



ELSEVIER

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

journal homepage: [www.jfda-online.com](http://www.jfda-online.com)

## Original Article

# Inhibitory activity of *Sargassum hemiphyllum* sulfated polysaccharide in arachidonic acid-induced animal models of inflammation

Pai-An Hwang<sup>a,\*</sup>, Yu-Lan Hung<sup>a</sup>, Shih-Yung Chien<sup>b</sup>

<sup>a</sup> Seafood Technology Division, Fisheries Research Institute, Council of Agriculture, Taiwan

<sup>b</sup> Institute of Food Science and Technology, National Taiwan University, Taiwan

## ARTICLE INFO

## Article history:

Received 16 January 2014

Received in revised form

7 May 2014

Accepted 7 May 2014

Available online 29 October 2014

## Keywords:

animal model

anti-inflammation

cytokines

myeloperoxidase

*Sargassum hemiphyllum*

## ABSTRACT

*Sargassum hemiphyllum* is a common plant found on the coasts of Taiwan; it has been used as an anti-inflammatory agent in traditional herbal medicine. This study aimed to evaluate the anti-inflammatory effects of *S. hemiphyllum* sulfated polysaccharide (SHSP) using two different mouse models. In both arachidonic acid-induced ear inflammatory gavage and paint models, SHSP decreased ear swelling and erythema. In addition, SHSP decreased the production of myeloperoxidase, nitric oxide, interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor- $\alpha$  in a dose-dependent manner. Histological examination results showed that SHSP reduced the area of neutrophilic infiltration in inflamed ears. The anti-inflammatory activity of SHSP has already been demonstrated *in vitro*. In this study, SHSP extracted from the same species of brown seaweed exhibited anti-inflammatory activity in both oral and topical applications *in vivo*. Therefore, SHSP may play a role in the treatment of inflammatory diseases.

Copyright © 2014, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan

LLC. Open access under [CC BY-NC-ND license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

Inflammation is a physical response that protects against injury, infection, and stress through multiple mechanisms. Inflammation can be classified into two separate conditions, namely, acute inflammation and chronic inflammation. Acute inflammation is the initial response to stress that brings about rapid and short-term immune responses [1]. Chronic inflammation is initiated by an inappropriate or excessive acute

inflammatory immune response and is a symptom of various pathological conditions [2]. It is well-known that inflammation can facilitate tumor progression [3], increase the risk of atherosclerosis [4], cause lesions of Alzheimer's disease [5], and promote insulin resistance and diabetes [6]. In such diseases, inflammation is a primary cause, and therefore, treatment of the inflammatory condition may be an effective therapeutic approach.

Prior to the 1950s, seaweeds were used as traditional and folk medicines [7]. However, biologically active compounds

\* Corresponding author. Seafood Technology Division, Fisheries Research Institute, Council of Agriculture, 199 Hou-I Road, Keelung 202, Taiwan.

E-mail address: [pahwang@mail.tfrin.gov.tw](mailto:pahwang@mail.tfrin.gov.tw) (P.-A. Hwang).  
<http://dx.doi.org/10.1016/j.jfda.2014.05.004>

1021-9498/Copyright © 2014, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. Open access under [CC BY-NC-ND license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

from brown seaweed were not discovered until the 1990s. Previous studies have shown that extracts from the brown seaweeds *Dictyota dichotoma* [8], *Ecklonia cava* [9,10], *Fucus vesiculosus* [11], *Sargassum hemiphyllum* [12,13], *Sargassum vulgare* [14], and *Eisenia bicyclis* [15] exhibit anti-inflammatory activity *in vitro*. Moreover, extracts from the brown seaweeds *Laminaria saccharina*, *Laminaria digitata*, *Cladosiphon okamuranus*, *Fucus evanescens*, *F. vesiculosus*, *Fucus serratus*, *Fucus distichus*, *Fucus spiralis*, *Ascophyllum nodosum* [16], *Sargassum fulvellum*, *Sargassum thunbergii* [17], *Sargassum wightii* [18], *Turbinaria ornata* [19], *Lobophora variegata* [20], *E. cava* [21], and *Padina tetrastratica* [22] also possess powerful anti-inflammatory activities *in vivo*. In two separate studies that used different extraction processes, *E. cava* elicited anti-inflammatory activity (separately) *in vitro* and *in vivo*; however, there is no evidence in previous reports indicating that single brown seaweed extract has anti-inflammatory activity in both *in vitro* and *in vivo*.

The brown seaweed *S. hemiphyllum* is commonly found in east Asian coastlines and its extracts (obtained by boiling the seaweed in water) are used as a traditional herbal anti-inflammatory medicine. Our previous study demonstrated that *S. hemiphyllum* sulfated polysaccharide (SHSP), which is extracted using hot water, is central to the anti-inflammatory activity of the plant. Inside the cell, SHSP causes decreased cytosolic and nuclear expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) p65, thereby inhibiting cytokine secretions of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and the nitric oxide (NO) and messenger RNA expression of IL- $\beta$ , inducible NO synthase, and cyclooxygenase-2. It was observed *in vitro* that the inhibitory effect of SHSP is through the NF- $\kappa$ B signaling pathway [13]. However, because *in vitro* studies are conducted in simulated organic conditions and are not in the living organism, their success is limited. To verify these observations *in vivo*, we determined the anti-inflammatory activity of SHSP using two arachidonic acid (AA)-induced ear inflammation animal models.

