Ameliorating Effects of *Banhasasim-tang* Extrat on the HCl/EtOH-induced Gastric Mucosa Damages in Mice

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The object of this study was to observe the effect of *Banhasasim-tang* (BHSST) on the HCl/EtOH-induced gastric ulcer in mice. Three different dosages of BHSST extract (400, 200 and 100 ㎎/㎏) were once orally administered 1 hr before HCl/EtOH mixture treatment. One hour after HCl/EtOH mixture single oral treatment, the changes on the gross hemorrhagic lesion scores, fundic histopathology, gastric nitrate/nitrite contents, lipid peroxidation and antioxidant defense system (catalase and superoxide dismutase (SOD) activities) were observed, and compared with that of ranitidine 100 ㎎/㎏. As results of all three different dosages of BHSST extract treatment in the HCl/EtOH-induced gastric ulcer mice, significant and dose-dependent decreased gastric damages. BHSST extracts also increased gastric nitrate/nitrite contents and strengthened the antioxidant defense systems, and increased the activities of catalase and SOD, respectively. BHSST extracts 200 ㎎/㎏ showed similar anti-ulcerative effect as compared with ranitidine 100 ㎎/㎏, in this experiment. BHSST has favorable protective effects against to the HCl/EtOH-induced gastric damages, through the strengthening of the body antioxidant defense systems. These gastroprotective effects of BHSST against HCl/EtOH-induced gastric ulcer considered as results of complicated synergic effects of their 8 kinds of herbal components, but exact synergic or individual herbal effects are difficult to discuss in this study. Therefore, more detail synergic effects between 8 kinds of individual herbal component of BHSST should be tested with screening of active anti-inflammatory chemical ingredients, in future.

keywords : *Banhasasim-tang* Extrat, Gastric Ulcer, Antioxidant effect, Ranitidine

Introduction

Gastric ulcer is an illness that affects a considerable number of people worldwide[1]. The pathogenesis of gastroduodenal ulcers is influenced by various aggressive and defensive factors, and gastric mucosal blood flow is an important factor regulating the gastric function[2], and mucosal damage can be easily produced by the generation of exogenous and endogenous reactive oxygen species (ROS) and free radicals[3]. Disorders or decreases of gastric mucosa antioxidant defense systems have been involved in the pathogenesis and progression of various gastric ulcers[4]. Apparently, the free radicals scavenging property of drugs might be contributing in protecting the oxidative damage to gastric mucosa that accelerates healing of gastric ulcers[1]. It is widely accepted that some free radical scavengers show a protective effect against the mucosal damage induced by free radicals[5].

EtOH-induced gastric ulcer models are commonly used to study both the pathogenesis of and therapy for human ulcerative disease[6]. In addition, to evoke severe and rapid gastric damages induced by EtOH, hydrochloric acid (HCl), which also involved in progression and pathogenesis of gastric damages, has been additionally treated[1], therefore, HCl/EtOH mixtures have been used as a valuable and simple animal models to study both the pathogenesis of and therapy for human ulcerative disease used in mouse gastric ulcer models, especially on natural products based on their potent antioxidant effects[1].

Banhasasim-tang (BHSST; Hange-shashin-to in Japanese Kampo Medicine; Banxia-xiexin-tang in Traditional Chinese Medicine) is one of the herbal formulas described in “Treatise on Cold Damage and Miscellaneous Diseases (Shan-han-za-bing-lin)”[7], a Chinese authoritative monograph. In traditional Korean medicine, this formula has been applied for treating the

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symptom “gastric stuffiness”, which is similar to dyspepsia and "burp, vomit, diarrhea, et al."[7] Recently, several studies have elucidated the gastric function and related mechanisms of BHSST[7-13]. Moreover, BHSST can be obtained as an over-the-counter herbal formula in Korea or prescribed for dyspeptic symptoms by the Traditional Korean Medicine doctors, and the safety and effectiveness of BHSST granules in patient suffering from functional dyspepsia are also reported through randomized, double-blind, placebo-controlled clinical trial[7], but the antioxidant effects on HCl/EtOH-induced gastric ulcer in mice did not researched, upon our knowledge.

