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Protective effect of Bojungikki-tang, a traditional herbal formula, against alcohol-induced gastric injury in rats

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ABSTRACT

Ethnopharmacological evidence: Oxidative stress plays an important role in the pathogenesis of ethanol-induced acute gastric mucosal injury. Bojungikki-tang (Hochuekkito in Japanese, Bu-zhong-yi-qi-tang in Chinese) is a traditional herbal formula used in Korea, Japan, and China to treat allergic diseases and gastrointestinal disorders. However, the mechanism responsible for its actions has not been investigated experimentally.

Aim of the study: The aims of this study were to investigate whether Bojungikki-tang water extract (BJITE) has protective effects against ethanol-induced acute gastric injury in rats and to perform an acute toxicity study to evaluate its safety.

Materials and methods: In this rat model, gastric mucosal injury was imposed by oral administration of 5 mL/kg body weight of absolute ethanol. BJITE at one of two doses (200 or 400 mg/kg body weight) was administered by gavage 2 h before ethanol administration. Gastric tissues were collected and analyzed to assess the gastric injury index, and content or activity of catalase, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), glutathione-*S*-transferase (GST), glutathione reductase (GR), and glutathione peroxidase (GPx).

Results: Acute administration of ethanol significantly increased the gastric injury index concomitantly with an increase in MDA and GSH content, and a decrease in the activities of catalase, GST, GR, GPx, and SOD. Pretreatment with 200 or 400 mg/kg BJITE attenuated ethanol-induced gastric mucosal injury; this was accompanied by an increase in the content or activity of PGE_2, catalase, GSH, GST, GR, GPx, and SOD, and a decrease in MDA content. In the acute toxicity study, no adverse effects of BJITE were observed at doses up to 2000 mg/kg body weight.

Conclusion: These results indicate that BJITE can partly protect the gastric mucosa from ethanol-induced acute gastric injury and suggest that these protective effects might be induced by increasing the antioxidant status. We suggest that BJITE can be developed as an effective drug for the treatment of acute gastric injury.

1. Introduction

Acute gastritis is induced by psychological or physical stress, alcohol, and drugs such as steroidal or nonsteroidal anti-inflammatory drugs. Ingestion of large amounts of ethanol in humans and rodents induces hemorrhagic gastric lesions, at least in part by increasing oxidative stress. These agents increase the production of reactive oxygen species (ROS) and decrease the activity of antioxidant enzymes, leading to acute gastric damage including hemorrhage, congestion, edema, erosion, and ulcers (Laine and Weinstein, 1988; Taylor and Rehm, 2005). Although the mechanism of alcohol-induced gastric injury is unclear, it occurs mainly by the production of ROS, modulation of nitric oxide system, reduction of mucosal blood flow, and depletion of sulfhydryl groups (Rao et al., 2004). Particularly oxidative stress is believed to be mediated by free radicals because ethanol administration depletes cells of major antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. The mechanisms responsible for the toxic and damaging actions of ethanol, although understood incompletely, seem to involve byproducts of oxidative stress (Gazzieri et al., 2007; Kwiecien et al., 2003). Oxidative stress, which refers to a state of elevated levels of reactive oxygen species (ROS), comprises a variety of conditions

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that stimulate either ROS production or a decline in antioxidant defenses. There is evidence that oxidative stress and lipid peroxidation play important roles in the pathogenesis of acute gastric lesions induced by ethanol (Pan et al., 2008). Alcohol intake increases the oxidative stress and then induces increased lipid peroxidation and reductions of antioxidant enzyme activities, resulting in gastric injury. Thus, treatment of gastric injury is focus on improving antioxidant status and reducing lipid peroxidation (Tuorkey and Karolin, 2009; Koyuturk et al., 2004).

Bojungikki-tang (Hochuekkito in Japanese, Bu-zhong-yi-qi-tang in Chinese) comprises eight component herbs; Angelicae Gigantis radix, Astragali radix, Atractylodis rhizome, Bupleuri radix, Cimicifugae rhizome, Citri unshii pericarpium, Ginseng radix alba and Glycyrrhizae radix. This formula has been identified as an effective drug for improving function of the digestive system and for strengthening the defensive defenses against various infections in Asia. Immunomodulatory effects of Bojungikki-tang include decreases in the recurrent attack rate of allergic rhinitis (Xie et al., 2011), decrease in IgE levels in animal models of atopic dermatitis (Kobayashi et al., 2003), antibacterial effect against *Helicobacter pylori* infection in mice (Yan et al., 2002), suppression of chronic contact hypersensitivity (Nakada et al., 2002), suppression of collagen-induced arthritis in mice (Hai et al., 2002), radioprotective effects in mice (Kim et al., 2002), inhibition of proliferation of hepatoma cell lines (Kao et al., 2001), modulation of allergic inflammation in a murine model of asthma (Ishimitsu et al., 2001), antiaging effects in mice (Shih et al., 2000) and suppression of IgE production in mice (Kaneko et al., 1997).