## 2. Materials and methods

### 2.1. Preparation of SHSP

SHSP was extracted and isolated as described earlier [13]. In brief, the dried *S. hemiphyllum* powder (100 g) was mixed with 5 L of distilled water and boiled at 100°C for 30 minutes. The hot-water extract was centrifuged at 10,000g for 20 minutes and the supernatant was lyophilized under reduced pressure. To the lyophilized hot-water extract, 4 volumes of 95% ethanol were added and the mixture was allowed to precipitate overnight at 4°C. The precipitated polysaccharides were collected by centrifugation and lyophilized. These lyophilized polysaccharides are the SHSP samples used in the experiments.

### 2.2. Chemical analysis

Sulfate content was determined according to the gelatin–barium method [23] using sodium sulfate (1 mg/mL) as standard and after acid hydrolysis of the polysaccharides (6N HCl, 100°C, 6 hours). Monosaccharide fractions of the

polysaccharide extract hydrolysates were separated by high-performance anion-exchange chromatography (Dionex BioLC, Sunnyvale, CA, USA) with an anion-exchange column (CarboPac PA10, 4.6 × 250 mm; Dionex BioLC). The monosaccharide was analyzed at an isocratic NaOH concentration of 18 mM at ambient temperature [24]. The fucose and sulfate compositions of SHSP are 221.40 ± 0.42 μmol/g and 44.2% ± 3.6% (wt/wt), respectively.

### 2.3. Mice

Male BALB/c mice (8 weeks old, weight, 20–25 g) were purchased from the National Taiwan University College of Medicine Laboratory Animal Center (Taipei, Taiwan). All animals aged 12 weeks at the start of the experiment and were housed in a normal, environmentally controlled animal room with free access to pathogen-free feed and water *ad libitum*.

### 2.4. AA-induced ear inflammatory animal models

The flowchart of AA-induced ear inflammatory animal models used in this study is shown in Fig. 1.

#### 2.4.1. Gavage model

The mice were given 500 μL of SHSP at doses of 20 mg/kg, 40 mg/kg, and 80 mg/kg body weight (BW) for 5 consecutive days (Day 1–5). Inflammation was induced on Day 5 by AA administration (2 mg AA dissolved in 5 μL acetone), 3 hours after SHSP gavage. AA was applied to both the anterior and posterior surfaces of the right ear of mice. Mice in the control group received the same volume of vehicle (500 μL) using the same schedule as that followed for the SHSP group. There were six mice in each group.

#### 2.4.2. Painting model

Both the anterior and posterior surfaces of the right ear of mice were painted with 500 μL of SHSP (doses: 1 mg/mL, 1.5 mg/mL, and 3 mg/mL). Inflammation was induced by AA injection, 10 minutes after painting with SHSP. Mice in the control group were painted with the same volume of vehicle (500 μL) using the same schedule as that followed for the SHSP group. There were six mice in each group.

