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EXPERIMENTAL STUDY

Extrusion process of Acanthopanax senticosus leaves enhances the gastroprotective effect of compound 48/80 on acute gastric mucosal lesion in rats

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Abstract

OBJECTIVE: To investigate the gastroprotective effects of Acanthopanax senticosus leaves (ASLs) extrusion on acute gastric mucosal lesion in rats induced by compound 48 / 80 (C48 / 80).

METHODS: Rats were divided into six groups: normal; C48/80-induced gastric lesion control; gastric lesion positive control (famotidine 4 mg/kg); gastric lesion administered with two levels of extruded ASLs (ASLE, 40 and 200 mg/kg); and gastric lesion treated with ASLs (ASL 200 mg/kg). Mucus secretion / damage was determined by immunohistological staining. Immunofluorescence and western blotting were performed to determine gastric mucosal Bax and Bcl-2 expression. Gastric mucosal oxidative-stress-related enzymes and malondialdehyde were determined.

RESULTS: C48/80-induced mucus depletion and inflammation in the gastric mucosa were significantly attenuated by ASLs. The increased serum serotonin and histamine concentrations in C48/80-treated rats were also attenuated by ASLs. Gastric mucosal Bax protein expression was increased and Bcl-2 expression was decreased after C48/80 treatment, and ASLs ameliorated Bax and Bcl-2 expression. The extrusion process significantly augmented the effects of ASLs in a dose-dependent manner. ASLEs at 200 mg/kg normalized mucus damage / secretion, C48 / 80-induced increases of mucosal myeloperoxidase activity (index of inflammation), xanthine oxidase, and malondialdehyde content (index of lipid peroxidation). The effects of ASLs on Bax and Bcl-2 expression were also enhanced by extrusion. Furthermore, these effects of ASLEs at 200 mg/kg were similar to those of famotidine, a histamine H_2-receptor antagonist commonly used to treat gastric ulcers.

CONCLUSION: ASLEs prevented acute gastric mucosal lesion progression induced by C48/80, possibly by inducing mucus production, and reduced inflammation and oxidative stress in gastric mucosa through an anti-apoptotic mechanism.

Key words: Acanthopanax senticosus leaves; Extrusion; Compound 48/80; Gastric mucosal lesion

INTRODUCTION

Acanthopanax senticosus (AS), also called Siberian

ginseng, is a widely used traditional medicine in East Asia. Since the first phytochemical and pharmacological studies of AS by Brekhman and his group in Russia in 1969,[1] the roots and stem barks of AS have been extensively studied, suggesting a variety of properties such as antibacterial, anti-inflammatory, anti-hepatitis, anti-hyperglycemic, anti-oxidant, immunostimulatory, hypocholesterolemic, and radioprotective effects.[2,3] However, its medical use has depleted its resources. Therefore, there is a current trend to develop a method to use the leaves for medical purposes. The leaves of this plant contain di- and triterpenoids, saponins, and flavonoids,[4-6] and recent pharmacological investigations of AS leaves (ASLs) report anti-oxidant, anti-diabetic, and anti-platelet aggregating activities.[7-9]

Gastric ulcers are common worldwide, and affect 5%-10% of people during their lifetime. Therapy for gastric ulcers includes control of acid secretion or histamine H_2 receptor blockers such as famotidine, as well as reversal of mucosal inflammation. The anti-inflammatory effects of ASLs are suggested by their nitric oxide scavenging activity and their suppression of lipopolysaccharide-induced nitric oxide synthesis.[10,11]

Extrusion is one of the most common industrial processes used to enhance the digestibility and bioavailability of food nutrients. Several studies have reported that extrusion has the potential to increase the yields of the effective components, such as saponins, ginsenosides, and flavonoids;[12,13] therefore, the extrusion process could be an interesting alternative to functional foods. The present study examined the protective effect of ASLs and extruded ASLs (ASLEs) on acute gastric mucosal lesions induced by compound 48 / 80 (C48/80) in rats.

MATERIALS AND METHODS

Preparation of plant samples

The leaves of Acanthopanax senticosus var. subinermis (ASLs) were collected in June 2008 from Yesan city, Korea. The air-dried and coarsely powdered ASLs were extruded using a twin-screw extruder (THK 31T; Inchon Machinery, Inchon, Korea). The extruder was operated at a feed rate of 110 g/min with a screw speed of 200 rpm, pressure of 5 bars, and temperature of 150℃. Samples were dried, soaked in hot water for 48 h, filtered through a 0.45-μm filter (Osmonics, Minnetonka, MN, USA), and lyophilized. The yields after vacuum evaporation were 9.7 and 10.4% for ASLs and ASLEs, respectively.

Animals and gastric mucosal lesion induction by C48/80

Six-week-old male Wistar rats were purchased from Samtako (Osan, Korea) and housed at a controlled temperature (23 ± 2) ℃ and relative humidity (60 ± 5) %, and 12-h light/dark cycle (7:00-19:00 h) with free access to water. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of Semyung University (smecae08-12-03). Rats were divided into six groups of 10: normal (no gastric lesion and administered distilled water); gastric lesion control (administered distilled water); gastric lesion positive control (administered 4 mg/kg famotidine; Nelson Korea, Seoul, Korea); gastric lesion administered with two levels of ASLEs (40 and 200 mg/kg); and gastric lesion treated with ASLs (200 mg/kg). The animals were maintained with free access to rat chow, and famotidine, ASL and ASLE extracts were orally administered *via* a stomach tube.

Five days after administration, C48/80 (0.75 mg/kg, Sigma-Aldrich, Rochester, NY, USA), dissolved in saline, was intraperitoneally injected to rats after fasting for 24 h. Normal control rats received saline injection. The animals were sacrificed by decapitation under ether anesthesia 3 h after C48/80 injection, and blood samples were obtained from the cervical wound.

Histological analysis

The isolated stomachs were cut open along the greater curvature and washed in ice-cold saline. Part of the mucosa was fixed with 10% formalin solution, and routinely processed for embedding in paraffin wax. Five-micrometer-thick sections were cut and stained with Periodic acid-Schiff (PAS) staining[16] to observe mucus secretion. Mucosal damage was analyzed with an image analyzer (Image-Pro Plus; Media Cybernetics, Rockville, MD, USA), and expressed as a percentage of PAS-negative mucosal surface.

The measurement of gastric mucosal adherent mucus is another indicator of gastric mucus secretion, and was assayed using Alcian blue staining.[17] A 50-mm^2 (~8 mm diameter) portion of the glandular region of the stomach was excised with a scalpel, and soaked in 0.1% Alcian blue dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate (pH 5.8) for 2 h. The unbound dye was removed and dye complex with mucus was extracted using 30% docusate sodium salt (Sigma-Aldrich) for 2 h. After centrifugation at 2060 ×*g* for 10 min, the optical density (OD) of the Alcian blue solution was measured at 620 nm, and calculated using the calibration curve. The adherent gastric mucosal mucus was expressed as the percentage of the Alcian blue adhering to the gastric mucosal surface of the gastric lesion control group.

Immunofluorescence analysis

Five-micrometer-thick sections were cut and mounted on glass slides. The immunofluorescence analysis was performed with mouse monoclonal anti-Bax antibody and rabbit monoclonal anti-Bcl-2 antibody (Santa Cruz Biotechnology, Dallas, TX, USA), and fluorescein-isothiocyanate-conjugated anti-mouse and