The effects of Bojungikki-tang water extract (BJITE) on acute gastritis have not been examined thoroughly in experimental studies. In the present study, we investigated the effects of BJITE on absolute ethanol-induced acute hemorrhagic gastric mucosal injury in rats. To evaluate the safety of BJITE, an acute toxicity study was conducted according to Organisation for Economics Co-operation and Development (OECD) Testing Guideline TG 423 (OECD, 2001).

2. Materials and methods

2.1. Reagents and materials

Liquiritin and nodakenin were purchased from NPC BioTechnology Inc. (Daejeon, Korea). Hesperidin and glycyrrhizin were purchased from Chengdu Biopurify Phytochemicals Ltd (Chengdu, China) and Wako (Osaka, Japan), respectively. The purity of all reference standards was $\geq$98.0%. High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid was of analytical reagent grade and was procured from Junsei (Tokyo, Japan). The materials forming BJITE were purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). A voucher specimen (2008-KE12-1—KE12-8) has been deposited at the Basic Herbal Medicine Research Group, Korea Institute of Oriental Medicine.

2.2. Preparation of standard solutions and calibration curves

Standard stock solutions of liquiritin, nodakenin, hesperidin, and glycyrrhizin (all at 1000 μg/mL) were prepared in methanol and stored at <4 °C. Standard solutions were determined according to the Korean Pharmacopeia published by Korea Food and Drug Administration. Working standard solutions were prepared by serial dilution of stock solutions with methanol. All calibration curves were obtained by assessing the peak areas for seven standard concentrations in the range of 3.91–250.00 μg/mL for liquiritin, hesperidin, and glycyrrhizin, and 1.95–125.00 μg/mL for nodakenin. The linearity of the peak area (y) versus concentration (x, μg/mL) curve for each component was used to calculate the contents of the main components in BJITE.

2.3. Preparation of sample solutions

A decoction of BJITE was prepared in our laboratory (Table 1) from a mixture of chopped crude herbs, extracted in distilled water at 100 °C for 2 h. The solution was evaporated to dryness and freeze-dried (extract: 890.6 g; yield: 25.4%). Lyophilized BJITE extract (250 mg) was dissolved in distilled water (25 mL) and mixed. The solution was filtered through a SmartPor GHP syringe filter (0.2 μm pore size, Woongki Science, Seoul, Korea).

2.4. Sample analysis

We performed simultaneous analyses using a Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan), comprising a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a Photo diode array detector (PDA) detector. The data processor used LCsolution software (Version 1.24). The analytical column included a SunFire C18 column (250 × 4.6 mm; particle size 5 μm; Waters, Milford, MA, USA) and was maintained at 40 °C. The mobile phases comprised 1.0% (v/v) aqueous acetic acid (A) and 1.0% (v/v) acetic acid in acetonitrile (B). The gradient flow was as follows: 0–40 min, 5%–70% B; 40–55 min, 70%–100% B; 55–60 min, 100% B; 60–65 min, 100%–5% B; 65–80 min, 5% B. The analysis was performed at a flow rate of 1.0 mL/min with PDA detection at 190–400 nm. The injection volume was 10 μL.

2.5. Acute toxicity study

Male and female 5-week-old Sprague Dawley rats were purchased from a specifically pathogen-free facility at Orient Bio Co. (Seoul, Korea) and were used after 1 week of quarantine and acclimatization. The animals were housed in a room maintained at 23 ± 3 °C and a relative humidity of 50% with artificial lighting from 08:00 to 20:00 and with 10 to 20 air changes per hour. The animals were allowed ad libitum access to a commercial pellet diet (PMI Nutrition International, Richmond, IN, USA) and tap water sterilized by UV irradiation and filtration. The acute toxicity study was performed in compliance with the test guidelines from the Korea Food and Drug Administration under the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (OECD, 1997), and the study protocol was approved by the Institutional Animal Care and Use Committee of the Korea Institute of Toxicology (earned by AALAC International).

In a preliminary study, a single oral administration of 2000 mg/kg BJITE did not induce any toxic effects. Based on these

Table 1
Herbal composition of BJITE.

Crude component	Amount (g)	Company of purchase	Source
Astragali radix	5.625	Omniherb	Jeongseon, Korea
Glycyrrhizae radix	3.75	HMAX	China
Ginseng radix alba	3.75	Omniherb	Geumsan, Korea
Atractylodis rhizoma	3.75	HMAX	China
Angelicae Gigantis radix	1.875	Omniherb	Yeongcheon, Korea
Citri unshii pericarpium	1.875	Omniherb	Jeju, Korea
Cimicifugae rhizoma	1.125	HMAX	China
Bupleuri radix	1.125	Omniherb	Hwasun, Korea
Total amount	22.875		

results, a dose of 2000 mg/kg was selected as the limited dose recommended by the OECD test guideline (2001). Ten rats of each sex were assigned randomly to two groups of five rats each, and the rats were given by gavage a single dose in the range of 0–2000 mg/kg. The vehicle control rats received an equivalent volume of distilled water alone. After oral administration of the dose, all abnormal clinical signs were recorded before and after dosing at least twice a day. Body weight was measured immediately before treatment on the day of dosing (day 1) and then on days 2, 4, 8, and 15 after treatment. On the scheduled termination day (day 15), all surviving animals were anesthetized by carbon dioxide and then sacrificed by exsanguinations from the aorta. Complete gross postmortem examinations were performed on all animals.

