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Original Article

Anti-Inflammatory Activity of Extract of *Ulva reticulata* Grown Fruit of C*yamopsis tetragonoloba* (l.) Taub

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ABSTRACT

The present study is an endeavour to evaluate anti inflammatory activity of extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba*. *In vivo* anti inflammatory activity was evaluated in Wistar albino adult rats by using carrageenan induced paw edema. Group I, Group II, III, IV, V and VI represented reference control (Carrageenan only), standard diclofenac (20 mg/kg p.o.), control plant extract (200 mg/kg p.o.), control plant extract (400 mg/kg p.o.), treated plant extract (200 mg/kg p.o.) and treated plant extract (400 mg/kg p.o.) respectively**.** Administration of diclofenac (20 mg/kg p.o.) induced time dependent (1st, 2nd., 3rd and 4 4th hours) significant anti-inflammatory effect. Oral administration of extract of *C. tetragonoloba* control fruit extract (Group III) gradually decreased paw edema in carrageenan induced rat and percentage inhibition of edema was 19, 31 and 43 at 2, 3 and 4 hrs respectively. However, when the dosage was increased (400 mg/kg body weight) percentage inhibition of edema was considerably increased. Further, it was noticed that in carrageenan induced rat administered with seaweed grown *C. tetragonoloba* extract, paw edema was reduced remarkably depending on extract concentration and duration. From the results it is concluded that the extract of *U. reticulata* grown fruit of *C. tetragonoloba* could be a new and potential source of anti-inflammatory drug.

Keywords: Carrageenan, Diclofenac, *Ulva reticulata*, *Cyamopsis tetragonoloba*, Edema.

1. INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, an uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses[1]. Pharmaceutical anti-inflammatory drugs are generally used

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to treat inflammation and pain. Nevertheless, reports on the increased risk of digestive, cardiovascular and renal diseases with long-term use of several non-steroidal anti-inflammatory drugs and the serious systemic side effects of glucocorticoids, have raised concerns about using the drugs[2]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary. Seaweeds have caused an emerging interest in the biomedical area, mainly due to their contents of bioactive substances which show great potential as anti-inflammatory, anti-microbial, anti-viral and anti-tumoral drugs[3]. Seaweeds are widely distributed in the marine world and these contain medicinal properties like medicinal plants. Over the past decades, seaweeds or their extracts have been shown to produce a novel compounds and some of them have been reported to possess biological activity of potential medicinal value[4,5]. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in green, brown and red algae[6]. Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and enhanced postharvest shelf-life of perishable products[7,8,9]. But the foliar application of extract of *U. reticulata* grown fruit of C. *tetragonoloba* has not been well studied yet. Therefore, the aim of the current study was to evaluate the anti-inflammatory activity of foliar application of extract of *U. reticulata* grown fruit of *C. tetragonoloba* against the inflammation diseases.

2. MATERIALS AND METHODS

2.1. Collection of seaweed

Green alga (*U. reticulata* Forsskal) was collected during low tide, at Hare Island, Thoothukudi from November 2014 to February 2015. The sample was washed thoroughly with seawater followed by fresh water to remove sand particles and macroscopic epiphytes. After draining, the seaweed was shade-dried, powdered, sieved and used for the preparation of seaweed concentrate.

2.1.2. Preparation of seaweed liquid fertilizer for foliar application

Seaweed extracts (SWEs) were prepared by adopting the method of [10] with certain modifications. About 20g dried seaweed powder with 200ml distilled water was heated to 60^{o}C and maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in air tight bottles at 4^{o}C (100% seaweed concentrate) for further use.

2.1.3. Experimental design

A pot culture experiment was conducted during February to April 2015 at Plant Research Centre, St. Mary's College Campus, Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 20 seeds of *C. tetragonoloba* were sown in each pot. After the emergence of seedlings, they were thinned to ten plants per pot and allowed to grow up to fruiting stage. Weeding and watering were done at regular intervals. 1% SWE was applied as foliar spray (along with 100ml of distilled water in the ratio of 1: 100) after expansion of first leaf and was continued till fruiting stage. Enough replicates were maintained.

2.2. Assay of Anti-inflammatory activity

2.2.1. Screening

For toxicity studies, two different groups of six albino rats of both sexes were administered orally with the test substances, in the range of doses 100 - 3000 mg/kg and the mortality rates were observed after 72 hrs. The methanol extracts of SWE treated and control fruit of *C. tetragonoloba* showed no mortality at 3000 mg/kg. Therefore, 3000 mg/kg dose was considered as LD_{50} cut off dose (safe dose) and hence 1/20 (200 mg/kg) 1/10 (400 mg/kg) of LD_{50} doses were selected as safe doses.