To this end, we have evaluated the healing effect of BHSST on the HCl/EtOH-induced gastric ulcer in mice. Three different dosages of BHSST extract (400, 200 and 100 ㎎/㎏) were once orally administered. One hour after single oral treatment of HCl/EtOH mixture was conducted and the changes on the gross hemorrhagic lesion scores (mm^2/gastricmucosa), fundic histopathology, gastric nitrate/nitrite contents, lipid peroxidation and antioxidant defense system (catalase and superoxide dismutase (SOD) activities) were observed, and compare the activity with that of the synthetic anti-ulcer drug, a representative histamine H2 receptor agonist, ranitidine 100 ㎎/㎏[14].

Materials and Methods

1. Test materials: BHSST and ranitidine

Light brown granules of BHSST (Hanzung Pharm. Co. Daejeon, Korea), produced according to Korean Good Manufacturing Practice (GMP) and permitted and regulated by the Korean Food & Drug Administration (KFDA; Seoul, Korea) were used in this experiment, and ranitidine (Sigma-Aldrich, St. Louise, MO, USA) was used as a reference drug as listed follows. Individual compositions of 8 kinds of herbs in BHSST were listed in Table 1.

2. Animals and husbandry

A total of 55, CrljOri:CD-1 (ICR), specific pathogen-free male mice (6-wk old upon receipt; OrientBio, Seungnam, Korea; Body weight ranged in 28.0~31.0 g upon receipt) were used after acclimatization for 7 days. Animals were allocated four per polycarbonate cage in a temperature (20-25℃)-and humidity (50-55%)-controlled room. Light : dark cycle was 12 hr : 12 hr and feed (Samyang, Seoul, Korea) and water were supplied free to access. Forty-seven mice were used as HCl/EtOH-induced gastric ulcer groups and 8 mice were used as sterilized distilled water treated intact control, instead of HCl/EtOH mixtures, in this study. All animals were fasted for 24 hrs before HCl/EtOH or test material administration, and they were treated according to the national regulations of the usage and welfare of laboratory animals, and approved by the Institutional Animal Care and Use Committee in Daegu Haany University (Gyeongsan, Gyeongbuk, Korea) [Approval No DHU2013-017]. Six groups, total 48 mice were selected based on the body weights (mean 35.01±1.46 g, ranged in 32.3-38.5 g at 10 days after acclimatization, and used in this experiment as follows(Table 2).

Table 1. Composition of BHSST Used in This Study

Herbs	Scientific Names	Amounts (g)
Pinelliae Rhizoma	*Pinellia ternata* (Thunb.) Breitenb.	1.34
Scutellariae Radix	*Scutellaria baicalensis* Georgi	1.80
Ginseng Radix Alba	*Panax ginseng* C.A.Meyer.	0.96
Glycyrrhizae Radix	*Glycyrrhiza uralensis* Fisch	1.08
Zingiberis Rhizoma Siccus	*Zingiber officinale* Roscoe	0.59
Coptidis Rhizoma	*Coptis japonica* (Thunb.) Makino	0.24
Zingiberis Rhizoma	*Zingiber officinale* Roscoe	0.26
Zizyphi Fructus	*Zizyphus jujuba* Miller var. *inermis* Rehder	1.67
Total	8 types	7.94

BHSST = *Banhasasim-tang* extracts, which were purchase from Hanzung Pharm. Co. (Daejeon, Korea)

Table 2. Experimental Design Used in This Study

Groups		Test article/Dose (㎎/㎏/day)
Control	Intact	Sterilized distilled water administered mice
	HCl/EtOH	Sterilized distilled water and HCl/EtOH mixture treated mice
Reference	Ranitidine	Ranitidine 100 ㎎/㎏ and HCl/EtOH mixture treated mice
BHSST	400	BHSST 400 ㎎/㎏ and HCl/EtOH mixture treated mice
	200	BHSST 200 ㎎/㎏ and HCl/EtOH mixture treated mice
	100	BHSST 100 ㎎/㎏ and HCl/EtOH mixture treated mice

Gastric ulcer was induced by single intragastric administration of HCl/EtOH mixtures (98% EtOH contains 150mM HCl) 5 ㎖/㎏, after 24 hrs fasting. All test materials were once orally administered in a volume of 10 ㎖/㎏ of sterilized distilled water as vehicle at 1 hr before HCl/EtOH mixture treatment. Eight mice per group, total 6 groups were used this study.