2.6. Experimental procedure of ethanol-induced acute gastric injury

Specific pathogen-free male Sprague Dawley rats, weighing 200–250 g (aged 5 weeks), were purchased from Orient Co. and used after 1 week of quarantine and acclimatization. The animals were maintained in a room at 23 ± 3 °C with relative humidity of 50% under a controlled alternating 12 h light/dark cycle. The rats were given a standard rodent chow and sterilized tap water. All experimental procedures were performed in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* and National Animal Welfare Law in Korea.

Acute gastric lesions were induced by intragastric administration of absolute ethanol in accordance with a previously described method (Robert et al., 1979; Kazuo et al., 2010). Thirty-five rats were divided into five groups and were fasted for 18 h before the experiment. The control group was given phosphate buffered saline (PBS) orally (5 mL/kg body weight) as the vehicle, and the ethanol group was given absolute ethanol (5 mL/kg body weight) by gavage. The positive control group was given omeprazole orally (50 mg/kg body weight) 2 h before administration of absolute ethanol. Omeprazole possesses anti-inflammatory and antioxidant activities, and is used widely in the treatment of gastritis (Lapenna et al., 1996; Sener-Muratoglu et al., 2001). The fourth and fifth groups received BJITE (200 or 400 mg/kg body weight, respectively) 2 h before absolute ethanol.

The animals were sacrificed with an overdose of 50 mg/kg pentobarbital 2 h after treatment with absolute ethanol. The stomach was removed, opened along the greater curvature, and rinsed gently in PBS. The stomach was stretched on a piece of cork with the mucosal surface facing up and was examined in a standard position to assess the degree of gastric mucosal lesions. The hemorrhagic erosions in the stomach were photographed with a Lecia digital camera. The total and injured gastric lesions were measured using an image analyzer (Leica Microsystems Imaging Solutions Ltd, Cambridge, UK) and are expressed in terms of the percent (%) of the gastric area with lesions. After photographing the gastric lesions, the stomach was stored at −70 °C for later biochemical analysis.

2.7. Biochemical analysis

The stomach was cut into small pieces and homogenized (1/10 w/v) in Tissue Lysis/Extraction reagent with protease inhibitor (Sigma, St Louis, MO, USA). The homogenates were centrifuged at 12,000 rpm for 10 min at 4 °C to remove cell debris, and the supernatant was used for the measurements of the content or activities of malondialdehyde (MDA), reduced glutathione (GSH), catalase, superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST). Total protein concentration was measured using a protein assay reagent (Bio-Rad Laboratories, Inc.).

Lipid peroxidation was estimated by measuring MDA level using a thiobarbituric acid reactive substances assay kit (BioAssay Systems, CA, USA). In brief, 100 μL of homogenate was mixed with 100 μL of 10% trichloroacetic acid and incubated for 15 min on ice. The mixture was centrifuged at 12,000 rpm for 5 min at 4 °C. Two hundred microliters of supernatant was mixed with 200 μL of thiobarbituric acid, and the mixture was incubated at 100 °C for 60 min. After cooling, the absorbance was measured at 535 nm. The results are expressed as nmol of MDA/mg protein.

The GSH content was measured using a GSH assay kit (Cayman Chemical, Ann Arbor, MI, USA) and the results are expressed as μmol/mg protein. The activities of antioxidative enzymes, including catalase, SOD, GR, GPx, and GST, were quantified using a commercial kit (Cayman) according to the manufacturer's protocols. The results are expressed as U/mg protein.

2.8. Measurement of prostaglandin E_2

The production of PGE_2 was determined in homogenate of gastric tissue using immuno-linked immunosorbent assay (ELISA) kit (Cayman), according to the manufacturer's instructions. Absorbance was measured at 450 nm with an ELISA microplate reader (Bio-Rad Laboratories, Ins.).

2.9. Histopathological assessment

The glandular face of the stomach was examined histologically. Tissue samples were preserved in 10% buffered formalin and processed for paraffin block preparation. Sections about 4 μm thick were cut and stained with hematoxylin and eosin. The extent of mucosal injury was evaluated using light microscopy by an experienced histologist blinded to the treatment regimen. The histopathological changes were assessed according to the criteria, as described previously (Laine and Weinstein, 1988), with simple modification. Quantitative analysis of gastric mucosal injury was performed using an image analyzer (Molecular Devices, Inc., CA, USA).