Wistar albino adult rats of both sexes with 150-180 g body weight were used for the present investigation. The animals were kept in individual cages under standard environmental conditions at temperature of 25±2^{o}C and a relative humidity of about 55%. The rats were provided with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water under *ad libitum* feeding and fasted for 16 hrs before the start of the experiment. Reference anti-inflammatory drug used in the study was diclofenac [2-(2-(2,6-dichlorophenylamino) phenyl) acetic acid] - Pharmco Pharmaceuticals Company. Carrageenan, type IV (Sigma, USA) was used for induction of inflammation.

Rats were divided into six groups and each group comprising five rats.

Group I : Control (negative) rats received normal saline 0.5 ml/kg body weight.

Group II: Rats received standard diclofenac 20 mg/kg body weight.

Group III : Rats administered with fruit extract of *C. tetragonoloba* (control) 200 mg/kg body weight.

Group IV : Rats administered with fruit extract of *C. tetragonoloba* (control) 400 mg/kg body weight.

Group V: Rats administered with fruit extract of *C. tetragonoloba* (SWE treated) 200 mg/kg body weight.

Group VI : Rats administered with fruit extract of *C. tetragonoloba* (SWE treated) 400 mg/kg body weight.

Paw edema was induced by injecting 0.1 ml of 1% W/V carrageenan in physiological saline into the sub-plantar tissues of the right hind paw of each rat[11]. The methanol extracts of SWE treated and control fruit of *C. tetragonoloba* were administered orally 30 minutes prior to carrageenan administration. The degree of edema formation at the right hind paw volume was measured by plethysnography at each hour, for 4 hrs after carrageenan was injected. The

percentage inhibition of edema has been calculated by the following formula

Percentage inhibition = $[(V_c - V_t)/V_c] \times 100$

Where, V_t represents the percentage difference in increased paw volume after administration of test drugs and V_c represents the difference in increased volume in control groups.

Table 1: Anti-inflammatory effect of fruit extract of *C. tetragonoloba*

Group	Drug and dose	Paw volume measuring in mm by Mercury Displacement Method															
		Normal		0 hour		1 hour			2 hours			3 hours			4 hours		
		L	R	L	R	L	R	D	L	R	D	L	R	D	L	R	D
I	Reference Control (Carageenan only)	0.405 ±0.005	0.405 ±0.005	0.405 ±0.003	0.567 ±0.005	0.405 ±0.004	0.59 ±0.002	0.185 ±0.002	0.405 ±0.02	0.607 ±0.02	0.202 ±0.002	0.405 ±0.005	0.617 ±0.003	0.212 ±0.005	0.405 ±0.03	0.62 ±0.01	0.215 ±0.02
II	Standard Diclofenac (20mg/kg p.o)	0.4 ±0.008	0.4 ±0.008	0.4 ±0.005	0.605 ±0.004	0.4 ±0.04	0.59 ±0.002	0.192 ±0.002	0.4 ±0.005	0.527 ±0.03	0.127 ±0.005 -37	0.4 ±0.005	0.472 ±0.02	0.072 ±0.002 -66	0.4 ±0.01	0.45 ±0.003	0.05 ±0.002 -77
III	Test (Carrageenan +control extract) (200mg/kg p.o)	0.4 ±0.005	0.4 ±0.005	0.4 ±0.002	0.595 ±0.008	0.4 ±0.01	0.59 ±0.005	0.19 ±0.003	0.405 ±0.005	0.567 ±0.002	0.162 ±0.001 -19	0.405 ±0.005	0.552 ±0.01	0.147 ±0.002 -31	0.402 ±0.002	0.525 ±0.01	0.122 ±0.002 -43
IV	Test (Carrageenan + control fruit extract) (400mg/kg p.o)	0.405 ±0.005	0.405 ±0.005	0.4 ±0.01	0.597 ±0.005	0.405 ±0.008	0.57 ±0.004	0.165 ±0.005 -11	0.4 ±0.005	0.557 ±0.01	0.157 ±0.02 -22	0.4 ±0.008	0.53 ±0.005	0.13 ±0.001 -39	0.517 ±0.008	0.517 ±0.01	0.112 ±0.005 -50
V	Test (Carrageenan +treated fruit extract) (200mg/kg p.o)	0.405 ±0.005	0.405 ±0.002	0.4 ±0.005	0.602 ±0.005	0.403 ±0.005	0.59 ±0.003	0.187 ±0.002	0.402 ±0.002	0.54 ±0.02	0.138 ±0.01 (31)*	0.402 ±0.002	0.527 ±0.002	0.125 ±0.001 (41)*	0.405 ±0.04	0.502 ±0.005	0.097 ±0.002 (55)*
VI	Test (Carrageenan +treated fruit extract) (400mg/kg p.o)	0.407 ±0.005	0.407 ±0.005	0.407 ±0.002	0.602 ±0.005	0.407 ±0.002	0.55 ±0.002	0.147 ±0.005 (21)*	0.407 ±0.002	0.52 ±0.006	0.115 ±0.001 (43)*	0.407 ±0.005	0.497 ±0.002	0.09 ±0.001 (58)*	0.408 ±0.003	0.49 ±0.002	0.082 ±0.001 (62)*