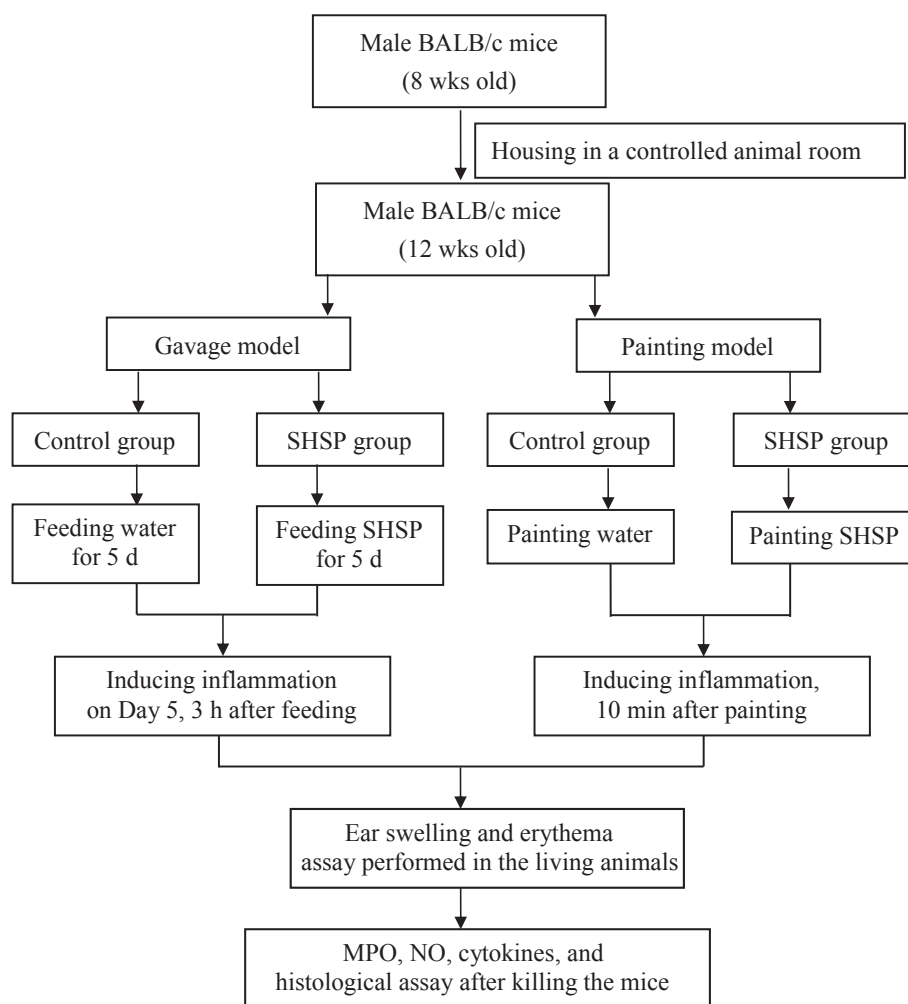
### 2.5. Ear swelling assay

The ear swelling was measured prior to the experiment and at 1-, 3-, 6-, and 24-hour time points after AA treatment. The ear thickness was measured using a micrometer (Model 293-561; Mitutoyo Digimatic, Veenendaal, The Netherlands) [25]. The ear swelling% is calculated as:

$$\left( \frac{\text{ear thickness after AA induction} - \text{ear thickness before AA induction}}{\text{ear thickness before AA induction}} \right) \times 100\%$$

### 2.6. Ear erythema assay

The ear erythema was measured prior to the experiment and at 1-, 3-, 6-, and 24-hour time points after AA treatment. The ear erythema was measured using the tissue viability (TiVi) polarized light spectroscopy method (Tissue Viability Imager



**Fig. 1 – Flowchart of arachidonic acid (AA)-induced ear inflammatory animal models. MPO = myeloperoxidase; NO = nitric oxide; SHSP = *Sargassum hemiphyllum* sulfated polysaccharide.**

TiVi600; WheelsBridge AB, Linköping, Sweden) to detect ear red blood cell concentration ( $RBC_{conc}$ ). The measurement was carried in a dark-room environment at an ambient temperature of 21–23°C and a relative humidity of 30–40%. The distance between the camera and ear surface was 25 cm [26]. The ear erythema% is calculated as:

$$\frac{[(\text{ear } RBC_{conc} \text{ after AA induction}) - (\text{ear } RBC_{conc} \text{ before AA induction})]}{(\text{ear } RBC_{conc} \text{ before AA induction})} \times 100\%$$

After the last measurement, the mice were sacrificed. The ears were collected for myeloperoxidase (MPO), NO, inflammatory cytokines, and histology assays.

## 2.7. MPO and NO assays

Ears were homogenized in 80 mM sodium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide for 45 seconds at 0°C, followed by centrifugation at 9000g for 20 minutes at 4°C. The supernatant was collected and stored at –80°C until MPO analysis. The concentration of MPO was determined using the InnoZyme Myeloperoxidase Activity

enzyme-linked immunosorbent assay (ELISA) kit (Calbiochem, Darmstadt, Germany). NO was determined using the Griess method [27].

## 2.8. Cytokines assay

The ears were homogenized and dissolved in 1% Triton X-100 lysis buffer (250 µL; Sigma, Zwijndrecht, The Netherlands) and enzyme inhibitor mix (Roche Diagnostics, Almere, The Netherlands) in phosphate-buffered saline. The homogenates were centrifuged at 13,500 rpm for 20 minutes at 4°C. The supernatants were collected and stored at –80°C until cytokine analysis. The concentration of IL-1β, IL-6, and TNF-α was determined using ELISA kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions.

## 2.9. Histological assay

The ears were fixed with 10% (v/v) paraformaldehyde, dehydrated, and embedded in paraffin. For histological analysis, 4-mm sections of fixed embedded tissues were cut on a microtome (Model 2165 rotary microtome; Leica, Nußloch, Germany). The sections were then placed on glass slides,

deparaffinized, and sequentially stained with hematoxylin and eosin (Richard-Allan Scientific, Kalamazoo, MI, USA).

### 2.10. Statistical analysis

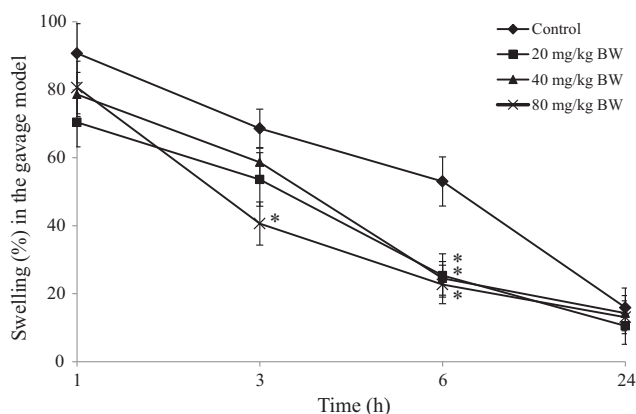
The data were presented as mean  $\pm$  standard deviation of three determinations. Significant between-group differences were determined by analysis of variance, and  $p < 0.05$  was considered as statistically significant.

## 3. Results and discussion

In traditional herbal medicine formulations, both oral and topical applications are common. To investigate differences between these two modes of application, oral gavage and topical paint models were used; in addition, we also determined whether SHSP is effective as both oral and topical applications using these two models.

