A Comparison between Water and Ethanol Extracts of *Rumex acetosa* for Protective Effects on Gastric Ulcers in Mice

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Abstract

*Rumex acetosa* is a perennial herb that is widely distributed across eastern Asia. Although the hot water extract of *R. acetosa* has been used to treat gastritis or gastric ulcers as a folk medicine, no scientific report exists for the use of this plant to treat gastric ulcers. Hence, the present study was undertaken to assess the anti-ulcer activity of water and 70% ethanol extracts obtained from *R. acetosa*, using an HCl/ethanol-induced gastric ulcer model in mice. Anti-inflammatory and free radical-scavenging activities of these two extracts were also evaluated and compared. As a result, the administration of *R. acetosa* extracts significantly reduced the occurrence of gastric ulcers. However, significant differences in protective activity against gastric ulcers were observed between the two samples. In the case of the group pretreated with an ethanol extract dosage of 100 mg/kg, the protective effect (90.9%) was higher than that of water extract (41.2%). Under histological evaluation, pretreatment with *R. acetosa* extracts reversed negative effects, such as inflammation, edema, moderate hemorrhaging and loss of epithelial cells, presented by HCl/ethanol-treated stomachs. Meanwhile, *R. acetosa* extracts showed potent DPPH radical-scavenging activity and decreased NO production in a murine macrophage cell line, RAW 264.7, in a dose-dependent manner without affecting cellular viability. The greater anti-ulcer and NO production inhibitory activities exhibited by ethanol extracts compared to water extracts could be ascribed to the higher emodin levels, a major anthraquinone component of this plant.

Key Words: *Rumex acetosa*, Gastric ulcer, Antioxidant activity, Anti-inflammatory activity

INTRODUCTION

The therapeutic management of peptic ulcers includes several classes of drugs, such as proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier, and prostaglandin analogs. However, there is still a need for the development of new anti-ulcer drugs because of the emergence of tolerance and side effects, which make the efficacy of these treatments questionable (Mard et al., 2008). 

*Rumex acetosa* L. (Polygonaceae) is a perennial herb that is widely distributed in eastern Asia (Anonymous, 1999; Lee, 2003). The leaf of this plant resembles that of spinach, and the plant is well known for strong acidity in Europe and America, where it is also known as sorrel. The extract of *R. acetosa* has been reported to have heat-cleaning, diuretic, insecticidal, antimicrobial, and anticancer activities (Lee et al., 2005; Gescher et al., 2011; Wegiera et al., 2011). Decoction of this plant has been used as a folk medicine in Korea to treat arthritis, gastritis and gastric ulcers, and as a substitute for rhubarb, which is an important crude drug for gastrointestinal problems. There are commercial herbal tonic products such as Essiace™ and Flor-Essence™ which contain *R. acetosa*, that are traditionally prepared as cancer therapy for natives in North America (Tamayo et al., 2000; Tai et al., 2004; Leonard et al., 2006). Flavones including vitexin, rutin, and kaempferol were isolated from the aerial parts of this plant with another flavone C-glycosides and flavonol O-glycosides (Kato and Morita, 1990). From the roots of *R. acetosa*, chrysophanein, hyperin, proanthocyanidin and chlorogluconol derivatives were isolated with anthraquinones, such as emodin and chrysophanol (Bicker et
Although hot water extracts of *R. acetosa* have been used to treat gastritis or gastric ulcers, no scientific reports exist concerning the use of this plant this manner. Meanwhile, it has been reported that emodin displays anti-ulcerogenic and anti-inflammatory activities, and in our pre-study, the emodin content in 70% ethanol extract (EER) was higher than in water extracts (WER) (Goel et al., 1991). Hence, the present study was undertaken to assess and compare the anti-ulcer activity of EER and WER obtained from *R. acetosa* using an HCl/ethanol-induced gastric ulcer model in mice. The free radical-scavenging and anti-inflammatory activities of these two extracts were also evaluated and compared.

### MATERIALS AND METHODS

#### General

Sucralfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), lipopolysaccharide (LPS), phosphoric acid, N-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, sodium nitrite, butylated hydroxytoluene (BHT), L-ascorbic acid (LAA) and emodin were purchased from the Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from the Merck Co. (Darmstadt, Germany). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS) and the antibiotic mixture (penicillin-streptomycin) were purchased from Hyclone (South Logan, UT, USA). All other chemicals were reagent-grade.