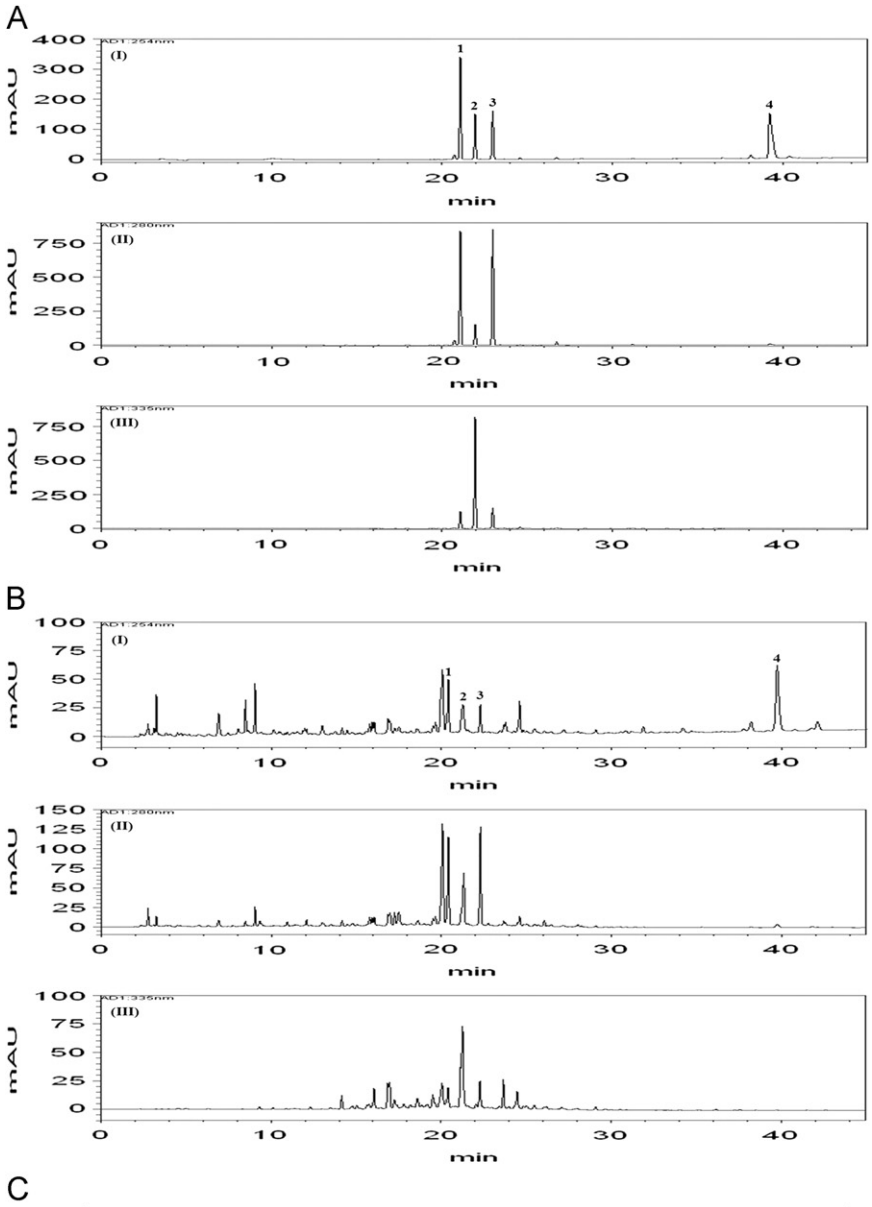
2.10. Statistical analysis

The data are expressed as means ± standard error. The data were analyzed using analysis of variance. If the tests showed a significant difference between groups, the data were analyzed by a multiple comparison procedure using Dunnett's test (Johansen et al., 2005). Statistical analysis was performed using the Path/Tox System (Version 4.2.2). The level of significance was defined as $p < 0.05$ or < 0.01.

3. Results

3.1. HPLC analysis of BJITE

The newly established analytical method was applied to the simultaneous determination of the four components of BJITE using the HPLC-PDA detector. The standard curves for the four components containing liquiritin, nodakenin, hesperidin, and glycyrrhizin were $y = 20{,}513.87x - 7654.64$ ($r^2 = 1.0000$), $y = 37{,}084.83x - 13{,}737.59$ ($r^2 = 0.9999$), $y = 20{,}889.32x - 7846.32$ ($r^2 = 1.0000$), and $y = 9010.06x - 6021.03$ ($r^2 = 0.9999$), respectively. Fig. 1 shows the results of HPLC analysis of BJITE with the detection of eluents at 230–400 nm. The retention times of the components were 21.04 min (liquiritin), 21.91 min (nodakenin), 22.96 min (hesperidin), and 39.81 min (glycyrrhizin). The content of each component was in the range 2.27–4.19 mg/g. We developed a simple and accurate HPLC method for the simultaneous



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Batch	Content (mg/g)											
	Liquiritin			Nodakenin			Hesperidin			Glycyrrhizin		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)
1	3.92	0.04	0.89	2.28	0.09	3.97	4.14	0.04	1.08	3.39	0.02	0.70
2	3.85	0.06	1.54	2.32	0.04	1.71	4.15	0.04	0.96	3.44	0.04	1.27
3	3.85	0.02	0.51	2.27	0.09	4.07	4.19	0.13	3.11	3.45	0.10	2.83

Fig. 1. HPLC analysis of BJITE. (A) chromatogram of a standard mixture, (B) chromatogram of BJITE, (C) the contents of marker compounds in BJITE. 254 nm (I), 280 nm (II) and 335 nm (III). Liquiritin (1), nokaenin (2), hesperidin (3), and glycyrrhizin (4). RSD, relative standard deviation.

separation and determination of four components to evaluate the quality of BJITE. The method will be helpful for improving quality control and fatten analysis of BJITE.