### 3.1. Effect of SHSP on AA-induced ear swelling

The AA metabolism pathway is one of the main mechanisms for the induction of inflammation. Therefore, AA was used to initiate inflammation in mouse ears. For the gavage model, SHSP was orally administered for 5 days. One hour after AA-induced inflammation, no significant difference between mice that received SHSP and the control group was noted. However, after 3 hours, treatment with SHSP administered at a dose of 80 mg/kg BW significantly inhibited ear swelling from  $68.64\% \pm 5.65\%$  (control group) to  $40.64\% \pm 6.32\%$ . In the majority of tested groups, inhibition of ear swelling was measured at the 6-hour time point. After 6 hours, SHSP administered at doses of 20 mg/kg, 40 mg/kg, and 80 mg/kg BW inhibited ear swelling from  $53.03 \pm 7.21\%$  (control group) to  $25.35 \pm 6.37\%$ ,  $24.52 \pm 8.94\%$ , and  $22.72 \pm 5.65\%$ , respectively (Fig. 2). This result complied with the test results obtained



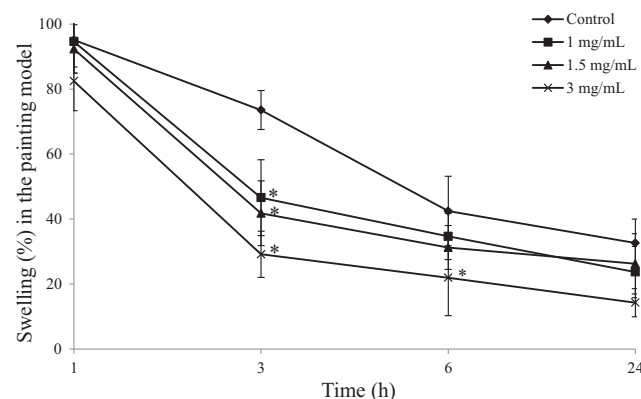
**Fig. 2** – Effect of *Sargassum hemiphyllum* sulfated polysaccharide (SHSP) on arachidonic acid-induced ear swelling in the gavage model. Vehicle (control), 20 mg/kg, 40 mg/kg, and 80 mg/kg body weight (BW) of SHSP were administered orally for 5 constitutive days. Results are shown as mean  $\pm$  standard deviation ( $n = 6$ ). \* $p < 0.05$  compared with the control.

using methanol extracts of *Sargassum swartzii* administered at doses of 175 mg/kg and 350 mg/kg BW [28] and water-soluble crude polysaccharide of *T. ornata* administered orally at a dose of 2.5–20 mg/kg BW [19], which showed inhibition of paw edema in carrageenan-induced rats.

To investigate the effects of topical SHSP on epidermis, SHSP was painted on the right ear 10 minutes after the administration of AA. Compared with the control group mice, significant inhibition of ear swelling was observed after 3 hours. At this time after AA-induced inflammation, 1 mg/mL, 1.5 mg/mL, and 3 mg/mL of SHSP inhibited ear swelling in a dose-dependent manner from  $73.56 \pm 5.99\%$  (control group) to  $46.59 \pm 11.67\%$ ,  $41.78 \pm 9.97\%$ , and  $29.16 \pm 7.12\%$ , respectively (Fig. 3). In further experiments, extracts of *S. thunbergii*, *S. fulvellum* [17], and *Turbinaria conoides* [29], which were obtained from boiling water, also inhibited ear edema and inflammation. The biological properties of these brown seaweed water extracts are quite similar to those of SHSP. These data, and those of previous studies, suggest that extraction by boiling in water effectively isolates an anti-inflammatory substance from *S. hemiphyllum*, and that SHSP may be the key active ingredient.

### 3.2. Effect of SHSP on AA-induced ear erythema

Erythema is one of the major visible inflammatory symptoms on skin, which is influenced by the regional blood flow rate, the concentration of RBC, and the thickness and pigmentation of skin. The clinical symptoms of skin erythema are usually analyzed subjectively with the naked eye. However, when clinical symptoms need to be graded (quantified), methods of skin reflectance spectrophotometry [30], erythema meter, and laser Doppler flow meter [31] have been used. These methods are noninvasive and touch free and can be used to measure erythema in living animals without physical interference. In this study, we used TiVi polarized light spectroscopy to investigate the effect of SHSP on ear erythema.



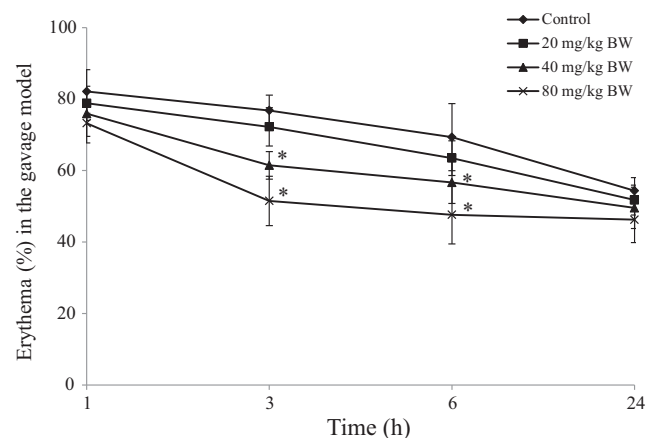
**Fig. 3** – Effect of *Sargassum hemiphyllum* sulfated polysaccharide (SHSP) on arachidonic acid (AA)-induced ear swelling in the painting model. Vehicle (control), 1 mg/mL, 1.5 mg/mL, and 3 mg/mL of SHSP were painted after the application of AA. Results are shown as mean  $\pm$  standard deviation ( $n = 6$ ). \* $p < 0.05$  compared with control.