Values are the mean of four replicates ±standard deviation. L (Left paw) – represent control without inflammation induction. R (Right paw) – represent inflammation induced by carrageenan. D- Difference. Group III and IV-Control = Plants irrigated with water. Group V and VI-Treated = Extract of U. reticulata (1%) was applied as foliar spray until fruit setting. Values within parenthesis indicate percentage reduction in paw inflammation. *p<0.05 Comparison made between control and treated.

2.3. Statistical analysis

All data were expressed as mean ± standard deviation (S.D.) The significance of difference between mean was determined by student's t-test where the values of $p<0.05$ were considered significant.

3. RESULTS AND DISCUSSION

The present study revealed that the effect of feeding (orally) methanolic extract of pods of *C. tetragonoloba* was investigated on inflammation or edema in normal and carrageenan induced rats and compared with diclofenac, a reference drug. Group I represented reference control (Carrageenan only). Group II, III, IV, V and VI represented standard diclofenac (20 mg/kg p.o.), control plant extract (200 mg/kg p.o.), control plant extract (400 mg/kg p.o.), treated plant extract (200 mg/kg p.o.) and treated plant extract (400 mg/kg p.o.) respectively (Table 1). Analyses revealed that paw edema was increased gradually in carrageenan induced rat (reference control) from 0.405 mm to 0.620 mm (Group I). Administration of diclofenac (20 mg/kg p.o.) induced time dependent significant anti-inflammatory effect. Oral administration of extract of *C. tetragonoloba* control fruit extract (Group III) gradually

decreased paw edema in carrageenan induced rat and percentage inhibition of edema was 19, 31 and 43 at 2, 3 and 4 hrs respectively. However, when the dosage was increased (400 mg/kg body weight) percentage inhibition of edema was considerably increased. Further, it was noticed that in carrageenan induced rat administered with seaweed grown *C. tetragonoloba* extract, paw edema was reduced remarkably depending on extract concentration and duration (Table 1). In group V (200 mg/kg body weight), the percentage inhibition of paw edema was 31, 41 and 55 at 2, 3 and 4 hrs respectively. However, when the dosage was increased (400 mg/kg body weight) percentage inhibition of edema was considerably increased (400 mg/kg body weight). The findings of the present study indicated that extract of *U. reticulata* grown fruit of *C. tetragonoloba* may be one of the alternative solution for synthetic anti-inflammatory drugs.

In our earlier study, we have shown that the methanolic extracts of foliar application of extract of *U. reticulata* grown fruit of *C. tetragonoloba* were found to be rich in various phytochemical components[12]. This is an essential step towards discovery of new drugs in pharmacological and pathological studies. Kang et al.[13] examined that the extracts obtained from marine green and brown seaweeds have been shown to inhibit the inflammation. Numerous studies have been reported that the beans of *C. tetragonoloba* can be used as anticancer, anticholesterol and antiinflammation agent[14, 15]. But this study may be the first endeavour on anti-inflammatory activity of foliar application of extract of *U. reticulata* grown fruit of *C. tetragonoloba*. Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatoy effect of drugs which has also frequently been used to assess the anti-edematous effect of natural products [16]. In the present study, reduction of paw edema in group III, IV, V and VI may be due to the implication of phytochemical compounds present in the *C. tetragonoloba* fruit extract [17,18,19].

4. CONCLUSION

The study revealed that extract of *U. reticulata* grown fruit of *C. tetragonoloba* is an excellent and fruitful anti-inflammatory representative and used as healthy green remedy against inflammatory diseases. However, further investigation is required for isolation and its mode of action for the development of a new drug in the treatment of inflammation diseases.

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