3. BHSST and ranitidine treatment

After subdivided into aforementioned six groups - eight mice/group, BHSST extracts were once orally administered at 1 hr before HCl/EtOH mixture treatment in a volume of 10 ㎖/㎏ dissolved in sterilized distilled water at dose levels of 400, 200 or 100 ㎎/㎏ by gastric

gavages using a stainless Zonde attached to 1 ㎖ -syringe, respectively. Ranitidine was also single orally administered at a dose level of 100 ㎎/㎏, dissolved in sterilized distilled water in a volume of 10 ㎖/㎏ in this experiment. In intact and HCl/EtOH control mice, only sterilized distilled water was administered, once orally, instead of BHSST or ranitidine (Table 2).

The dosage of ranitidine was selected based on the previous efficacy test[14], in which 100 ㎎/㎏ of ranitidine showed enough protective effects against acute experimental gastric ulcer, and the lowest dosage of BHSST extracts was selected as 100 ㎎/㎏ based on the previous brief efficacy test on the cisplatin-induced gastric dysmotility[8] in which, 93 ㎎/㎏ of BHSST effectively improve gastric empty on the various chemical-induced delayed gastric empty in mice including cisplatin. In addition, 400 and 200 ㎎/㎏ were also selected as higher and middle dosages of BHSST extracts in this experiment using common ratio 2, respectively.

4. HCl/EtOH-induced gastric ulcer

One hr after administration of vehicle, three different dosages of BHSST extracts or ranitidine 100 ㎎/㎏ on 24 hrs fasted mice, HCl/EtOH mixtures (98% EtOH contains 150 mM HCl) was single orally administered in a volume of 5 ㎖/㎏ according to previous report[1]. In intact control mice, only sterilized distilled water was once treated by gastric gavages instead of HCl/EtOH mixtures, in the present study.

5. Quantification of gross lesions

The animals were sacrificed at 6 hrs after HCl/EtOH or vehicle, sterilized distilled water treatment by cervical dislocation, the abdomen was opened with a median incision, and then stomachs were excised. Excised stomach was opened out along with greater curvature and fixed in 10% neutral buffered formalin for 24 hrs and acquired digital images. Ulcer areas on the stomachs' surface were examined macroscopically and measured by computer based automated image analysis process (iSolution FL ver 9.1, IMT i-solution Inc., Quebec, Canada) according to the methods of Morais et al[15] and Oyagi et al.[1] with some modifications. Any macroscopically visible lesions were measured to calculate the gastric damage score. For this purpose, the total areas of the ulcerous stomach regions were calculated as ㎟(Fig. 1).

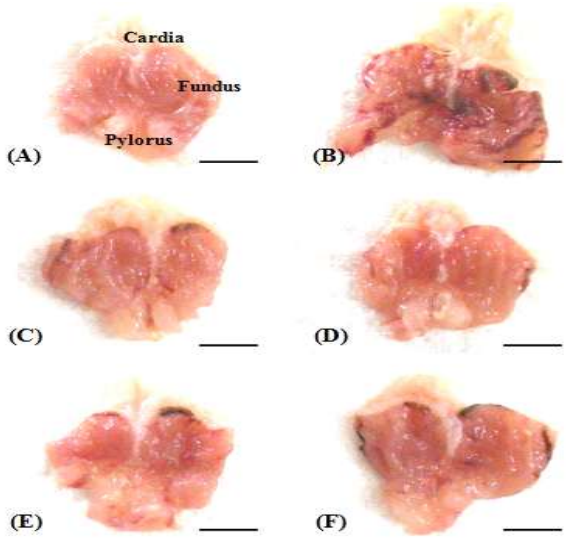

Fig. 1. Representative Gross Images, Taken from Intact or HCl/EtOH-treated Mice. Note that focal hemorrhagic ulcerative lesions were dispersed throughout whole gastric mucosa in all HCl/EtOH treated mice, but slight negligible restricted ulcerative lesions were grossly observed in vehicle treated intact control mice. However, noticeable and marked inhibitions of the gross gastric damages were observed in ranitidine and all three different dosages of BHSST, dose-dependently. A = Intact control mice, B = HCl/EtOH control mice, C = Ranitidine treated mice, D = BHSST 400 ㎎/㎏ treated mice, E = BHSST 200 ㎎/㎏ treated mice, F = BHSST 100 ㎎/㎏ treated mice, Scale bars = 5 mm