In the gavage model, SHSP administered at doses of 40 and 80 mg/kg BW inhibited erythema from  $76.81 \pm 4.31\%$  (control group) to  $61.49 \pm 3.83\%$  and  $47.64 \pm 6.94\%$ , respectively, at the 3-hour time point. At the 6-hour time point, SHSP administered at doses of 40 mg/kg and 80 mg/kg BW also inhibited erythema from  $69.35 \pm 9.36\%$  (control group) to  $61.67 \pm 5.84\%$  and  $51.52 \pm 8.16\%$ , respectively. However, no inhibition of erythema was observed when SHSP was administered at the dose of 20 mg/kg BW (Fig. 4). In the paint model, 3 mg/mL of SHSP inhibited erythema from  $73.67 \pm 5.35\%$  (control group) to  $63.81 \pm 2.66\%$  at the 3-hour time point, and 1.5 mg/mL of SHSP showed slight inhibition at the 6-hour time point; however, 1 mg/mL of SHSP had no effect on erythema (Fig. 5).

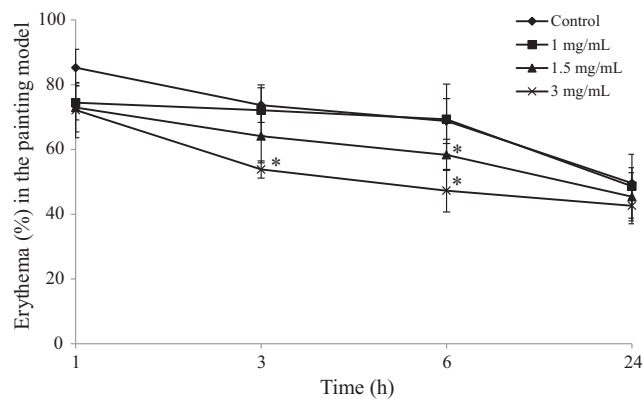
Some types of inflammatory tissue injury are mediated by free radicals. These free radicals injure cells and tissues directly by causing oxidative degradation of essential cellular components and by exacerbating inflammation [32]. Antioxidants, such as sulfated polysaccharides from brown seaweed, protect against oxidant-mediated inflammation by scavenging free radicals. Indeed, the anti-inflammatory effect of sulfated polysaccharide is improved with the degree of sulfation [33]. Our previous study indicated that *S. hemiphysllum* contains compounds with potent antioxidant activity [34] and high sulfate content [13]. Thus, it was suggested that the effect of SHSP on animal models might be due to the presence of such compounds with high antioxidative activity and sulfate content.

### 3.3. Effect of SHSP on inflammatory MPO, NO, and cytokine levels in ears

The neutrophil leukocyte marker MPO is known to indicate chemotaxis of neutrophil leukocytes in inflamed tissues [35]. Elevated MPO release in the inflammation site is correlated



**Fig. 4** – Effect of *Sargassum hemiphysllum* sulfated polysaccharide (SHSP) on arachidonic acid-induced ear erythema in the gavage model. Vehicle (control), 20 mg/kg, 40 mg/kg, and 80 mg/kg body weight (BW) of SHSP were administered orally for 5 constitutive days. Results are shown as mean  $\pm$  standard deviation ( $n = 6$ ). \* $p < 0.05$  compared with control.



**Fig. 5** – Effect of *Sargassum hemiphysllum* sulfated polysaccharide (SHSP) on arachidonic acid (AA)-induced ear erythema in the painting model. Vehicle (control), 1 mg/mL, 1.5 mg/mL, and 3 mg/mL of SHSP were painted after the application of AA. Results are shown as mean  $\pm$  standard deviation ( $n = 6$ ). \* $p < 0.05$  compared with control.

with NO, which is derived from MPO [36]. Therefore, we analyzed the effect of SHSP on MPO and NO production. In the gavage model, MPO and NO were present in AA-induced inflamed tissues at levels of  $34.22 \pm 3.55$  ng/mL and  $29.35 \pm 2.97$   $\mu$ M, respectively. Among mice treated with an SHSP dose of 80 mg/kg BW, MPO and NO levels decreased to  $20.63 \pm 2.49$  ng/mL and  $18.42 \pm 3.38$   $\mu$ M, respectively. Treatment with SHSP at doses of 40 mg/kg and 80 mg/kg BW was more effective than at a dose of 20 mg/kg BW in causing inhibition of MPO and NO production. Moreover, SHSP showed an inhibitory effect on these molecules in the paint model. We also evaluated the production of the proinflammatory cytokines IL-1, IL-6, and TNF- $\alpha$  in inflamed ears. These data also demonstrate that SHSP inhibits IL-1, IL-6, and TNF- $\alpha$  production in both gavage and paint models (Table 1). The results comply with those from previous studies in which *C. okamuranus* ameliorated inflammation by inhibiting MPO, IL-4, IL-6, and IL-10 [37]. Therefore, the anti-inflammatory activity of SHSP can be attributed to suppression of the release of proinflammatory cytokines, low production of MPO and NO, and maybe inhibition of leukocyte influx [38].

