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Full Length Research Paper

Studies on the analgesic and anti-inflammatory activities of *Sargassum swartzii* (Turner) C. Agardh (Phaeophyta) and *Ulva reticulata* Forsskal (Chlorophyta) in experiment animal models

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The analgesic and anti-inflammatory effects of methanolic extracts of the brown seaweed *Sargassum swartzii* (Turner) C. Agardh (Phaeophyta) and green seaweed *Ulva reticulata* Forsskal (Chlorophyta) were examined. *S. swartzii* and *U. reticulata* extracts at the dose of 500 mg/kg body weight showed analgesic effects in both acetic acid-induced writhing and hot plate-induced pain models. *S. swartzii* extract showed acute anti-inflammatory effect in both edemas in hind paw induced by carrageenan and peritonitis models, while *U. reticulata* extract only showed this effect on peritonitis. This only *S. swartzii* extract showed chronic anti-inflammatory effects (at the dose of 175 and 350 mg/kg body weight). Effects of these seaweed extracts were similar to those commonly used as analgesic and anti-inflammatory reference drugs as aspirin (100 mg/kg), morphine (100 mg/kg), indomethacin (25 mg/kg) and prednisolon (5 mg/kg). No acute toxicity was observed after oral administration of each extract (up to 66 g/kg body weight of tested extracts). These results are in agreement with the claims of the health care industry and indigenous medicine that the stated seaweeds could be used as an effective remedy for inflammation-related symptoms and they can be good criteria for reducing effect of inflammation as well as inducing analgesic effect.

Key words: Analgesic, anti-inflammatory, methanol extract, *Sargassum swartzii*, *Ulva reticulata.*

INTRODUCTION

Many studies for Vietnamese seaweeds had been published on nutritional values of some significant seaweeds of Viet Nam, for example, *Ulva* sp., *Porphyra* sp., *Sargassum* sp., *Gracilaria* sp., *Kappaphycus alvarezii*, etc. (Hong and Hoang, 2004; Dang et al., 2007; Huynh and Nguyen, 1998; Pham et al., 2002; Tran and Chau 2005).

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Abbreviations: ω-3 PUFAs, ω-3 Polyunsaturated fatty acids; **HPLC,** high performance liquid chromatography; **ABC,** animal blood counter.

Seaweeds have been used as function food for long years, direct food for man and animals, remedies, organic fertilizer, industrial materials (agar, alginate, carrageenan), production of biofuel, etc. (Algo rythme, 2000; Wikfors and Ohno, 2001; Smith, 2004). In the last decades, seaweed metabolites presenting biological activities have been increasingly discovered. Such compounds have shown antibacterial, cytotoxic and anticoagulant activeties, capability of agglutinating red blood cells and stimulating cell migration, anticancer properties, effects on the immune response and anti-inflammatory activity (Smith, 2004).

Seaweed is one of 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 (Kijjoa et al., 2004; Haefner, 2003; Mayer and Hamann, 2004; Kuramoto et al., 2004; Faulkner, 2000; Hong and Detmar, 2003). Some marine algae which can be used as foods are reported to contain high levels of ω-3 PUFAs. The extracts of the brown seaweeds *Sargassum fulvellum* and *Sargassum thunbergii* were examined for antipyretic, analgesic and anti-inflammatory activities in mice (Kang et al., 2008). The anti-inflammatory substances with different nature have been separated from marine algae (sterol glycoside from *Undaria pinnatifida* and *Enteromorpha linza)* (Honda et al., 1998). Nowadays, searches of natural medicinal herbs against inflammatory diseases, especially from marine organisms including marine algae with certain advantages are attracting the attention of many scientists and other countries in the world.

In previous study, twenty species of Vietnamese seaweeds had been used to investigate the anti-inflammatory ability according to paper published by Hoang et al. (2007). *Inter alia*, *Sargassum swartzii* and *Ulva reticulata* exhibited high potential in anti-inflammatory activity. The present study investigated the analgesic and anti-inflammatory effects of methanolic extracts of *S. swartzii* (Turner) C. Agardh and *U. reticulata* Forsskal.