3.2. Acute toxicity of BJITE

We investigated the potential toxic effects of BJITE, the first step in establishing a safe and effective dose to provide gastroprotective activity. BJITE given orally up to 2000 mg/kg did not produce any signs of evident toxicity and there were no treatment-related deaths within 14 day. No BJITE treatment-related clinical signs or abnormal gross pathological findings were observed at dose levels up to 2000 mg/kg body weight (Fig. 2).

3.3. Effects of BJITE on ethanol-induced acute gastric injury

Ethanol-induced gastric mucosal injury was evaluated by gross examination of the gastric mucosa and the injury index. As shown in Fig. 3A and B, ethanol treatment markedly increased severity of injury, and this injury was prevented by pretreatment with BJITE at 200 or 400 mg/kg before administration of ethanol.

3.4. Effects of BJITE on lipid peroxidation and GSH content in ethanol-induced acute gastritis

As shown in Fig. 4, BJITE decreased the gastric GSH content in this model of ethanol-induced gastric injury. Animals treated with 400 mg/kg of BJITE before ethanol administration had a significantly higher GSH content (39.2 ± 6.5 μmol/mg protein) compared with the ethanol-only group (23.9 ± 4.32 μmol/mg of protein). The omeprazole group also had a significantly higher GSH content (31.9 ± 7.11 μmol/mg of protein) than the ethanol group. The content of MDA, an end product of lipid peroxidation, was higher in the ethanol group (175.7 ± 18.15 μmol/mg protein) than in the control group (109.1 ± 17.52 μmol/mg protein) and BJITE-treated groups (200 mg/kg, 121.5 ± 29.19 μmol/mg protein; 400 mg/kg, 119.6 ± 26.67 μM/mg of protein).

3.5. Effects of BJITE on antioxidant enzymes activities

Catalase activity was significantly lower in the ethanol-only group than in the control group. Catalase activity was similar to that in the control rats pretreated with 400 mg/kg (but not 200 mg/kg) of BJITE in (Fig. 5A). GR activity was lower in the ethanol-treated group (71.5 ± 4.44 U/mg of protein, Fig. 5A) than in the control group (86.0 ± 2.73 U/mg protein) and in the group pretreated with the higher dose of BJITE (400 mg/kg, 82.4 ± 6.26 U/mg protein). The activities of GPx, GST and SOD were lower in the ethanol group than in the control group and were higher in the BJITE-pretreated group than in the ethanol-only group (Fig. 5B).

3.6. Effects of BJITE on production of PGE_2

The production of PGE_2 was lower in the ethanol group (8.3 ± 2.38 ng/mg protein) than in the control group (12.4 ± 1.74 ng/mg protein) (Fig. 6). Although Omeprazole (10.8 ± 1.07 ng/mg protein) and 200 mg/kg of BJITE-treated groups (10.7 ± 2.08 ng/mg protein) increased the production of PGE_2 compared with the ethanol group. The 400 mg/kg of BJITE group (12.9 ± 2.06 ng/mg

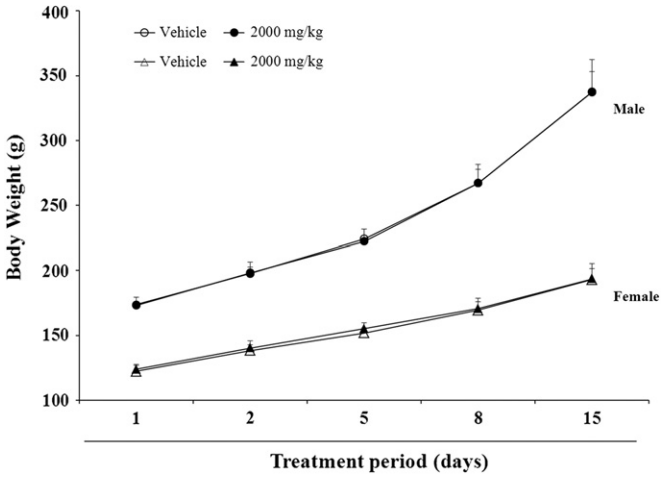

Fig. 2. Body weight changes in animals treated with BJITE at dose levels of 0 (●) and 2000 mg/kg (○) in males and at 0 (△) and 2000 (▲) mg/kg in females. Body weight did not differ significantly between the BJITE-treated and control groups.

