

## A preliminary study on the anti-inflammatory activity of methanol extract of *Ulva lactuca* in rat

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(Received: June 15, 2007; Revised received: October 20, 2007; Re-revised received: February 25, 2008; Accepted: July 28, 2008)

**Abstract:** Anti-inflammatory drugs presently available for the treatment of various inflammatory disorders have diverse and undesirable side effects. In recent years; active principles of varied chemical structures have been isolated from plants possessing anti-inflammatory activity. Sulfated polysaccharides present in algae were shown to possess 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 oedema at the 4<sup>th</sup> day of the experiment compared with the positive control drug and control. Microscopic examination of the elite organs did not show any alteration compared with the control and reference group. Moreover, the hematological parameters were found normal compared with the control. The present study suggests the need for further studies for the development of anti-inflammatory drug of marine origin with proper clinical trials.

**Key words:** Anti-Inflammatory, *Ulva Lactuca*, Tuticorin Coast, Drug  
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### Introduction

Many non-infectious diseases are known to be treated by herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role as therapeutic remedies in many developing countries (Ody, 1993; Khan *et al.*, 2008). As a consequence of increasing demand for diversity in screening programmes, seeking therapeutic agents from natural products, there is a great interest now in studying marine organisms. Marine halophytic plants which include mangroves, seaweeds, sea grasses and blue green algae are adapted for high saline conditions. They are also known to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential (Ravikumar *et al.*, 2002; Sureshkumar *et al.*, 2002; Ostensvik, 1998). Several marine algae have been reported to possess medicinal value and are traditionally used in many countries. Many bioactive and pharmacologically active substances have been isolated from algae. The phycocolloid present in *Ulva* species is used in jam preparation. *Ulva lactuca* is a rich source of iron and vitamin C and has been consumed by people in Scotland (Chapman and Chapman, 1980). A polysaccharide compound, isolated from *Ulva lactuca*, has been shown to have considerable antiviral effects, reducing replication rates of a number of strains of human and avian influenza viruses (Ivanova *et al.*, 1994). Studies indicate that the bioactive principles having antioxidant property might also have other effects too. However, the anti-inflammatory effect of *Ulva lactuca* has not been studied so far and hence the present study has been undertaken.

### Materials and Methods

**Collection of seaweeds:** Fresh seaweed samples of *Ulva lactuca* were collected from the Tuticorin coast at Hare Island, South West coast of India. Healthy and well grown plants were cleaned with seawater and then freshwater to remove adhering debris and associated fauna and further dried at room temperature. The dried samples were powdered using an electric mixer for the extraction of active compounds.

**Preparation of extract:** The dried material of *Ulva lactuca* was taken for extraction of bioactive compounds with methanol using Soxhlet apparatus. The solvent was removed under reduced pressure and a semi-solid mass was obtained. The extract at different doses of 50, 100 and 200 mg kg<sup>-1</sup> was suspended in aqueous solution was used for the treatment. Positive control was maintained with commercially available anti-inflammatory drug aspirin.

**Carrageenan-induced paw oedema in rats:** Male Wistar albino rats, weighing 150-200 g maintained under standard husbandry conditions (temperature 23±2°C, relative humidity 55±10% and 12 hr light: 12 hr dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by institutional animal ethics committee, Ultra College of pharmacy, Madurai, Tamil Nadu. Pedal inflammation in rats was induced as described by Winter *et al.* (1962). A suspension of 0.1 ml of 1% carrageenan was injected into the right hind foot of each rat under the subplantar aponeurosis. The test groups of 8 rats were

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treated with seaweed extracts intraperitoneally at a concentration of 500 mg kg<sup>-1</sup> of rat 1 hr before carrageenan injection.

The control group received only the vehicle (0.2 ml of normal saline) and the positive control group received aspirin (i.p) at a concentration of 150 mg kg<sup>-1</sup> of rat. All the treatments were carried out with 8 rats in each group. Paw volume measurement was done by wrapping a piece of cotton thread round the paw of each rat and the circumference was measured on a meter rule (Hess and Milonig, 1972; Bamgbose and Noamesi, 1981). Values are taken in cm for calculation. This procedure was done prior to irritant injection, and afterwards on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day.