#### Plant material

The whole plant of *Rumex acetosa* L. (Polygonaceae) was collected from the fields of the Sancheong province in May 2011. After cleaning with water, samples were dried at room temperature in the shade. These plant materials were identified by Prof. J. H. Park of College of Pharmacy, Pusan National University, Korea. The voucher specimen (APG-1103) was deposited in the Herbarium of the College of Pharmacy, Gyeongsang National University.

#### Sample preparation and HPLC conditions

The dried plant material was ground into powder with a mill. The finely pulverized samples were weighed (5 g), and 500 ml of water or 70% ethanol solution in water was added, respectively. The mixtures were extracted with a soxhlet extractor for 3 hr at 100°C and 80°C, respectively. The extract was later filtered using filter papers (Whatman No. 40), and centrifuged for 10 min at 4°C, 5,000 rpm. The filtrate was lyophilized, and the extract amount was weighed and suspended in water for the following bioassays. A portion of the filtrate was pre-treated with Sep-Pack catridge (C₁₈, Waters, USA) prior to HPLC analysis.

The Agilent 1100 HPLC system used for analysis (Avondale, CA, USA) consisted of a temperature-controlled autosampler, column oven, and a binary pump. A 10 μl volume of sample solutions, or standard, was directly injected on a YMC J’sphere ODS-H80 column (4 μm, 4.6×150 mm; YMC, Kyoto, Japan) using a gradient acetonitrile-water solvent system. The step gradient elution was as follows: 10% acetonitrile for the first 5 min, 10% to 80% acetonitrile for a further 25 min, and then, 80% to 90% for the next 5 min, followed by maintaining the condition for another 5 min. A conditioning phase (40-45 min) was subsequently used to return the column to its initial state. The flow rate was 1.0 ml/min, and the column temperature was set at 35°C. The eluent was detected at 254 nm using a DAD detector. Chemstation software (Hewlett-Packard, Avondale, CA, USA) was used to operate this HPLC system. Emodin was used as a standard compound.

#### Animals

Male ICR mice (4 weeks old; weighing 24-28 g; Koatech, Korea) were used in the *in vivo* experiments. The animals were housed in an air-conditioned room (24 ± 2°C), under a 12 hr light/dark cycle. Each animal was used for one experiment only. All procedures relating to animal care and treatment conformed to the Animal Care Guidelines of the Animal Experiment Committee of Gyeongsang National University.

#### HCl/ethanol-induced gastric ulcer

The protective activity against gastric ulcer in mice was evaluated according to the method described previously with slight modifications (Ogawa et al., 2011). After 16 hr of fasting, the mice were divided randomly into five groups (5 mice per a group). Sham-operated and vehicle-treated groups were given 0.2 ml/25 g of water. Another group was treated with sucralfate (100 mg/kg, p.o.), that was suspended in 0.25% sodium carboxymethylcellulose solution (Samchun Chemicals, Korea) as a positive control. The other two groups were treated with WER and EER in water (100 mg/kg, p.o.). The administration dosage (100 mg/kg, p.o.) in mice has been decided according to the related published reports (Karimi et al., 2004; Ogawa et al., 2011). The mice, except for those belonging to the sham-operated group, were administered 150 mM HCl/ethanol (0.2 ml/25 g body weight) after 1 hr of sample treatment. The mice were euthanized using carbon dioxide after 1 hr of HCl/ethanol treatment, and their stomachs were removed and fixed with 10 ml of 1% formalin for 30 min. Next, the stomachs were opened, along the greater curvature, and were gently rinsed with saline. The gastric ulcers were evaluated according to the area of gastric lesion with specific image analysis software (Isolation Lite, IMT i-solution Inc., Vancouver, Canada).

#### Histological analysis

Histological evaluation was performed on the glandular stomach of the mice. The tissue samples were preserved in 10% neutral buffered formalin and processed for routine paraffin block preparation. Sections approximately 8 μm in thickness were cut and stained with hematoxylin and eosin. Mucosal injury evaluation was performed under light microscopy, and the histopathological alterations were assessed by comparing lesions.

#### DPPH radical scavenging activity

Based on the method introduced by Gordon et al. (2001), 200 μl of the 0.2 mM DPPH solution in methanol (w/v) was mixed with 800 μl sample solution of different concentrations (2.5, 5.0, 10, 25 and 50 μg/ml) in an Eppendorf tube and the solution was incubated at room temperature for 30 min. After incubation, absorbance of mixtures was measured at 517 nm against the blank, methanol. DPPH radical scavenging activity was calculated with the following formula: % activity = [(Ac-As)/Ac]×100, where Ac and As are absorbance of the control and sample, respectively. BHT and L-ascorbic acid were used as positive controls.