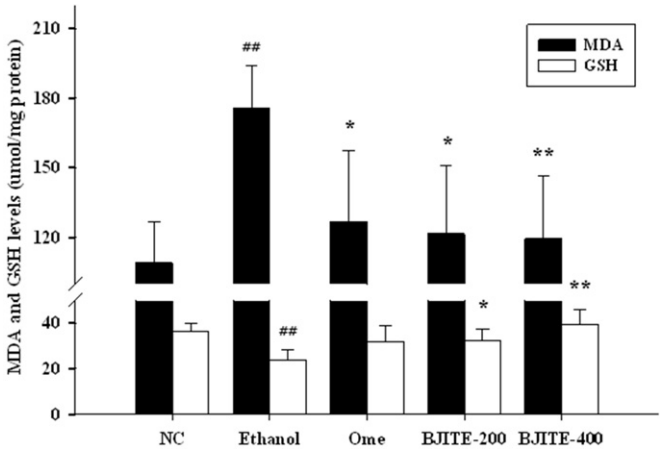

Fig. 4. Effects of BJITE on gastric GSH and MDA contents in absolute ethanol-induced gastric injury in rats. Normal control group (NC); ethanol, absolute ethanol-treated group; BJITE-200, ethanol + BJITE (200 mg/kg); BJITE, ethanol + BJITE (400 mg/kg); Ome, ethanol + omeprazole (50 mg/kg). Each bar represents the mean ± SD of six rats. ##Significant difference at $p < 0.01$ compared with the control group. *Significant difference at $p < 0.05$ and **at $p < 0.01$ compared with the ethanol-only group.

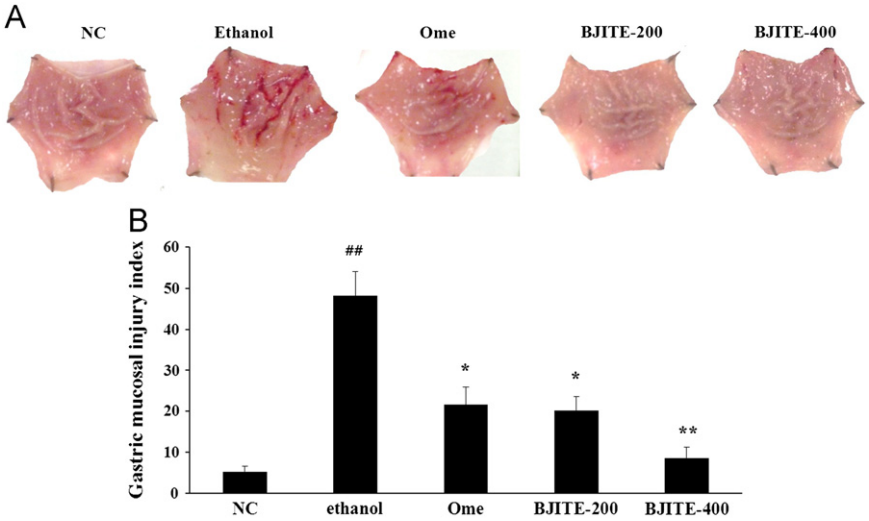

Fig. 3. Effects of BJITE on absolute ethanol-induced gastric mucosal injury. (A) representative photographs of the gastric mucosa (B) gastric mucosal injury index. Normal control group (NC); ethanol, absolute ethanol treatment group; BJITE-200, ethanol + BJITE (200 mg/kg); BJITE-400, ethanol + BJITE (400 mg/kg); Ome, ethanol + omeprazole (50 mg/kg). Absolute ethanol induced hemorrhage and hyperemia in the gastric mucosa, whereas BJITE attenuated the gastric mucosal injury induced by absolute ethanol. ##Significant difference at $p < 0.01$ compared with the control group. *Significant difference at $p < 0.05$ and **at $p < 0.01$ compared with the ethanol-only group.