The percentage of inhibition in drug treated rats versus control was calculated using the following formula:

$$\% \text{ of Inhibition} = \frac{200 \times (Ct - Co) \text{ Control} - (Ct - Co) \text{ Treated}}{(Ct - Co) \text{ Control}}$$

Where Ct is paw size after carrageenan injection and Co is paw size before carrageenan injection. The experiment was carried out at Ultra College of Pharmacy, Madurai, Tamilnadu, India.

**Histopathological analysis:** After the treatment, treated rat was sacrificed and subjected to histopathological examination. The elite organs such as lung, liver, kidney and heart were aseptically removed and the tissue samples were fixed in 10% buffered formalin and processed with paraffin wax for histopathological examination. Sections of 5 µm size were made and stained with hematoxylin and eosin. The extent and depth of change and alteration were evaluated.

**Haematological analysis:** Haematological parameters were determined in treated and control animals using automatic analyzer (COBOS MICROS OT). All the data was statistically analyzed by using student 't' test.

**Table - 1:** Effect of *Ulva lactuca* extract on the percentage inhibition of carrageenan-induced inflammation

Treatment groups	% of inhibition			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day
Seaweed extract of <i>U. lactuca</i> 500 mg kg <sup>-1</sup> i.p.	10	50	63	80*
Aspirin 150 mg kg <sup>-1</sup> i.p. (positive control)	5	10	40	60
Normal saline 0.2 ml (Control)	-	-	-	-

\* = Values are found significant at 1% level over control and aspirin (n=6)

**Table - 2:** Effect of *Ulva lactuca* extract on the blood and serum parameters in carrageenan-induced inflammatory in rat

Parameter	Normal	Control	Positive control Aspirin	<i>Ulva lactuca</i>
Glucose (mg dl <sup>-1</sup> )	80-120 mg%	149±1.63	196±1.63	404±2.49
Cholesterol (mg dl <sup>-1</sup> )	150-250 mg%	33±2.05	40±1.63	53±1.63
SGOT (IU l <sup>-1</sup> )	40 U l <sup>-1</sup>	434±1.63*	233±2.05*	435±2.05*
SGPT (IU l <sup>-1</sup> )	37 U l <sup>-1</sup>	140±1.24*	131±1.24*	215±1.24*
Alkaline phosphate (IU l <sup>-1</sup> )	100-290	114±1.63*	212±1.69*	363±1.63*
Total protein (g dl <sup>-1</sup> )	6-8 g dl <sup>-1</sup>	7.89±0.02	6.36±0.02	7.31±0.02
Albumin (g dl <sup>-1</sup> )	3.5-5.0 g dl <sup>-1</sup>	4.33±0.02	3.39±0.02	3.77±0.02
Total bilirubin (mg dl <sup>-1</sup> )	0.2-1 mg%	-	0.25±0.02	-
Direct (mg dl <sup>-1</sup> )	0.0-0.5 mgs%	-	0.20±0.02	-
Total leucocytes count (cells mm <sup>-3</sup> )	4000-11000	2500±5.2	5200±5.2	2100±5.2
Hemoglobin (g dl <sup>-1</sup> )	13-15 g men <sup>-1</sup> 12-15 g women <sup>-1</sup>	12.7±0.16	12.0±1.24	10.3±0.24
Red blood cells (Million.m.mm)	4.5-6.5-men 3.9-5.6 - women	4.01±0.02	3.96±0.02	3.42±0.02
Platelet count (lakhs.m.mm)	1.5-4.5	2.80±0.01	0.90±0.01	0.85±0.01
PCV (%)	40 -54%-men 36-47%-women	36±1.63	35±1.6	30±1.63
ESR 30 mt (mm) 60 mt. (mm)	0-5 mm-men 0-7 mm- women	2±0.02 4±0.02	4±0.02 4±0.02	2±0.02 4±0.02

#### Differential count

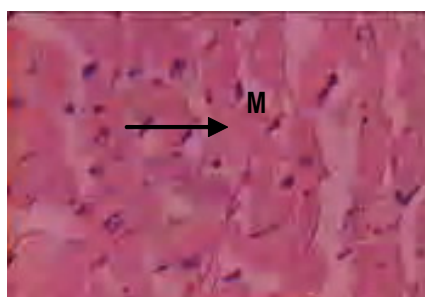
Parameter	Normal	Control	Aspirin	<i>Ulva lactuca</i>
Neutrophil (%)	40 - 65	22±1.63	45±1.24	35±1.63
Lymphocyte (%)	30 - 50	77±1.63	55±1.63	65±1.63
Eosinophil (%)	2 - 6	1±0.09	-	-

