In Vivo Antipyretic, Analgesic, and Anti-inflammatory Activities of the Brown Alga Ecklonia cava Extracts in Mice

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Abstract

Dichloromethane, ethanol, and boiling water extracts of the brown alga Ecklonia cava were examined \textit{in vivo} for their antipyretic, analgesic, and anti-inflammatory activities in mice. These activities were evaluated by yeast-induced pyrexia, tail-flick test, and phorbol myristate acetate-induced inflammation (edema, erythema, and blood flow). Ethanol extract of \textit{E. cava} (0.4 mg/ear) inhibited the inflammatory symptoms of mouse ear edema, erythema, and blood flow by 82.6%, 69.0%, and 65.4%, respectively. This extract also demonstrated potent analgesic activity. No acute toxicity was observed after \textit{p.o.} administration of each extract (5 g/kg bw). These \textit{in vivo} data are in agreement with the claims of the health care industry and indigenous medicine that \textit{E. cava} is an effective remedy for inflammation-related symptoms.

Key words: Ecklonia cava, Analgesic, Anti-inflammation, Antipyretic, Phaeophyta, \textit{In vivo} assay

Introduction

The brown seaweed \textit{Ecklonia cava} Kjellman is abundant in the subtidal region of Jeju Island and the southern coast of Korea. It is used as abalone feed (Kang, 1968) and in treatments of hemorrhoids and gastroenteritis, as well as insecticide, as recorded in the Oriental medical textbook Donguibogam published in 1613 (Donguibogam Committee, 1999). \textit{E. cava} is also used as a source for an immune-booster, which has been claimed to contain antitumor, anticoagulant, and antithrombin polysaccharides (Koyanagi et al., 2003). The seaweed contains marine polyphenols known as phlorotannins (Li et al., 2009), which are found only in brown algae, synthesized via an acetate-melonate pathway and formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) (Ragan and Glombitza, 1986). Recently, accumulating evidence suggests that \textit{E. cava} exhibits matrix metalloproteinase inhibitory activity (Kim et al., 2006), bactericidal activity (Jin et al., 1997), protease inhibition (Ahn et al., 2004), and effects on osteoarthritis (Shin et al., 2006), asthma (Kim et al., 2008), and melanogenesis (Heo et al., 2009). Numerous studies have examined the antioxidant properties of extracts (Jung et al., 2009) or chemical components (Kang et al., 2004; Heo et al., 2009; Li et al., 2009). \textit{E. cava} polysaccharide also showed anti-inflammatory activity mediated by inhibition of NO and prostaglandin \textit{E}\(_2\) production (Jung et al., 2009; Kang et al., 2011). The underlying mechanism may involve repression of inflammation by antioxidant substances (Garrett and Grisham, 2005). Thus, to evaluate the anti-inflammatory activities of \textit{E. cava}, we assayed \textit{in vivo} the anti-inflammatory activities of dichloromethane, ethanol, and boiling water extracts against hyperpyrexia, algesthesia, edema, erythema, and local blood flow in mice.
Materials and Methods

Seaweed materials

Thalli of the brown seaweed *E. cava* were collected from the coast of Kijang, Korea, from August 2008 to 2010. The scientific name of the seaweed described in the Donguibogam was identified from its common or local names (Suh, 1997). A voucher specimen is deposited in our laboratory (Y. K. Hong). For convenience, the seaweed tissue was completely dried for 1 week at room temperature and then ground to powder for 5 min by a coffee grinder. The powder was stored at -20°C until use. Dichloromethane or ethanol (1 L) was used to extract 20 g of the seaweed powder by shaking at room temperature for 1 h. For the water-soluble fraction, distilled water was boiled for 1 h. The crude extract was evaporated under vacuum and dried under nitrogen. To remove salt from extracts, extractions were repeated several times (from the previous solvent-soluble fraction) until the amount of salt was visibly negligible.

Animals

BALB/c mice (8-10 weeks old; 20-25 g body weight) were used to assay various anti-inflammatory activities. The animals were kept at room temperature (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 U. S. NIH Guidelines for the Care and Use of Laboratory Animals.

Antipyretic activity

A brewer’s yeast-induced pyrexia mouse model was used to determine the antipyretic activity (Teotino et al., 1963). When the rectal temperature peaked after 24 h, extracts (4 g) 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 temperature suppression (%) is expressed as [\((\text{value of the control} - \text{value of the extract})/\text{value of the control}\) × 100]. Acetaminophen (APAP, 75 mg/kg, *p.o.*) was used as a standard.

Analgesic activity

In the tail-flick test (Gray et al., 1970), extracts (1.5 g/10 mL of 5% Tween-80/kg body weight) or control was administered *i.p.* to mice, and tail-flick latency time(s) was measured 1 h later using a tail-flick unit (Ugo Basile, Varese, Italy). Relative latency (%) was expressed as [\((\text{value of the extract} - \text{value of the control})/\text{value of the control}\) × 100]. Acetaminophen (APAP, 150 mg/kg, *p.o.*) in the same volume of vehicle was used as a standard.

Anti-inflammatory activity

Stock solutions of the 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−1 ear−1) was combined with the seaweed extracts in ethanol (0.4 mg 10 μL−1 ear−1) 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) (Grisswold et al., 1998). Ear earthema was determined at 10 h by digital photography, adjusted to balance white, and Photoshop 7.0 (Adobe, San Jose, CA, USA) was used to measure the magenta value (Khan et al., 2008). To confirm the anti-inflammatory activity of the seaweed, local blood flow in the mouse ear was measured by 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 \((I_e - I_o)/I_o\) where \(I_o\) is the measurement 10 h after PMA application and \(I_e\) is the measurement at 0 h. The relative inhibition rate (%) was expressed as [\((\text{value of the control} - \text{value of the extract})/\text{value of the control}\) × 100]. Indomethacin (0.3 mg 10 μL−1 ethanol−1 ear−1) was used as a standard.

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 Tween-80 served as the control.

Statistical analysis

All animal experiments were performed with at least seven mice per group, and the highest and lowest values were discarded. Data are presented as means ± SE. The significance of the results was calculated using Student’s *t*-test, and differences were deemed statistically significant at *P* < 0.01.

Results and Discussion

When preparing traditional medicines and health care foods, the materials are commonly boiled in water or soaked in beverage alcohol. To undertake more detailed investigations of the active substances in *E. cava*, we prepared boiling water-, alcohol-, and dichloromethane-soluble seaweed extracts. Brownish extracts of boiling water (yield, 15.6%), ethanol (yield, 1.7%), and dichloromethane (yield, 0.3%) were obtained. The antipyretic activity was evaluated by measuring changes in rectal temperature. When the mice were injected with brewer’s yeast, the rectal temperature peaked at 39.19 ± 0.07°C, which was above the normal 38.45 ± 0.06°C, at 24 h.