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Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice

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Abstract

Dichloromethane, ethanol, and boiling water extracts of the brown seaweeds *Sargassum fulvellum* and *Sargassum thunbergii* were examined for antipyretic, analgesic, and anti-inflammatory activities in mice. The activities were evaluated against yeast-induced pyrexia, tail-flick test, and phorbol myristate acetate-induced inflammation (edema, erythema, and blood flow). The dichloromethane extract (0.4 mg/ear) of *Sargassum fulvellum* inhibited an inflammatory symptom of mouse ear edema by 79.1%. The ethanol extract (0.4 mg/ear) of *Sargassum thunbergii* also inhibited edema by 72.1%. No acute toxicity was observed after p.o. administration of each extract (5 g/kg bw). These findings are consistent with various claims that these seaweeds can be used as remedies for inflammation-related symptoms.

Keywords: Analgesic; Anti-inflammation; Antipyretic; Brown seaweed; *Sargassum fulvellum*; *Sargassum thunbergii*

1. Plant

Thalli of the brown seaweed *Sargassum fulvellum* (Turner) C. Agardh were collected from Wando aquaculture farm (Jeonam, Korea) in January 2006. Thalli of the *Sargassum thunbergii* (Roth) Kuntze were collected from the coast of Kijang, Korea in January 2006 and January 2007. Scientific names of the seaweed species have been authenticated by their common or local names (Suh, 1997). Voucher specimens (SF18-2006 and ST22-2006) are deposited in our laboratory (Y.K. Hong). They are perennially growing on rocks near low tide-level on calm open and sheltered coasts, with mature fronds growing to lengths of 1–3 m in *Sargassum fulvellum* and 0.3–1 m in *Sargassum thunbergii*, respectively. Annual production of *Sargassum fulvellum* in 2006 was estimated at 13 tonnes (wet wt) for food additives in Korea (MOMAF, 2007).

2. Uses in traditional medicine

Sargassum fulvellum known as Hejo is used as a food additive (Kang, 1968), and recorded many uses in treatment of lump, dropsy, swollen and painful scrotum, and urination problems with no side effects in the Oriental medical textbook Donguibogam, published in 1613 (Donguibogam Committee, 1999). *Sargassum thunbergii* known as Hede is used as a food additive, an anti-helminthic (Kang, 1968), and to treat lump, dropsy, swollen and painful scrotum (Donguibogam Committee, 1999).

3. Previously isolated classes of constituents

Sargassum fulvellum has been reported to inhibit oxidation (Heo et al., 2005) and breast carcinoma (Jo et al., 2005). It also contains alginate (Fujihara et al., 1984) and anti-coagulant fucoidan (Koo et al., 2001). *Sargassum thunbergii* has anti-helminthic (Lee and Min, 1970), antioxidant (Heo et al., 2005; Park et al., 2005), and hepatoprotective effects (Park et al., 1997), and inhibits DNA polymerase (Jin et al., 1997) and xanthine oxidase (Kim et al., 1996). In addition, *Sargassum thunbergii* contains diacylglycerylhydroxymethyltrimethyl-β-alanine (Araki et al., 1991), glyceroglycolipids (Son et al., 1992), antitumor fucoidan (Zhuang et al., 1995), peroxynitrite-scavenging sargahydroquinoic acid, sargaquinoic acid, and sargachromenol (Seo et al., 2004).

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4. Materials and methods

4.1. Tested materials

Dark greenish extracts of dichloromethane (yield: 0.9%) and ethanol (4.6%) were obtained from 20 g of *Sargassum fulvellum* powder with 1 l of each solvent by 1-h shaking at room temperature. The brownish water extract (8.9%) was obtained by boiling in water for 1 h. Brownish yellow extracts of dichloromethane (yield: 1.0%) and ethanol (4.0%) were obtained from 20 g of *Sargassum thunbergii* powder with 1 l of each solvent by 1-h shaking at room temperature. The brownish water extract (9.6%) was obtained by 1-h boiling.

4.2. Animals

BALB/c mice (8–10 weeks old, 20–25 g body weight) were used for assaying various activities and anti-inflammatory effects. They were maintained in standard environmental conditions, with free access to food and water. Animal experiments were performed in accordance with the U.S. NIH Guidelines for the Care and Use of Laboratory Animals.