\* = Values are found significant at 5% level between drug and control

**Results and Discussion**

Herbal medicine records of various countries show a number of plant species have been recognized for their medicinal value however, only about 20% of which claimed bioactivity bioassay screening (Houghton, 2001). Seaweeds have proved for antimicrobial activity against some human pathogens (Ravikumar *et al.*, 2002; Sureshkumar *et al.*, 2002; Ravikumar *et al.*, 2005). Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents In the experimental condition used in this study, the methanolic extract of *Ulva lactuca* shows maximum inhibition of carrageenan paw oedema in rats. The development of carrageenan induced inflammation is a biphasic event, the first phase occurs within an

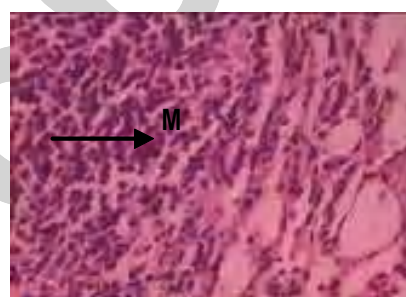
hour of injection is attributed to the release of histamine, 5-HT and kinins, while the second phase which can be measured around 3-4 hr time is related to the release of prostaglandins (Vane and Botting, 1998). The presence of prostaglandin E2 in inflammatory exudates from the injected foot can be demonstrated at three hr time period and thereafter (Vineagar *et al.*, 1987). Aspirin is used as standard reference drug as it is reported to inhibit inflammation by its effect upon plasma exudation associated with carrageenan mediated inflammation. Methanolic extract of *Ulva lactuca* showed a maximum of 80% edema inhibition at 4<sup>th</sup> day at the dose of 500 mg kg<sup>-1</sup>. This result indicated that extract with a dose of 500 mg kg<sup>-1</sup> body weight showed a maximum anti-inflammatory activity as compared to the reference drug Aspirin, which showed only 60% inhibition.



0.2 ml of saline (i.p) without administration of plant extract or aspirin (control). Myocardium (M). H & E stain X10

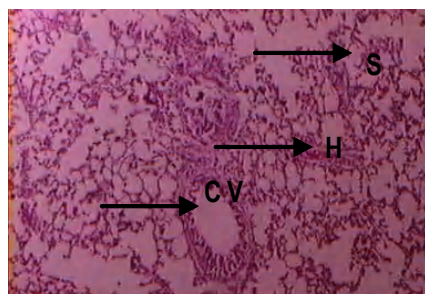


Aspirin (150mg/Kg, single dose i.p) + 0.1 ml of 1% carrageenan. Myocardium (M). Aschoff body (A). H & E stain X10

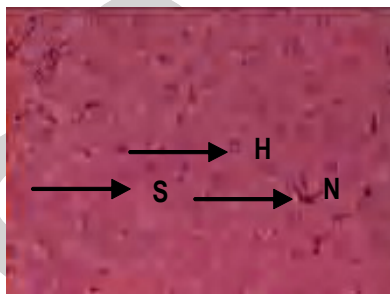


Extract of *Ulva lactuca* (500 mg/Kg, single dose I.P)+ 0.1 ml of 1% carrageenan. Myocardium(M), Reduction in Aschoff bodies. H & E stain X10

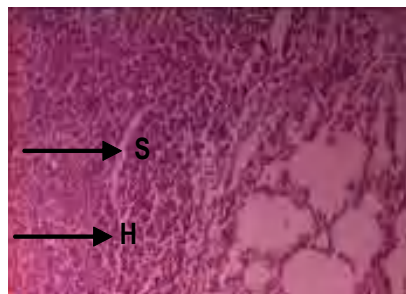
**Fig. 1:** Histopathological observation of heart tissue in rats (magnification 100x)



0.2 ml of saline (i.p) without administration of plant extract or aspirin (control). Central vein (CV), Sinusoids (S) and Hepatocytes (H). H & E stain X10