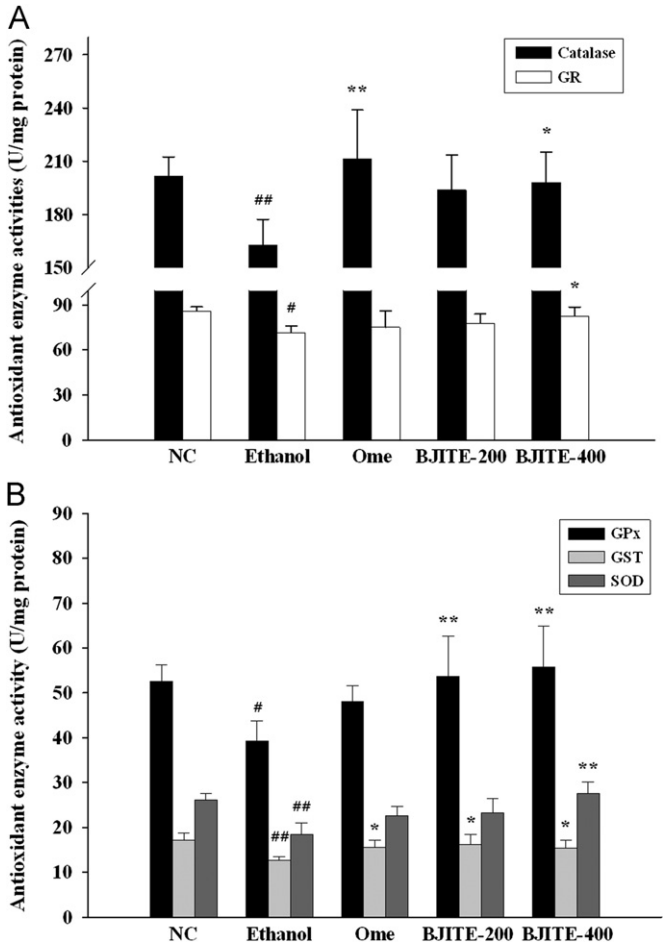

Fig. 5. Effects of BJITE on activities of gastric antioxidant enzymes. (A) catalase and GR activities, (B) GPx, GST and SOD activities. Normal control group (NC); ethanol, absolute ethanol treatment group; BJITE-200, ethanol+BJITE (200 mg/kg); BJITE, ethanol+BJITE (400 mg/kg); Ome, ethanol+omeprazole (50 mg/kg). #Significant difference at $p<0.05$ and ##at $p<0.01$ compared with the control group. *Significant difference at $p<0.05$ and **at $p<0.01$ compared with the ethanol-only group.

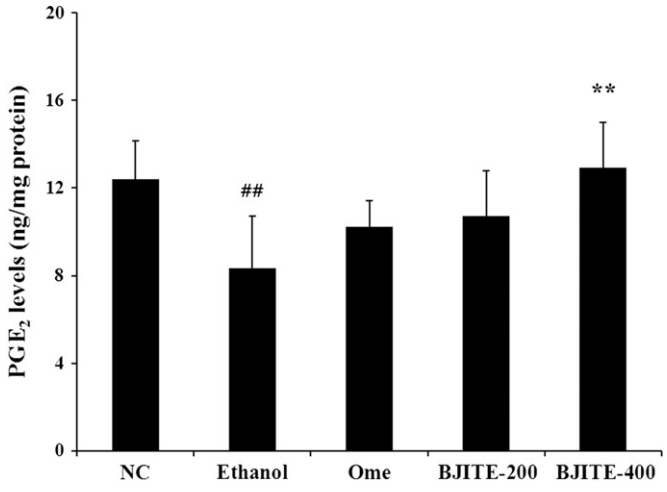

Fig. 6. Effects of BJITE on the production of PGE_2 in absolute ethanol-induced gastric injury in rats. Normal control group (NC); ethanol, absolute ethanol treatment group; BJITE-200, ethanol+BJITE (200 mg/kg); BJITE, ethanol+BJITE (400 mg/kg); Ome, ethanol+omeprazole (50 mg/kg). ##Significant difference at $p<0.01$ compared with the control group. **Significant difference at $p<0.01$ compared with the ethanol-only group.

protein) significantly increased the production of PGE_2 compared with the ethanol group.

3.7. Effects of BJITE on histological results

Administration of ethanol produced severe hemorrhagic injury lesions in the gastric mucosa. In the group given ethanol, the following were observed extensive hemorrhage in the mucosaand hyperemia and inflammatory cell infiltration in the mucosa and submucosa. However, mucosa and submucosa healing were higher in the BJITE-pretreated group than in the ethanol-only group (Fig. 7).

4. Discussion

We investigated the gastroprotective activity of BJITE in a model of ethanol-induced experimental gastric injury. Rats treated with BJITE did not show any signs of acute toxicity up to a dose of 2000 mg/kg. These data suggest that this is a safe dose range for in vivo acute experimental protocols. We observed ethanol-induced gastric lesions, as shown by an increase in the gastric injury index, evidence of lipid peroxidation, and decreased content or activities of antioxidants such as GSH, catalase, GST, SOD, and GPx. By contrast, BJITE-treated animals showed a lower gastric injury index, less lipid peroxidation, and increased antioxidant activities compared with the ethanol-only group.

Ethanol-induced acute gastric lesions are characterized by pathological changes such as hemorrhage, edema, inflammatory infiltration, and loss of epithelial cells (Medeiros et al., 2008; Silva et al., 2009). Even a single episode of heavy drinking can induce mucosal inflammation and hemorrhagic lesions. In the present experiments, a single oral administration of ethanol caused acute gastric bleeding and hemorrhagic lesions in the rat stomach, confirming the detrimental effect of ethanol on the gastric mucosa. BJITE pretreatment at both doses (200 and 400 mg/kg) dose-dependently prevented ethanol-induced gastric mucosal injury.

Alcohol causes severe oxidative stress in gastric tissue, which is manifested as increased lipid peroxidation via increasing MDA content and reducing gastric GSH content. GSH is a well-known antioxidant and is usually present as the most abundant low-molecular mass thiol in most organisms. This study showed that BJITE significantly increased GSH content and reduced MDA content in a rat model of alcohol-induced gastric injury. Our results are consistent with the results of earlier studies that reported an increase in MDA content and decrease in GSH content in rat gastric tissues following exposure to intragastric alcohol (Bilici et al., 2002; Lutnicki et al., 1992). Another study reported a decrease in GSH content and an increase in both SOD activity and lipid peroxidation in animals exposed to ethanol (Rukkumani et al., 2004). The results from our experiments are consistent with ethanol-induced gastric damage described in the literature.