6. Determination of lipid peroxidation or malondialdehyde (MDA) formation

The concentrations of gastric mucosal lipid peroxidation were determined by estimating malondialdehyde using the thiobarbituric acid test[16]. The mouse stomachs were promptly excised and rinsed with cold saline. To minimize the possibility of hemoglobin's interference with free radicals, any blood adhering to the mucosa was carefully removed. The corpus mucosa was scraped, weighed, and homogenized in 10 ㎖ of 100 g/ℓ KCl (Sigma-Aldrich, St. Louise, MO, USA). The homogenate (0.5 ㎖) was added to a solution containing 0.2 ㎖ of 80 g/ℓ sodium lauryl sulfate (Sigma-Aldrich, St. Louise, MO, USA), 1.5 ㎖ of 200 g/ℓ acetic acid (Merck, West Point, PA, USA), 1.5 ㎖ of 8 g/ℓ 2-thiobarbiturate (Sigma-Aldrich, St. Louise, MO, USA), and 0.3 ㎖ of distilled water. This mixture was heated at 98℃ for 1 hr and after it had cooled, 5 ㎖ of n-butanol:pyridine (15:1) (Sigma-Aldrich, St. Louise, MO, USA) was added. The mixture was vortexed for 1min and centrifuged for 30 min at 4000 rpm. The supernatant's absorbance was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, St. Louise, MO, USA). The recovery was over 90%. The results were

expressed as nM MDA per gram of wet tissue (nM/g tissue).

7. Tissue catalase activity

Catalase was determined according to the method of Evans and Diplock[17]. Homogenate of mouse gastric mucosa was diluted with buffer, as described before, in order to obtain an adequate dilution of the enzyme. Then, 2 ㎖ of the enzyme dilution were added to the cuvette and mixed with 1 ㎖ of 30 mM H_2O_2, measuring the absorbance at 240 nm for 100 sec. Initial absorbance of the reaction mixture must be around 0.5. The enzyme activity is expressed as the first order constant that describes the decomposition of H_2O_2 at room temperature, mM/min/㎎ tissue.

8. Tissue SOD activity

Gastric SOD activity was determined by the modified version from the method of Minami and Yoshikawa[18]. Briefly, 15 ㎕ of gastric homogenate were mixed with 450 ㎕ of cold deionized water, 125 ㎕ of chloroform, and 250 ㎕ of ethanol. The mixture was then, centrifuged at 8000 g for 2 minutes at 4℃. 500 ㎕ of the extracts were added to the reaction mixture containing 500 ㎕ of 72.4 mM triscacodylate buffer with 3.5 mM diethylene pentaacetic acid (pH 8.2; Sigma-Aldrich, St. Louise, MO, USA), 100 ㎕ of 16% Triton X-100, and 250 ㎕ of 0.9 mM nitroblue tetrazolium (Sigma-Aldrich, St. Louise, MO, USA). The reaction mixture was incubated for 5 minutes at 37℃ before adding 10 ㎕ of 9 mM of pyrogallol (Sigma-Aldrich, St. Louise, MO, USA) dissolved in 10 mM HCl. Then, it was incubated for exactly 5 minutes at 37℃. The reaction was stopped with the addition of 300 ㎕ of 2 M formic buffer (pH 3.5) containing 16% Triton X-100 (Sigma-Aldrich, St. Louise, MO, USA). The absorbance was measured at 540 nm in a spectrophotometer. One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitroblue tetrazolium by 50% (mM/min/㎎ tissue).

9. Gastric nitrate/nitrite contents

Gastric nitric oxide levels were measured as total nitrate/nitrite levels with the use of the Griess reagent[19]. The stomach was homogenized in 50 mM potassium phosphate buffer (pH 7.8; Sigma-Aldrich, St. Louise, MO, USA) and centrifugated at 11,000×g for 15 min at 4℃. 100 ㎕ of the supernatant was mixed with 100 ㎕ Griess reagent (0.1% N-(1-naphthyl) ethylenediamide dihydrochloride, 1% sulfanilamide in 5% phosphoric acid; all obtained from Sigma-Aldrich, St. Louise, MO, USA) and after 10 min the absorbance was measured at 540 nm. The standard curve was obtained by using sodium nitrite. The results were expressed as ㎛M nitrate/nitrite per g of protein. The protein concentration of the sample was determined by the Bradford assay[20].