MATERIALS AND METHODS

Seaweed materials

The brown seaweed (*S. swartzii* (Turner) C. Agardh) and green seaweed (*U. reticulata* Forsskal) were collected from Bai Tien (Nha Trang, Khanh Hoa province with coordinates of 12°18' 21''N in latitude and 109°14' 23''E in longitude) in the central part of the Vietnamese coast during March and June of 2006 and 2007. The seaweed species were identified based on their scientific names with authentication done by Dr. Huynh Quang Nang, (Research Institute of Application and Technology, Nha Trang, Vietnam). The seaweeds were washed with fresh water to remove epiphytes and salts. A voucher specimen of each was deposited in our laboratory (Department of algal biotechnology, Institute of Biotechnology, Vietnam Academy of Science and Technology, Vietnam). For convenience, the seaweed tissue was dried at room temperature until moisture content was reduced to 20 to 30% and then, ground to powder for 5 min using a coffee grinder. The powder was stored at -20°C until use. For 10 g of each seaweed powder, 500 ml of methanol was used to exhaustively extract solvent-soluble fraction by shaking at room temperature for 2 h and filtered. The crude extracts were concentrated *in vacuo* at 45 to 50°C to dryness. To remove salt from the seaweed extracts, the extraction was repeated several times until the amount of salt was visibly negligible. The *S. swartzii* and *U. reticulata* extracts was a greenish brown residue (2.1and 1.9% yields, respectively) (Kang et al., 2008).

Animals

Wistar albino rats (140 to 180 g) and Swiss albino mice (18 to 22 g) of either sex supplied from National Institute of Hygiene and Epidemiology (Hanoi, Vietnam) were used for experiments. Animals were kept under standard animal housing conditions (at temperature of 24 ± 1°C on a 12 h light/dark cycle) with free access to food and water. Animal experiments were performed in accordance with the US NIH guidelines for the care and use of laboratory animals (Bethesda, MD, USA).

Chemicals and apparatus used in this study

Unless otherwise stated, chemicals and reagents were purchased from Sigma (St. Louis, MO, USA), including carrageenan, formaldehyde, natri chloride, aspirin, indomethacin, morphine, prednisolon, acetic acid, etc. All solvents and chemicals used in this study were of high performance liquid chromatography (HPLC) grade.

Automatic hematology apparatus of animal blood counter (ABC) from Ugo-Basile Company, Italy; automatic blood biochemistry test apparatus of screenmaster from Hospitex Diagnostic Company, Italy; hot-plate apparatus (Ugo- Basile, Italy); Plethysmometer no. 7250 from Ugo- Basile (Italy) were used in this study.

Acute toxicity test

Mice were divided into five groups, each containing five animals. *S. swartzii* and *U. reticulata* extracts were administered orally at possible maximum dose (66 g/kg body weight) following a standard method (Turner, 1965). Animals were continuously observed for 2 h to detect changes in the autonomic or behavioral responses and then, monitored for any mortality for the following 7 days. A group of animals treated with the vehicle (0.5% carboxyl methyl cellulose sodium) served as control. Based on the results of preliminary toxicity test, the doses of 175, 250, 350 and 500 mg/kg body weight of *S. swartzii* and *U. reticulate* extracts were chosen for further experiments.

Analgesic activity

Acetic acid-induced writhing in mice

For peripheral analgesic activity, the writhing test was performed as described by Koster et al. (1959). Six different groups of six mice were used in this experiment. Among them, four different groups of six mice each received the test extracts at 250 and 500 mg/kg body weight of *S. swartzii* and *U. recticulata*. After 60 min of receiving an oral dose of the extract, 0.6% acetic acid (10 ml/kg body weight) was intraperitoneally injected to each mouse and then, placed in an observation box. The numbers of writhes were counted for 25 min, after 5 min of the acetic acid injection to each mouse. The number of writhes in each treated group was compared with that of a control distilled water-treated group (10 ml/kg body weight) and a control aspirin-treated group (100 mg/kg body weight).

Hot plate-induced pain in mice

For central analgesic activity, the hot-plate method of Eddy and Leimbach (1953) and Vogel (2002) was used. As in writhing method, six different groups of six mice were used. Among them, four different groups of six mice were used for tested extracts of *S. swartzii* and *U. reticulata*. The test extract were orally administered in mice at different doses (250 and 500 mg/kg body weight). The animals were placed on a hot plate maintained at 56 ± 0.5°C for a maximum time of 30 s. Latency to exhibit the nociceptive response such as licking their fore- and hind paws or jumping was determined before and 30 min after oral administration of the extract. Morphine sulphate (10 mg/kg, s.c.) was used for comparison. A cut-off time of 60s was selected to avoid tissue damage.