### 3.4. Effect of SHSP on histological changes in ears

In the inflammatory process, neutrophils are the first type of leukocytes to infiltrate inflammatory sites, thereby the most common histological feature of inflammation [39]. Thus, we conducted a histological evaluation of AA-induced inflammatory neutrophilic infiltration (Fig. 6A). After oral administration of SHSP at a dose of 80 mg/kg BW or topical administration by painting with 3 mg/mL SHSP, neutrophilic infiltration was clearly decreased (Fig. 6B and C). Importantly, these histological data correspond with the observed dose–response relationship to ear swelling,



**Table 1 – Effect of SHSP on AA-induced cytokine production in the ear.**

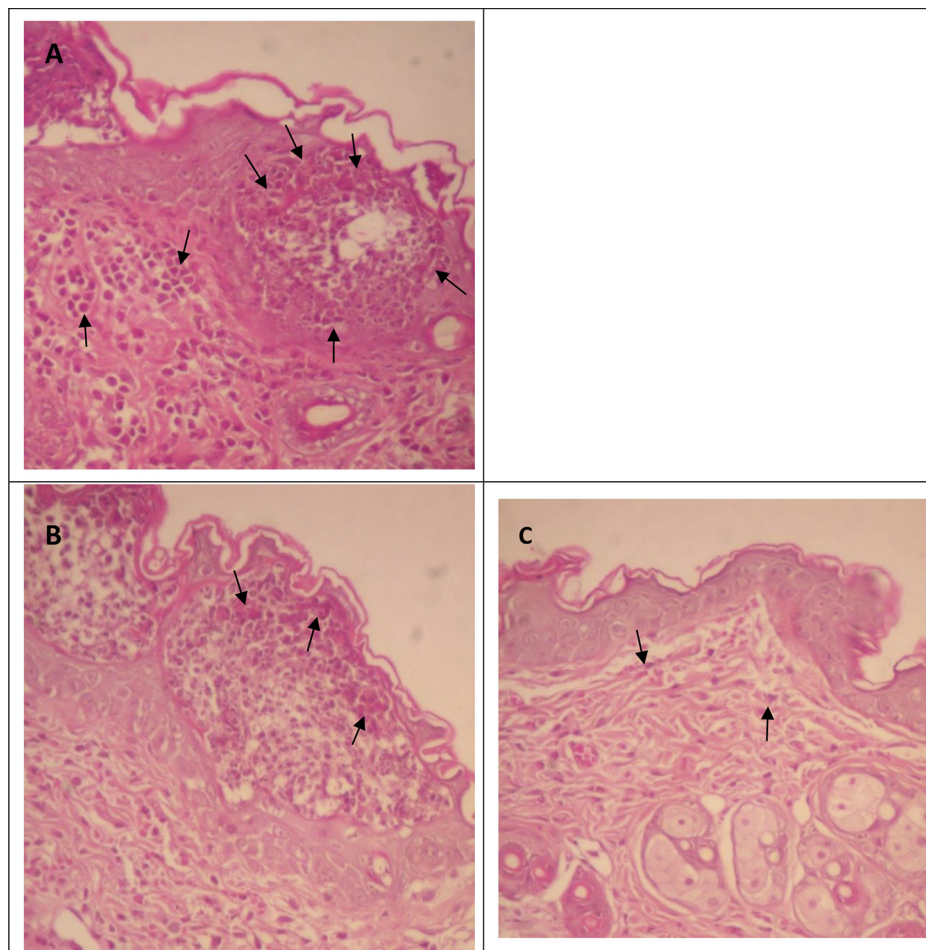
	Gavage model				Painting model			
	Control	20	40	80	Control	1	1.5	3
	mg/kg BW							
MPO (ng/mL)	34.22 ± 3.55	32.40 ± 4.37	22.34 ± 4.16*	20.63 ± 2.49*	39.14 ± 3.14	36.84 ± 2.58	22.76 ± 1.94*	18.76 ± 2.14*
NO (μM)	29.35 ± 2.97	29.11 ± 3.41	20.58 ± 3.71*	18.42 ± 3.38*	30.13 ± 1.78	27.12 ± 2.56	19.94 ± 3.62*	16.86 ± 3.23*
IL-1β (ng/mL)	5.17 ± 0.23	5.72 ± 1.29	5.15 ± 1.01	3.21 ± 0.84*	5.68 ± 0.88	5.41 ± 0.71	3.21 ± 0.84*	2.01 ± 0.44*
IL-6 (ng/mL)	0.92 ± 0.04	0.84 ± 0.10	0.62 ± 0.09*	0.60 ± 0.10*	0.95 ± 0.03	0.82 ± 0.19	0.85 ± 0.11	0.71 ± 0.02*
TNF-α (ng/mL)	0.22 ± 0.04	0.21 ± 0.01	0.22 ± 0.03	0.11 ± 0.05*	0.25 ± 0.02	0.24 ± 0.05	0.22 ± 0.04	0.23 ± 0.06

Results are shown as mean ± standard deviation (n = 6).  
 \*p < 0.05 compared with control.  
 AA = arachidonic acid; BW = body weight; IL = interleukin; MPO = myeloperoxidase; NO = nitric oxide; SHSP = *Sargassum hemiphyllum* sulfated polysaccharide; TNF-α = tumor necrosis factor-α.

erythema, and measurements of MPO, NO, and pro-inflammatory cytokines.