10. Histopathology

Approximated regions of individual stomach (between cardiac and pylorus, the fundus) were sampled. Then they were crossly trimmed based on the lumen. All trimmed fundus were fixed in 10% neutral buffered formalin for 24 hrs, at least. After paraffin embedding, 3-4 ㎛ sections were prepared. Representative sections were stained with hematoxylin and eosin (H&E) for light microscopically examination. After that the histological profiles of individual cross trimmed fundus tissues were observed. To more detail changes, the total thicknesses of fundic mucosa, from luminal mucosal surface to muscularis mucosa on the periulcerative regions of the crossly trimmed histological specimens, were measured using computer based automated image analysis process as described by Ku et al.[35]. In addition, lesion invasive percentages in fundus (%) were also calculated as follow Equation [1] according to the method of Ku et al.[21], and semiquantative scoring as divided into four degrees; 0 = normal intact mucosa, 1 = slight surface erosive damages, 2 = moderate muocsa damages and 4 = severe total mucosa damages, based on general and histomorphometrical analysis, aforementioned in this experiment.

EQUATION [1].
Invasive Percentages of Lesions (%)
= (Length of lesions on the crossly trimmed fundic walls / total thickness of crossly trimmed fundic walls) × 100

The histopathologist was blindes to group distribution when this analysis was made.

11. Statistical Analyses

Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the obtain data were analyzed by one way ANOVA test followed by least-significant differences (LSD)

multi-comparison test to determine which pairs of group comparison were significantly different. In case of significant deviations from variance homogeneity were observed at Levene test, a non-parametric comparison test, Kruskal-Wallis H test was conducted. When a significant difference is observed in the Kruskal-Wallis H test, the Mann-Whitney U (MW) test was conducted to determine the specific pairs of group comparison, which are significantly different. Statistical analyses were conducted using SPSS for Windows (Release 14.0K, IBM SPSS Inc., Armonk, NY, USA). In addition, the percent changes between intact and HCl/EtOH control were calculated to observe the severities of gastric mucosa damages including ulcerative lesions induced in this study, and the percent changes as compared with HCl/EtOH control and BHSST or ranitidine treated mice were also calculated to help the understanding of the efficacy of test substances as follow Equation [2] and [3], respectively.

EQUATION [2].
Percentage Changes as Compared with Intact Control (%)
= [(((Data of HCl/EtOH control – Data of intact control mice)/Data of intact control mice) × 100]

EQUATION [3].
Percentage Changes as Compared with HCl/EtOH Control (%)
= [(((Data of test substance treated mice – Data of HCl/EtOH control mice)/Data of HCl/EtOH control mice) × 100]

Results

1. Changes on the gastric mucosa gross lesions

Focal hemorrhagic ulcerative lesions were dispersed throughout whole gastric mucosa in all HCl/EtOH treated mice, but slight negligible restricted ulcerative lesions were grossly observed in vehicle treated intact control mice. However, noticeable and marked inhibitions of the gross gastric damages were observed in ranitidine and all three different dosages of BHSST, dose-dependently. Accordingly, significant ($p<0.01$) increases of gastric mucosa gross lesion areas were detected in HCl/EtOH control as compared with intact control mice, but they were significantly ($p<0.01$) and dose-dependently decreased by treatment of BHSST extracts and also by ranitidine 100 ㎎/㎏ as compared with HCl/EtOH control mice, respectively(Fig. 2).

2. Effects on the lipid peroxidation

Significant ($p<0.01$) increases of gastric lipid peroxidation, the increases of MDA contents were detected in HCl/EtOH control mice as compared with intact control mice. However, significant ($p<0.01$) and dose-dependent decreases of MDA contents were demonstrated in all three different dosages of BHSST treated mice, respectively. In addition, gastric lipid peroxidation induced by HCl/EtOH were also significantly ($p<0.01$) inhibited after single oral administration of ranitidine 100 ㎎/㎏, in this experiment (Fig. 3).