Anti-inflammatory activity

Acute inflammatory test

Carrageenan-induced hind paw edema in rats:

Inflammation in rat hind paw was produced by the method described by Winter et al. (1962) and Vogel (2002). The test groups of rats were treated with *S. swartzii* and *U. recticulata* extracts (175 and 350 mg/kg body weight). The animals in the reference control group were administered indomethacine at a dose of 25 mg/kg body weight as the standard drug. The control group of rats was given 9‰ sodium chloride at the dose of 1 ml/100 g body weight. After 1 h, they were administered orally, 0.1 ml of 1% carrageenan suspension in 9‰ NaCl solution was injected into the sub-plantar tissue of the right hind paw of the rat and the paw volume was measured at 2, 4, 6 and 24 h after the carrageenan injection using a plethysmometer. The results were calculated from Equations 1 and 2 as shown below (Dang et al., 2008). Equation 1 as the increasing of hind paw's volume:

$$\Delta V\% = \frac{V_t - V_0}{\overline{V}_0} \times 100 \quad (1)$$

Where V_0, Hind paw's volume before inflammation; V_t: hind paw's volume after inflammation.

Equation 2 as the anti-inflammatory effects of drugs (or seaweed extracts) were evaluated by edema inhibited ability (I %):

$$I\% = \frac{\Delta\overline{V}_c\% - \Delta\overline{V}_t\%}{\Delta\overline{V}_0\%} \times 100 \quad (2)$$

Where, $\Delta\overline{V}_c\%$, Average of the increasing of hind paw's volume in control group; $\Delta\overline{V}_t\%$, average of the increasing of hind paw's volume in experimental group

Induction of peritoneal inflammation (Peritonitis):

Groups of rats were prepared similar to edema in hind paw model. Rats were injected into peritoneum with 2 ml of 0.05% carrageenan and 1.4% formaldehyde suspension in 9‰ sodium chloride. After 24 h, the rats were killed by ether inhalation and the peritoneal cavity exposed. Peritoneal exudates were collected by aspiration and the volumes recorded; amount of leucocytes per 1 ml exudation was counted and protein in the exudation quantified.

Chronic inflammation

The method of Goldstein et al. (1967) was used with slight modification. Groups of rats were prepared as the peritonitis model. A sterilized amiant fiber weight 6 mg (soaked in 1% carrageenan) was implanted in the back of the neck of rats in different groups. They were treated with *S. swartzii* and *U. recticulata* extracts (175 and 350 mg/kg body weight) daily for five consecutive days, whereas, 9‰ NaCl was given to the control animals. Prednisolon (5 mg/kg body weight) was used as a standard anti-inflammatory drug.

Then, the granulomas were removed and after removing extraneous tissues, they were dried overnight at 56°C and weighed.

Statistical analysis

For each independent assay, the experiments were replicated at least five times. The mean values of the indexes were compared with the control using student's t-test.

RESULTS

Acute toxicity

The methanolic extracts of *S. swartzii* and *U. reticulata*, when orally administered in the possible maximum dose of 66 g/kg body weight to mice, did not produce any significant change in the autonomic or behavioral responses during observation period. No mortality was observed up to the 7th day of monitoring in any group. According to WHO (1992), herbal medicine is said to be toxic if the LD_{50} is lower than 5 g/kg body weight. From this assertion, it can be said that the extracts are not toxic and can be safely used by humans.

Analgesic effect

On the acetic acid-induced writhing model

The results showed that, the methanolic extract of *S. swartzii* statistically significantly ($P < 0.001$) reduced analgesic effect in the number of acetic acid-induced writhes in mice with a protection 55 and 56% at the doses of 250 and 500 mg/kg body weight, respectively, at time of 5 to 10 min, 10 to 15 min and 15 to 20 min after acetic acid injection. Among them, high dose of *S. swartzii* extract had better and stronger effect compared with aspirin at the dose of 100 mg/kg body weight. The methanol extract of *U. reticulata* at the dose of 250 mg/kg body weight showed similar effect to aspirin at the dose of 100 mg/kg body weight, whereas, at the dose of 500 mg/kg body weight, exhibited significant ($P < 0.01$) protection of 44% at time 15 to 20 min after acetic acid injection (Table 1).

On the hot plate-induced pain model

The results presented in Table 1 show that, *S. swartzii* and *U. reticulata* extracts significantly increased the hot plate reaction time of mice up to 30 min at the dose of 500 mg/kg body weight when compared with times before and after using drugs ($P < 0.05$). It means that, at this dose, both extracts possessed analgesic effect. Otherwise, at the dose of 250 mg/kg body weight of *S. swartzii* and *U. reticulata* extracts showed no analgesic effect. However, the analgesic effects of *S. swartzii* and *U. reticulata* extracts were less than morphine at a dose

Table 1. Effect of the methanolic extracts of *S. swartzii* and *U. reticulata* on acetic acid-induced writhing and hot plate methods in mice[a].