In conclusion, SHSP is the major anti-inflammatory agent present in *S. hemiphyllum* preparations, and it acts by

suppressing the production of inflammatory mediators. To our knowledge, this is the first study to demonstrate that SHSP, obtained from a brown seaweed using a single extraction process, has anti-inflammatory activity in an animal



**Fig. 6 – Effect of *Sargassum hemiphyllum* sulfated polysaccharide (SHSP) on histological changes as a result of arachidonic acid-induced inflammation. (A) Control, (B) 80 mg/kg body weight of SHSP in the gavage model, and (C) 3 mg/mL of SHSP in the painting model. Sections of the ears were stained by hematoxylin and eosin staining (200×). The arrows indicate neutrophilic infiltration.**

model and is effective in both oral and topical applications. Hence, SHSP may have promise in the treatment of inflammatory disease.

### Conflicts of interest

All contributing authors declare no conflicts of interest.

### Acknowledgments

We would like to thank Enago ([www.enago.tw](http://www.enago.tw)) for the English language review. This research was kindly supported by grants from the Council of Agriculture, Executive Yuan, Taiwan, R.O.C.

### REFERENCES

- [1] Ulich TR, Yin SM, Guo KZ, del Castillo J, Eisenberg SP, Thompson RC. The intratracheal administration of endotoxin and cytokines. III. The interleukin-1 (IL-1) receptor antagonist inhibits endotoxin- and IL-1-induced acute inflammation. *Am J Pathol* 1991;138:521–4.
- [2] Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol* 2001;22:199–204.
- [3] Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J Immunol* 2006;176:284–90.
- [4] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–43.
- [5] McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. *Neurobiol Aging* 2001;22:799–809.
- [6] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111–9.
- [7] Lincoln RA, Strupinski K, Walker JM. Bioactive compounds from algae. *Life Chem Rep* 1991;8:97–183.
- [8] Yoon WJ, Ham YM, Kim KN. Anti-inflammatory activity of brown alga *Dictyota dichotoma* in murine macrophage RAW 264.7 cells. *J Med Plants Res* 2009;3:1–8.
- [9] Kang S-M, Kim K-N, Lee S-H, Ahn G, Cha S-H, Kim A-D, Yang X-D, Kang M-C, Jeon Y-J. Anti-inflammatory activity of polysaccharide purified from AMG-assistant extract of *Ecklonia cava* in LPS-stimulated RAW 264.7 macrophages. *Carbohydr Polym* 2011;85:80–5.
- [10] Lee S-H, Ko C-I, Ahn G, You SG, Kim J-S, Heu MS, Kim J, Jee Y, Jeon Y-J. Molecular characteristics and anti-inflammatory activity of the fucoidan extracted from *Ecklonia cava*. *Carbohydr Polym* 2012;89:599–606.
- [11] Kang JW, Yoon SY, Oh SJ, Kim SK, Kang KW. Bifunctional effects of fucoidan on the expression of inducible nitric oxide synthase. *Biochem Biophys Res Commun* 2006;346:345–50.
- [12] Na HJ, Moon PD, Ko SG, Lee HJ, Jung HA, Hong SH, Seo Y, Oh JM, Lee BH, Choi BW, Kim HM. *Sargassum hemiphyllum* inhibits atopic allergic reaction via the regulation of inflammatory mediators. *J Pharmacol Sci* 2005;97:219–26.
- [13] Hwang PA, Chien SY, Chan YL, Lu MK, Wu CH, Kong ZL, Wu CJ. Inhibition of lipopolysaccharide (LPS)-induced inflammatory responses by *Sargassum hemiphyllum* sulfated polysaccharide extract in RAW 264.7 macrophage cells. *J Agric Food Chem* 2011;59:2062–8.
- [14] Dore CM, das C Faustino Alves MG, Will LS, Costa TG, Sabry DA, de Souza Rêgo LA, Accardo CM, Rocha HA, Filgueira LG, Leite EL. A sulfated polysaccharide, fucans, isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydr Polym* 2013;91:467–75.
- [15] Kang YM, Eom SH, Kim YM. Protective effect of phlorotannins from *Eisenia bicyclis* against lipopolysaccharide-stimulated inflammation in HepG2 cells. *Environ Toxicol Pharmacol* 2013;35:395–401.
- [16] Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI, Usov AI, Ustyuzhanina NE, Grachev AA, Sanderson CJ, Kelly M, Rabinovich GA, Iacobelli S, Nifantiev NE, Consorzio Interuniversitario Nazionale per la Bio-Oncologia, Italy. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology* 2007;17:541–52.
- [17] Kang JY, Khan MN, Park NH, Cho JY, Lee MC, Fujii H, Hong YK. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice. *J Ethnopharmacol* 2008;116:187–90.
- [18] Sarithakumari CH, Renju GL, Kurup GM. Anti-inflammatory and antioxidant potential of alginic acid isolated from the marine algae, *Sargassum wightii* on adjuvant-induced arthritic rats. *Inflammopharmacology* 2013;21:261–8.
- [19] Ananthi S, Raghavendran HR, Sunil AG, Gayathri V, Ramakrishnan G, Vasanthi HR. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (marine brown alga). *Food Chem Toxicol* 2010;48:187–92.
- [20] Medeiros VP, Queiroz KC, Cardoso ML, Monteiro GR, Oliveira FW, Chavante SF, Guimaraes LA, Rocha HA, Leite EL. Sulfated galactofucan from *Lobophora variegata*: anticoagulant and anti-inflammatory properties. *Biochemistry (Mosc)* 2008;73:1018–24.
- [21] Kim SK, Lee DY, Jung WK, Kim JH, Choi I, Park SG, Seo SK, Lee SW, Lee CM, Yea SS, Choi YH, Choi IW. Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: role of suppressor of cytokine signaling. *Biomed Pharmacother* 2008;62:289–96.
- [22] Mohsin S, Kurup GM, Mahadevan R. Effect of ascophyllan from brown algae *Padina tetrastratica* on inflammation and oxidative stress in carrageenan-induced rats. *Inflammation* 2013;36:1268–78.
- [23] Dodgson KS, Price RG. A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem J* 1963;84:106–10.
- [24] Lee IH, Huang RL, Chen CT, Chen HC, Hsu WC, Lu MK. *Antrodia camphorata* polysaccharides exhibit anti-hepatitis B virus effects. *FEMS Microbiol Lett* 2002;209:63–7.
- [25] Hartog A, Leenders I, van der Kraan PM, Garssen J. Anti-inflammatory effects of orally ingested lactoferrin and glycine in different zymosan-induced inflammation models: evidence for synergistic activity. *Int Immunopharmacol* 2007;7:1784–92.
- [26] O'Doherty J, Henricson J, Anderson C, Leahy MJ, Nilsson GE, Sjöberg F. Sub-epidermal imaging using polarized light spectroscopy for assessment of skin microcirculation. *Skin Res Technol* 2007;13:472–84.
- [27] Di Rosa M, Ialenti A, Iannaro A, Sauterin L. Interaction between nitric oxide and cyclooxygenase pathways. Prostaglandins Leukot Essent Fatty Acids 1996;54:229–38.
- [28] Hong DD, Hien HM, Anh HTL. Studies on the analgesic and anti-inflammatory activities of *Sargassum swartzii* (Turner) C. Agardh (Phaeophyta) and *Ulva reticulata* Forsskal