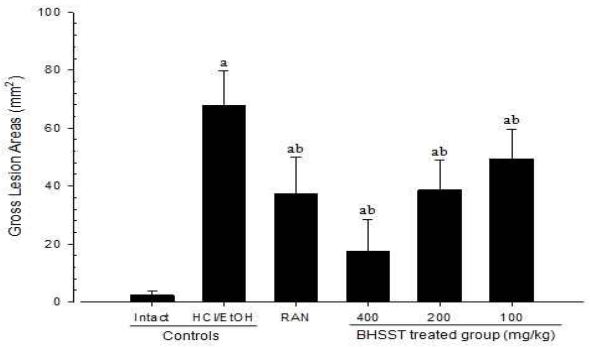

Fig. 2. Changes on the Gastric Mucosa Gross Lesion Areas in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ as compared with intact control by LSD test. b $p<0.01$ as compared with HCl/EtOH control by LSD test. RAN = Ranitidine.

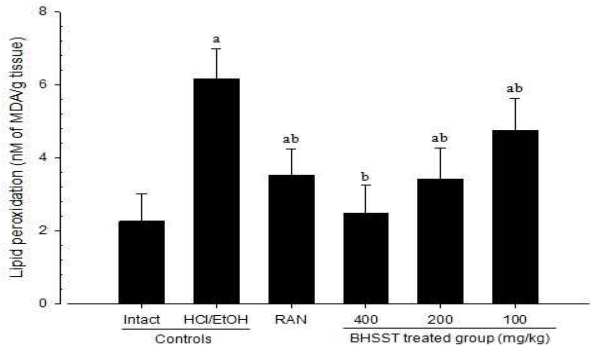

Fig. 3. Changes on the Gastric Lipid Peroxidation in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ as compared with intact control by LSD test. b $p<0.01$ as compared with HCl/EtOH control by LSD test. RAN = Ranitidine.

3. Changes on the catalase activities

Significant ($p<0.01$) decreases of gastric catalase activities were demonstrated in HCl/EtOH control mice as compared with intact control mice, but these decreases of catalase activities induced by treatment of HCl/EtOH were significantly ($p<0.01$) inhibited after single oral administration of ranitidine, and also dose-dependently after single oral administration of BHSST 400 and 200 ㎎/㎏, respectively. BHSST 100 ㎎/㎏ treated mice also showed marked but non-significant elevated gastric catalase activities as compared with HCl/EtOH mice in this experiment(Fig. 4).

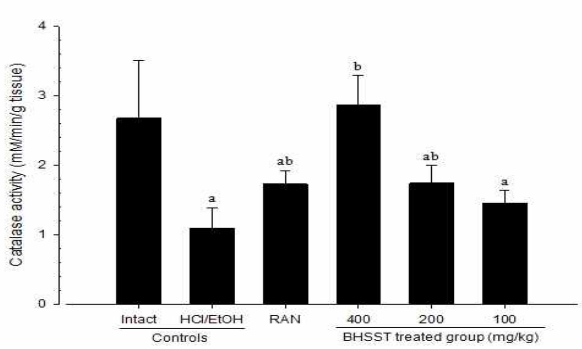

Fig. 4. Changes on the Gastric Catalase Activities in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ as compared with intact control by LSD test. b $p<0.01$ as compared with HCl/EtOH control by LSD test. RAN = Ranitidine.

4. Effects on the SOD activities

Significant ($p<0.01$) decreases of gastric SOD activities were detected in HCl/EtOH control mice as compared with intact control mice. However, significant ($p<0.01$ or $p<0.05$) and dose-dependent increases of SOD activities were demonstrated in all three different dosages of BHSST treated mice, respectively. In addition, gastric SOD activities induced by HCl/EtOH were also significantly ($p<0.01$) inhibited after single oral administration of ranitidine 100 ㎎/㎏, in this experiment(Fig. 5).

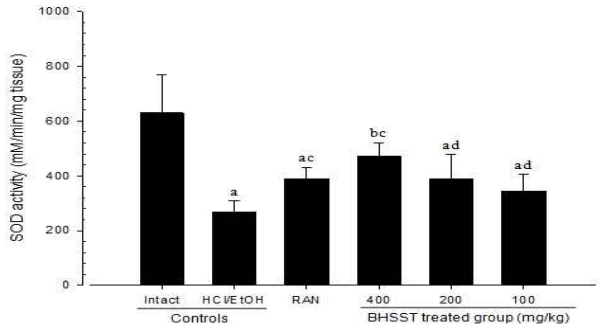

Fig. 5. Changes on the Gastric SOD Activities in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ and b$p<0.05$ as compared with intact control by MW test. c $p<0.01$ and d$p<0.05$ as compared with HCl/EtOH control by MW test. RAN = Ranitidine.