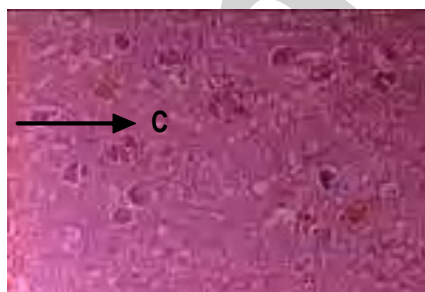


Aspirin (150mg/Kg, single dose i.p) + 0.1 ml of 1% carrageenan. Sinusoids (S) and Hepatocytes (H), Necrosis (N). H & E stain X10

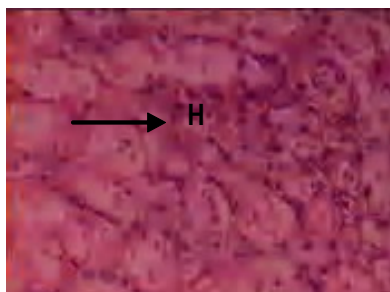


Extract of *Ulva lactuca* (500 mg/Kg, single dose I.P)+ 0.1 ml of 1% carrageenan, Showing reduction in necrosis. H & E stain X 10

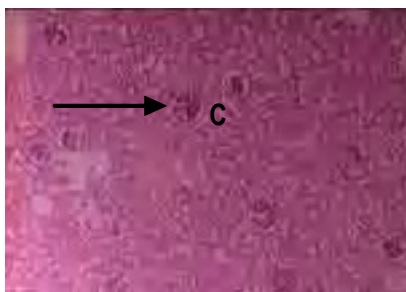
**Fig. 2:** Histopathological observation of liver tissue in rats (magnification 100x)



0.2 ml of saline (i.p) without administration of plant extract or aspirin (control). Bowmanian capsule (C). H & E stain X10



Aspirin (150mg/Kg, single dose i.p.) + 0.1 ml of 1% carrageenan. Hydropic change. H & E stain X10



Extract of *Ulva lactuca* (500 mg/Kg, single dose I.P)+ 0.1 ml of 1% carrageenan. H & E stain X10. Bowmanian capsule (C) Reduction in Hydropic change.

**Fig. 3:** Histopathological observation of kidney tissue in rats (magnification 100x)

Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induce accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility (Meade *et al.*, 1986). Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility.

This inhibition decreased significantly on the 4<sup>th</sup> day of the experiment compare with the positive control drug and control (Table 1). However, elevated levels of serum marker enzymes such as SGOT, SGPT, ALP and also the hematological parameters such as platelets and neutrophils (Table 2) is an indication of liver damage. The levels of SGOT and SGPT in liver tissues are found in significant in higher concentrations in cytoplasm and SGOT in particular also exists in mitochondria (Wells, 1988). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (Zimmerman *et al.*, 1970), thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like SGOT normally located there, will also be similarly released. The elevated activities of SGOT and SGPT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Drotman *et al.*, 1978). Histopathological examination of liver, kidney and heart challenged with normal saline group showed normal cell architecture. In case of extract pretreated groups liver showed reduced necrosis and fatty changes compare with the aspirin treated rats. In the kidney reduced hydrophic changes were observed in the extract treated group compare with the aspirin treated group. Numbers of ascoff body were reduced in heart tissues where the extract treated group compare with the aspirin treated group. (Fig. 1-3). Although serum enzyme levels are not a direct measure of hepatic injury they show the status of liver. Earlier findings reported that the alcoholic extract of *Ulva fasciata* collected from Gujarat coast exhibited antiviral and anti-inflammatory activity (Panday *et al.*, 1988). Significantly high anti-inflammatory activity of methanolic extract of *Ulva lactuca* (500 mg kg<sup>-1</sup>) may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The present study shows that the methanolic extract of *Ulva lactuca* collected from Tuticorin Coast exhibit the anti-inflammatory effect. Further development of anti-inflammatory drug from the seaweed species are in progress.

#### Acknowledgements

The authors are grateful to the authorities of respective instituteion. The author R. Jothibai, Margret is grateful to the director, Southern Regional Office, University Grants Commission (UGC).

Hydrabad for providing financial assistance under Faculty Improvement Programme (FIP).

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