- (Chlorophyta) in experiment animal models. *Afr J Biotechnol* 2011;10:2308–14.
- [29] Boonchum W, Peerapornpisal Y, Kanjanapothi D, Pekkoh J, Amornlerdpison D, Pumas C, Sangpaiboon P, Vacharapiyasophon P. Antimicrobial and anti-inflammatory properties of various seaweeds from the Gulf of Thailand. *Int J Agric Biol* 2011;13:100–4.
- [30] Bjerring P, Andersen PH. Skin reflectance spectrophotometry. *Photodermatol* 1987;4:167–71.
- [31] Lahti A, Kopola H, Harila A, Myllylä R, Hannuksela M. Assessment of skin erythema by eye, laser Doppler flowmeter, spectroradiometer, two-channel erythema meter and Minolta chroma meter. *Arch Dermatol Res* 1993;285:278–82.
- [32] Conner EM, Grisham MB. Inflammation, free radicals, and antioxidants. *Nutrition* 1996;12:274–7.
- [33] Ji SL, Du HY, Chi YQ, Cui HF, Cao JC, Geng MY, Guan HS. Effects of dermatan sulfate derivatives on platelet surface P-selectin expression and protein C activity in blood of inflammatory bowel disease patients. *World J Gastroenterol* 2004;10:3485–9.
- [34] Hwang P-A, Wu C-H, Gau S-Y, Chien S-Y, Hwang D-F. Antioxidant and immune-stimulating activities of hot-water extract from seaweed *Sargassum hemiphyllum*. *J Mar Sci Tech* 2010;18:41–6.
- [35] Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. *J Leukoc Biol* 1994;56:672–86.
- [36] Hazen SL, Zhang R, Shen Z, Wu W, Podrez EA, MacPherson JC, Schmitt D, Mitra SN, Mukhopadhyay C, Chen Y, Cohen PA, Hoff HF, Abu-Soud HM. Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation *in vivo*. *Circ Res* 1999;85:950–8.
- [37] Matsumoto S, Nagaoka M, Hara T, Kimura-Takagi I, Mistuyama K, Ueyama S. Fucoidan derived from *Cladosiphon okamuranus* Tokida ameliorates murine chronic colitis through the down-regulation of interleukin-6 production on colonic epithelial cells. *Clin Exp Immunol* 2004;136:432–9.
- [38] Montanher AB, Zucolotto SM, Schenkel EP, Fröde TS. Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. *J Ethnopharmacol* 2007;109:281–8.
- [39] Engeman T, Gorbachev AV, Kish DD, Fairchild RL. The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. *J Leukoc Biol* 2004;76:941–9.