5. Effects on the gastric nitrate/nitrite levels

Significant ($p<0.01$) decreases of gastric nitrate/nitrite contents were observed in HCl/EtOH control mice as compared with intact control mice, but they were significant ($p<0.01$ or $p<0.05$) and dose-dependent normalized by treatment of all three different dosages of BHSST 400, 200 and 100 ㎎/㎏, respectively. In addition, ranitidine 100 ㎎/㎏ also favorably and significantly ($p<0.01$) inhibited the HCl/EtOH-induced depletion of gastric nitrate/nitrite contents as compared with HCl/EtOH control mice, in this experiment(Fig. 6).

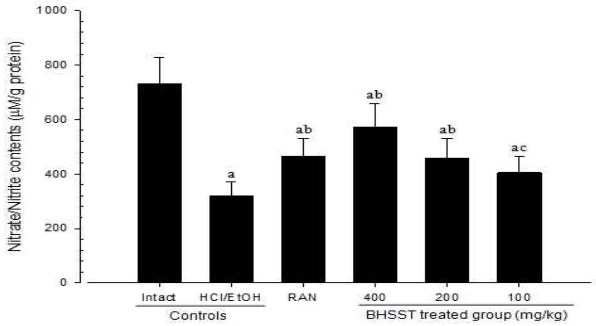

Fig. 6. Changes on the Gastric Nitrate/Nitrite Levels in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ as compared with intact control by LSD test. b $p<0.01$ and c$p<0.05$ as compared with HCl/EtOH control by LSD test.

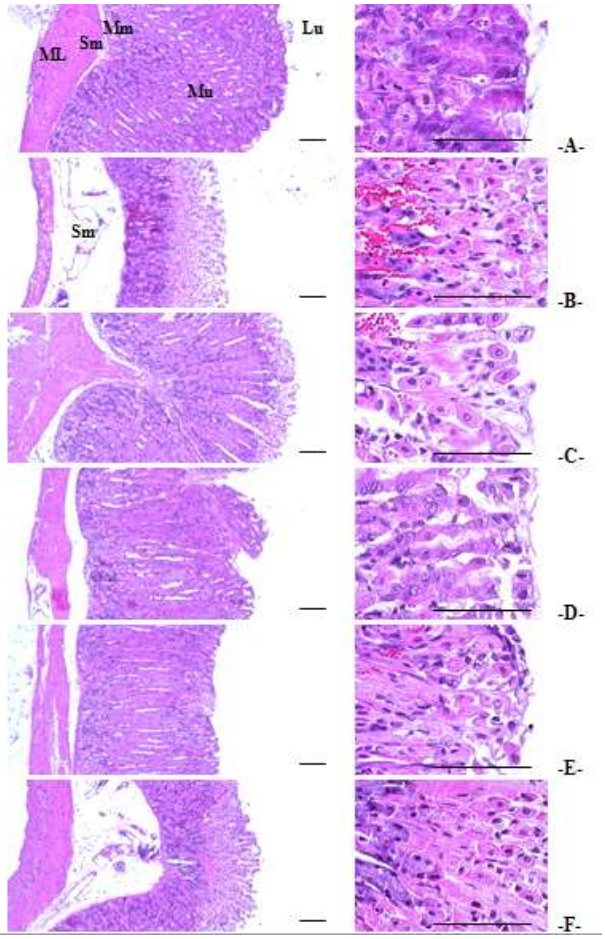

Fig. 7. Representative Histological Images of Fundus, Taken from Intact or HCl/EtOH-treated Mice. A = Intact control mice, B = HCl/EtOH control mice, C = Ranitidine treated mice, D = BHSST 400 ㎎/㎏ treated mice, E = BHSST 200 ㎎/㎏ treated mice, F = BHSST 100 ㎎/㎏ treated mice, Lu = lumen; Mu = mucosa; Mm = muscularis mucosa; Sm = submucosa; ML = muscle layer, All H&E stain, Scale bars = 160 μm

6. Changes on the gastric mucosa histopathology

Severe focal extensive superficial epithelial damage, desquamation of focal epithelium, congestion/hemorrhages, inflammatory cell infiltrations and necrosis of gastric glands, the ulcerative lesions were detected on the fundus after treatment of HCl/EtOH mixtures. However, these microscopic ulcerative lesions were markedly inhibited by pre-treatment of ranitidine 100 ㎎/㎏, and also dose-dependently by treatment of all three different dosages of BHSST, respectively(Fig. 7).

