of methanolic extract of *Gracilaria corticata* J.Ag. at the dose of 400mg/kg orally showed 0.6mm, 0.5mm, 0.5mm and 0.4mm within 1h, 2h, 3h and 4h respectively. Similar results were obtained by other researchers also. In the previous research, *Gracilaria foliifera* showed high anti-inflammatory activity (89.12%) which is almost equal to 250mg/kg. They concluded that this significant anti-inflammatory activity may be due to the presence of UFA [15]. Seaweeds are rich in PUFA [16] and are of potential value as source of essential fatty acids, important in the nutrition of humans and animal [17,18].

Gracilaria corticata is red algae and they are said to be rich in sulfated polysaccharides and ω- 3 fatty acids which are commonly associated with anti-inflammatory activity. Extracts as well as structurally diverse compounds obtained from marine red algae have been shown to inhibit inflammation. The important seaweed *Gracilaria marginata* displayed anti-inflammatory activity in its apolar extract which was ten-fold more potent than the apolar substance obtained from *Liagora farinose* [19, 20]. Sulfated polysaccharides present in algae were shown to posses anti-inflammatory properties. *Ulva lactuca* the green alga available in Tuticorin coast was found to show anti-inflammatory effect as evidenced by the reduction in the inhibition of edema at the 4th day of the experiment compared with the positive control drug and control [21].

Table 1. Anti-inflammatory activity of methanolic extract of *Gracilaria corticata* J.Ag.

Animal groups	Normal		Paw volume measuring in mm by Mercury Displacement Method			
	Left	Right	1st h	2nd h	3rd h	4th h
Control	0.6±0.04	0.6±0.04	0.6±0.01	0.6±0.01	0.6±0.02	0.6±0.02
20mg/kg Diclofenac	0.6±0.01	0.6±0.01	0.5±0.01	0.4±0.01	0.3±0.01	0.3±0.01
200mg/kg Methanol extract	0.6±0.08	0.6±0.08	0.5±0.03	0.4±0.03	0.4±0.03	0.35±0.03
400mg/kg Methanol extract	0.6±0.02	0.6±0.04	0.6±0.01	0.5±0.01	0.5±0.02	0.4±0.02

Figure 2. Anti-inflammatory activity of methanolic extract of *Gracilaria corticata* J.Ag.



CONCLUSION

The anti-inflammatory effect of the methanolic extract of *Gracilaria corticata* J.Ag. was evaluated using the well recommended animal protocols. The extract showed a well marked anti-inflammatory efficacy. Among the two concentration of methanolic extracts tested, the maximum effect was noticed by higher dose of the methanol extract (400mg/kg) compared to the lower dose of the extract (200mg/kg) of *Gracilaria corticata* J.Ag. It means that the effect is based on the dose dependent. The anti-inflammatory activity of *Gracilaria corticata* J.Ag. may be due to the presence of various secondary metabolites.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Ravikumar S, Anburajan L, Ramanathan G, Kaliaperumal N. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogen. *Seaweed Res. Utiln*, 24, 2002, 95-99.
2. Sureshkumar S, John JA, Ravikumar S. Antimicrobial activity of acetone extracts of seaweeds against human pathogens. *Seaweed Res. Utiln*, 24, 2002, 111-115.
3. Smith AJ. Medicinal and pharmaceutical uses of seaweed natural products, a review. *J. Appl. Phycol*, 16, 2004, 245-262.
4. Serhan CN, Novel ω-3-derived local mediators in anti-inflammation and resolution. *Pharmacol. Ther*, 105, 2005, 7-21.
5. Zedler S, Faist E. The impact of endogenous triggers on trauma associated inflammation. *Current Opinion in Critical Care*, 12, 2006, 595-601.
6. Mariathasan S, Monack DM. Inflammasome adaptors and sensors, intracellular regulators of infection and inflammation. *Annual Review of Immunology*, 7, 2007, 31-40.
7. Choy CS, Hu CM, Chiu WT, Lam CS, Ting Y, Tsai SH, Wang TC. Suppression of lipopolysaccharide induced of inducible nitric oxide synthase and cyclooxygenase-2 by *Sanguis draconis*, a dragon's blood resin, in RAW 264.7 cells. *Journal of Ethnopharmacology*, 115, 2008, 455-462.
8. Kijjoa A, Sawangwong P. Drugs and cosmetics from the sea. *Mar. Drugs*, 2, 2004, 73-82.
9. Honda D, Yokochi T, Nakahara T, Erata M, Higashihara T. *Schizochytrium limacinum* sp. nov., a new thraustochitrid from a mangrove area in the west Pacific Ocean. *Phycol. Res*, 102(4), 1998, 439-448.
10. Iniya Udhaya C, John Peter Paul J, Shri Devi SDK. Anti-diabetic activity of methanolic extract of *Gracilaria corticata* J.Ag. (Red Seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *International Journal of Pharmacy & Therapeutics*, 7(3), 2016, 135-139.
11. Muthu Sheeba M, John Peter Paul J. Diuretic activity of methanol extract of *Enteromorpha linza* (L.) J.Ag. in Hare Island, Thoothukudi, Tamil Nadu, India. *International Journal of Phytopharmacology*, 7(2), 2016, 80-84.
12. Zimmerman M, Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1989, 16, 109-110.
13. Ecobichon DJ. The Basis of Toxicology Testing. CRC press, New York, 1997, 43-86.
14. Winter CA, Rissey EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drug. *Proc. Soc. Exp. Biol. Med*, 11, 1962, 544-547.
15. Haefner B. Drugs from the deep - Marine natural products as drug candidates. *Drug Discovery Today*, 8(12), 2003, 536-644.
16. Kayama M, Iijima N, Kumarahara M, Sado T, Araki S, Sakurai T. Effect of water temperature on the fatty acid composition of *Porphyra*. *Nippon Suisan Gaikkashi*, 51, 1985, 687.
17. Floreto EAT, Teshima S, Koshio S. The effect of seaweed diet on the lipids and fatty acids of the Japanese disc abalone *Haliotis discushannai*. *Fish.Sci*, 62, 1996, 582-588.
18. Newton IS, Long chain fatty acids in health and nutrition. *J. Food Lipids*, 3, 1996, 233-249.
19. Mayer AM, Paul VJ, Fenical W, Norris JN, Carvalho MS. Jacobs RS. Phospholipase A_2 inhibitors from marine algae. *Hydrobiologia*, 260/261, 1993, 521-529.
20. Mendonca P, Freitas JC. Topical anti-edematous activity of the organic extract of *Liagora farinosa* algae (Rhodophyta, Nemaliales). *Cienc. Cult*, 52, 2000, 175-178.
21. Jothibai MR, Kumaresan S, Ravikumar S. A preliminary study on the anti-inflammatory activity of methanol extract of *Ulva lactuca* in rat. *Journal of Environmental Biology*, 30(5), 2009, 899-902.