4.3. Antipyretic activity

A brewer's yeast-induced pyrexia model in female mice was used to test the antipyretic activity of seaweed extracts (Teotino et al., 1963). When the rectal temperature peaked after 24 h, either 4 g of extracts in 10 ml of 5% Tween-80 or 10 ml of 5% Tween-80 (control) per kg body weight were administered orally, and the rectal temperature (°C) was recorded after an additional 45 min, using an electric thermometer connected to a probe, inserted 2 cm into the rectum. Relative antipyrexia (%) was expressed as [(value of the control − value of the extract)/value of the control] × 100. Acetyl salicylic acid (150 mg/kg, p.o.) was used as a standard.

4.4. Analgesic activity

In the tail-flick test (Gray et al., 1970), either extracts (1.5 g in 10 ml of 5% Tween-80 per kg) or control was administered i.p. to mice, and the tail-flick latency time (s) was measured 1 h later using a tail-flick unit (Ugo Basile, Varese, Italy). Relative latency (%) was expressed as [(value of the extract − value of the control)/value of the control] × 100. Acetyl salicylic acid (150 mg/kg, p.o.) in the same volume of vehicle was used as the standard.

4.5. Anti-inflammatory activities

Stock solutions of extracts were prepared by adding ethanol (1 ml) to dried seaweed extracts (40 mg). Phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO, USA) in acetone (0.2 μg/10 μl/ear) was combined with the seaweed extracts in ethanol (0.4 mg/10 μl/ear) and topically applied to the whole inner side of the mouse's ear. Ear edema was measured after 10 h using a spring-loaded micrometer (Mitutoyo Corp., Tokyo, Japan) (Griswold et al., 1998). Ear erythema was determined at 10 h using digital photography, adjusted to balance white, and Photoshop 7.0 (Adobe, San Jose, CA, USA) to measure the magenta value (Khan et al., 2007). To confirm the anti-inflammatory activity of the seaweeds, local blood flow in the mouse ear was measured using laser speckle flowgraphy (Inflameter LFG-1; SoftCare, Fukuoka, Japan) (Lee et al., 2003). Edema (AU), erythema (AU), and blood flow (AU) values were calculated as $(I_{10}–I_0)/I_0$, where I_{10} is measurement 10 h after PMA application and I_0 is the measurement at 0 h. The relative inhibition rate (%) was expressed as [(value of the control − value of the extract)/value of the control] × 100. Indomethacin (0.3 mg/10 μl-ethanol/ear) was used as the standard.

4.6. Acute toxicity test

Mice were fasted for 6 h, with water provided ad libitum. Extracts (5 g/10 ml of 5% Tween-80/kg bw) were administered orally to mice ($n = 5$, each). The animals were then observed for any abnormal behavior for 3 h, and mortality was noted for up to 2 weeks. A group of animals treated with the Tween-80 served as the control.

4.7. GC–MS analysis

To identify major constituents in seaweed extracts, the extracts were analyzed on a GC–MS-QP5050A (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector, and compared to the spectral data from the database.

4.8. Statistical analysis

All animal experiments were performed with at least seven mice for each group, and the highest and lowest values were discarded. Data are reported as means ± S.E.M. The significance of the results was calculated using Student's *t*-test and was deemed statistically significant at $P < 0.01$.

5. Results

In preparing traditional medicines and health care foods, it is common to boil the materials in water or to soak them in beverage alcohol. To understand more detailed investigations of the active substances, we prepared boiling water-, alcohol-, and dichloromethane-soluble extracts of the seaweeds, and determined their antipyretic, analgesic, and anti-inflammatory activities in mice. From the *Sargassum fulvellum* seaweed, oral administration of extracts marginally lowered rectal temperatures in hyperthermic mice (Table 1). Tail-flick behavior in mice was used to evaluate the analgesic activity of the seaweed extracts. As controls, mice injected with 5% Tween-80 responded by tail flicking in 3.08 ± 0.04 s on average. i.p. injection of dichloromethane extract of the *Sargassum fulvellum* seaweed showed significant ($P < 0.001$) activity to the analgesia, with the latency increasing by 3.74 ± 0.17 s (i.e., 21.4%).