Treatment group	Dose (mg/kg body weight)	Acetic acid induced writhing (Number of writhing of mice - individual/5 min)						Hot plate reaction time in seconds	
		0 to 5 min	5 to 10 min	10 to 15 min	15 to 20 min	20 to 25 min	Protection (%) in 15 to 20 min	Pre-drug	30 min post-drug
Control (vehicle, 10 ml/kg bw)[b]		5.6 ± 0.52	18.2 ± 4.71	16.5 ± 3.75	13.0 ± 3.30	9.1 ± 2.51		13.67 ± 4.01	12.42 ± 3.71
Morphine	10							14.08 ± 2.01	21.99 ± 6.93*
Aspirin	100	1.2 ± 0.42***	11.6 ± 3.63***	15.5 ± 3.47	12.1 ± 2.77*	6.3 ± 1.95	7		
S. swartzii	250	5.2 ± 1.55	11.7 ± 4.06	10.4 ± 3.13***	5.8 ± 1.95 ***	4.6 ± 1.35***	55	13.37 ± 3.90	15.22 ± 3.40
S. swartzii	500	2.9 ± 0.57***	8.8 ± 1.93***	8.9 ± 3.11***	5.7 ± 1.23 ***	3.1 ± 0.88***	56	12.61 ± 2.60	16.5 ± 4.80*
U. reticulata	250	6.0 ± 1.15	12.1 ± 3.87*	13.8 ± 2.57	11.4 ± 3.37 *	7.0 ± 2.21	12	14.39 ± 3.81	15.96 ± 4.50
U. reticulata	500	6.2±1.62	8.8 ± 1.93*	13.3 ± 2.45*	7.3 ± 1.77 **	6.2 ± 1.62*	44	13.27 ± 2.06	17.87 ± 5.88*

[a] Values are mean ± S.E.; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001 versus control; student's t-test; [b] vehicle, distilled water.

of 10 mg/kg body weight.

Acute anti-inflammatory effect

On the carrageenan - induced edema in hind paw model

The extracts were tested at two different dose levels. The methanolic extract of *S. swartzii* at both of 175 and 350 mg/kg body weight doses showed significant inhibition on carrageenan induced rat right hind paw edema at 2, 6 and 24 h (P < 0.05). The acute anti-inflammatory effects of *S. swartzii* extract (52.12% at 175 mg/kg body weight dose and 45.84% at 350 mg/kg body weight dose) were better than indomethacine at 25 mg/kg body weight (33.145%) for 24 h after inflammation. Conversely, methanolic extract of *U. reticulata* with two different doses did not reduce the acute inflammation on the carrageenane- induced edema in right hind paw model (P > 0.05) (Table 2).

On the peritonitis model

The effects of *S. swartzii* and *U. reticulata* extracts on the volume, protein levels and amount of leucocytes of inflammatory exudates are shown in Table 3. The *S. swartzii* extract at the doses of 175 and 350 mg/kg body weight reduced significantly the exudates volume compared with control (P < 0.05), while *U. reticulata* extract only showed this effect at the dose of 350 mg/kg body weight (P < 0.05).

The *S. swartzii* and *U. reticulata* extracts at both of 175 and 350 mg/kg body weight doses did not affect protein levels in inflammatory exudates compared with control (P > 0.05), but reduced significantly the amount of leucocytes in inflammatory exudates compared with control (P < 0.05). The effects of these two extracts for decrease of amount of leucocytes trended better than using indomethacine at 25 mg/kg body weight (Table 3).

Chronic anti- inflammatory effect

The obtained results of chronic anti-inflammatory effect of both *S. swartzii* and *U. reticulata* extracts in rats are shown in Table 3. Methanolic extract of *S. swartzii* at the doses of 175 and 350 mg/kg body weight possessed chronic anti-inflammatory effect because they significantly reduced the weight of granulomas in rat compared with control (P < 0.05) (Table 3). This effect trended better than using prednisolon at 5 mg/kg dose. *U. reticulata* extract have no chronic anti-inflammatory effect at any of the used doses (P > 0.05).

DISCUSSION

Extracts as well as structurally diverse compounds obtained from marine brown and green seaweeds have been shown to inhibit inflammation (Kang et al., 2008; Hoang et al., 2007). In the present work, the results of acute toxicity testing indicate that, the methanolic extracts of *S. swartzii* and *U. reticulata* are safe even up to an oral dose of 66 g/kg body weight. Known for their anti-inflammatory effects, compounds in the seaweed extracts may play as competitive inhibitors of cyclooxygenase and/or lipoxygenase in an inflammation reaction, resulting in decreased production of prostaglandins and leukotrienes

Table 2. The acute anti-inflammatory effect of *S. swartzii* and *U. reticulata* extracts on the carrageenan-induced edema in hind paws model[a].