Pathological conditions induced by various chemicals and stress could lead to an increased production of ROS, decreasing the activities of antioxidant enzymes including catalase, GST, GPx, GR, and SOD (Johansen et al., 2005; Valko et al., 2007). Of the antioxidant enzymes, catalase is one of the antioxidant enzymes that control the accumulation of ROS generated through numerous metabolic processes such as the breakdown of ethanol in the stomach (Chelikani et al., 2004). Inhibition of catalase activity leads to lipid formation by increasing the generation of hydroxyl radicals (Das et al., 1997). SOD catalyzes the breakdown of hydrogen peroxide to water and oxygen (Bannister et al., 1987). GSH-bound enzymes in tissues, particularly GPx, GST, and GR have been proposed as potential chemopreventive agents because

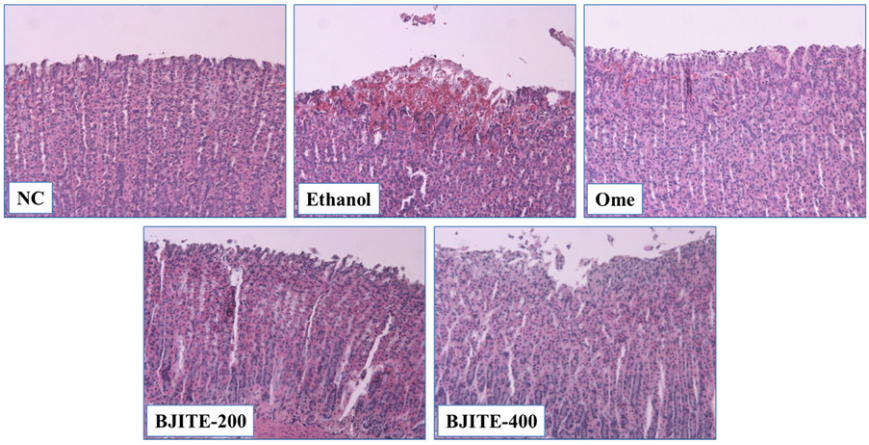

Fig. 7. Effects of BJITE on histopathological lesions in absolute ethanol-induced gastric injury in rats. Histological sections were stained with H&E and photographs were taken at 50x. Ethanol-induced gastric tissue exhibits hemorrhage and loss of epithelial cells in gastric mucosa. In contrast, administration of BJITE reduces ethanol-induced gastric mucosal injury. Normal control group (NC); ethanol, absolute ethanol treatment group; BJITE-200, ethanol + BJITE (200 mg/kg); BJITE, ethanol + BJITE (400 mg/kg); Ome, ethanol + omeprazole (50 mg/kg).

of their antioxidant and detoxification properties (Hayes and Pulford, 1995). GPx reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Muller et al., 2007). GST is a soluble protein located in the cytosol and the endoplasmic reticulum that catalyzes conjugation of GSH with many xenobiotics and their reactive metabolites (Johansen et al., 2005) This activity detoxifies endogenous compounds such as peroxidized lipid and promotes the breakdown of xenobiotics. GR reduces glutathione disulfide (GSSG) to the sulfhydryl form, GSH, which is an important cellular antioxidant (Valko et al., 2007). Several studies have shown that absolute ethanol increases lipid peroxidation and reduces the activities of antioxidant enzymes in gastric tissue (Tuorkey and Karolin, 2009; Koyuturk et al., 2004). In this study showed that pretreatment of BJITE at a dose of 200 or 400 mg/kg increased the activities of antioxidant enzymes in the rat stomach. These results are consistent with the previous studies that evaluated the protective effects of antioxidants against ethanol-induced gastric injury (Koyuturk et al., 2004; Kanter et al., 2005). Thus, our data suggest that BJITE attenuated ethanol-induced acute gastric injury via enhancement of antioxidant status. These protective effects of BJITE are considered to be closely related to pharmacological effects of BJITE and its component herbs. BJITE is composed of 8 different herbs that possess various pharmacological effects such as anti-inflammatory and anti-oxidative effects (Kim and Kim, 2000; Yokozawa et al., 2005; Sugiura et al., 2006; Li et al., 2007; Kim et al., 2009; Wu et al., 2011; You et al., 2011). In particular, Atractylodis rhizome, Bupleuri radix, Glycyrrhizae radix and Ginseng radix alba showed the protective effects against experimentally induced gastric injury (Sakurai et al., 1994; Goso et al., 1996; Matsumoto et al., 2002; Yeo et al., 2008). In addition, PGE_2 is considered major protective agents in the gastric tissue (Nassini et al., 2010). PGE_2 has been reported to protect gastric mucosal cells from ethanol-induced damage in a concentration-dependent manner (Nagy et al., 2000). In present study, administration of BJITE increased the production of PGE_2 in gastric mucosa compared with the ethanol only group. Thus, these results strongly supported the protective effects of BJITE against ethanol-induced gastric injury.

In conclusion, our data show that BJITE has gastroprotective effects and antioxidant properties. Although the exact mechanism underlying these actions is unclear, the effects on acute gastric lesions suggest a multifactorial mechanism probably involving the antioxidant properties of BJITE. BJITE may be a new alternative for the clinical management of gastric ulcer diseases and as an antioxidant against oxidative stress.

Conflicts of interest

No competing financial interests exist.

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