Treatment and dose (mg/kg, body weight)	Edma rate (%) (inhibition %)			
	2 h	4 h	6 h	24 h
Control (9‰ NaCl)	40.44 ± 14.06	53.90 ± 12.25	55.97 ± 14.12	28.97 ± 11.96
S. swartzii (175)	26.87 ± 12.08 * (33.56)	45.43 ± 13.24 (15.71)	40.50 ± 11.32 * (27.64)	13.87 ± 6.86 * (52.12)
S. swartzii (350)	25.03 ± 10.29 (38.11)	44.20 ± 16.58 (17.99)	40.08 ± 9.0 (28.39)	15.69 ± 4.71 (45.84)
U. recticulata (175)	40.52 ± 20.49 (0)	64.48 ± 16.86 (0)	51.52 ± 16.04 (7.95)	17.56 ± 8.67 (4.87)
U. recticulata (350)	37.31 ± 21.56 (7.74)	66.51 ± 33.28 (0)	55.03 ± 25.25 (1.68)	22.71 ± 10.07 (21.61)
Indomethacin (25)	24.92 ± 14.43 * (38.38)	24.92 ±14.43 ** (53.77)	37.31 ± 16.42 * (33.34)	19.37 ± 11.24 *(33.14)

[a]Values are mean ± S.E.; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001 versus control; student's t-test.

Table 3. The effects of *S. swartzii* and *U. reticulata* extracts on volume, protein content, amount of leucocytes in inflammatory exudation and chronic anti-inflammatory[a].

Treatment and dose (mg/kg, body weight)	Acute anti-inflammatory effect			Chronic anti-inflammatory effect
	Inflammatory exudation volume (ml)	Protein content (mg/dl)	Amount of leucocytes (g/l)	Weight of granuloma (mg)
Control (9‰ NaCl)	3.62 ± 1.30	50.64 ± 7.81	25.07 ± 8.76	105.5 ± 14.84
Indomethacin (25)	2.3 ± 0.73*	47.45 ± 5.17	18.70 ± 3.85*	-
Prednisolon (5)	-	-	-	86.67 ± 7.35*
S. swartzii (175)	1.62 ± 0.39**	51.77 ± 6.54	17.73 ± 4.57*	84.25 ± 12.89*
S. swartzii (350)	2.22 ± 0.68**	49.44 ± 2.67	11.72 ± 1.45 **	72.0 ± 18.09**
U. recticulata (175)	3.9 ± 0.81	48.55 ± 4.17	13.67 ± 3.15 **	105 ± 23.18
U. recticulata (350)	1.76 ± 0.32**	49.75 ± 2.51	13.47 ± 0.88 **	108.4 ± 20.53

[a] Values are mean ± S.E.; n = 6; *P < 0.05; **P < 0.01 versus control; student's t-test.

(James et al., 2000). In the present study, two animal models for investigation of the analgesic and anti-inflammatory effects of the selected seaweeds were used. The hot plate thermal stimulation and the acetic acid induced writhes in mice were selected to investigate the central and peripheral analgesic effects. Carrangeenan-induced hind paw edema in rats, peritonitis and the amiant-induced granuloma in mice were selected to represent models of acute (exudative phase) and chronic (proliferate phase) inflammation, respectively. We have known that, carrageenan-induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug (Ratheesh and Helen, 2007). The present results show that, 500 mg/kg body weight of *S. swartzii* and *U. reticulata* extracts induced analgesic protective effect against both thermal stimuli and the writhing syndrome indicating central and peripheral effects. On the other hand, the present results demonstrate that Methanolic extracts of *S. swartzii* possess a dose-dependent anti-inflammatory effect against both acute (exudative) and chronic (proliferate) inflammation. The activity of methanolic extract of *U. reticulata* against acute inflammation (carrageenan-induced edema) only indicates possible anti-phlogistic but not anti-proliferative effect. All the tested extracts have variable degrees of both analgestic and anti-inflammatory effects. *S. swartzii* has both analgestic and anti-inflammatory effects. Isolation of the main anti-inflammatory compounds from *S. swartzii* and *U. reticulata* crude extracts is now in progress.

In conclusion, the present study demonstrated that extracts of the *S. swartzii* and *U. reticulata* have potent analgesic and anti-inflammatory effects, without any serious toxic effect at highest possible doses. These findings reinforce the claims of the health care industry and indigenous medicine that those seaweeds can be used as remedies for inflammation related symptoms.

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