At the semiquantative analysis, significant ($p<0.01$) increases of semiquantative histological scores were observed in HCl/EtOH control as compared with intact control mice, but they were significantly ($p<0.01$ or $p<0.05$) normalized by treatment of BHSST 400, 200 and 100 ㎎/㎏, respectively. In addition, significant decreases of semiquantative histological scores were noticed in ranitidine 100 ㎎/㎏ treated mice as compared with HCl/EtOH control mice in the present study (Fig. 8).

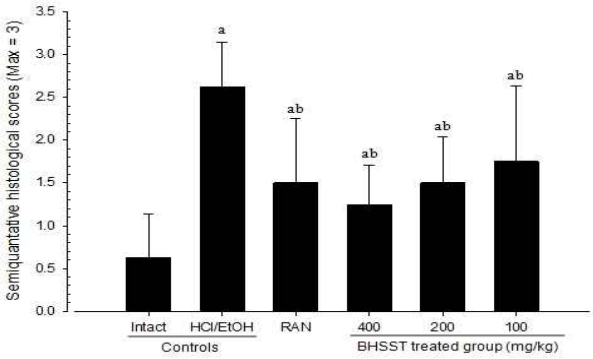

Fig. 8. Changes on the Gastric Semiquantative Histological Scores in HCl/EtOH-treated Mice. Values are expressed as mean ± S.D. of eight mice. a $p<0.01$ as compared with intact control by LSD test. b $p<0.01$ as compared with HCl/EtOH control by LSD test

Discussion

Excessive EtOH intakes leads to erosion, ulcerative lesions, and petechial bleeding in the mucosa of the stomach, rapidly absorbed through gastric mucosa and also generate harmful ROS, superoxide anion and hydroperoxy free radicals, involved in pathogenesis of gastric mucosa damages after metabolized in body[1]. Gastric mucosa damage can be easily produced by the generation of exogenous and endogenous ROS and free radicals[3], and disorders or decreases of gastric mucosa antioxidant defense systems have been involved in the pathogenesis and progression of gastric ulcers[4]. EtOH-induced gastric ulcer models are commonly used to study both the pathogenesis of and therapy for human ulcerative disease[6] and also in this experiment.

Various synthetic anti-ulcer drugs are presently available and some of these like ranitidine, a representative histamine H2 receptor antagonist used as a reference drug in this study are specifically used to cure gastric ulcer. However, each of these drugs confers simpler to severe side effects[22], warranting a search for non-toxic and inexpensive antiulcer medication[23]. Investigation on dietary plants that are also valued in the traditional systems of medicine might provide efficient formulation for prolonged use[5]. Especially, the antioxidants are advocated to offer effective protection against induction and progression of gastric ulcer[24]. The reported medicinal attributes and antioxidant property of the chosen plants triggered us to assess their possible protective effects against HCl/EtOH-induced gastric lesions in mice[1].

Ranitidine is a representative histamine H2 receptor antagonists that inhibits stomach acid production[14]. It is commonly used in treatment of peptic ulcer disease and gastroesophageal reflux disease. In this experiment, ranitidine 100 ㎎/㎏ was selected as a positive reference drug to compare the anti-ulcerative effects based on the previous reports[14].

BHSST has been applied for treating the symptom of gastric stuffiness, which is similar to dyspepsia and burp, vomit, diarrhea, et al[7]. Recently, several studies have elucidated the gastric function and related mechanisms of BHSST[10-16], and according to Jang's research[12] BHSST has favorable inhibitory effects for early reflus esophagitis. Li et al.[25] researched about the effects of BHSST on stress-induced gastric ulcers by measuring ulcer lesions and mucin contents. According to wang et al.[26] BHSST increased epidermic growth factors(EGF) on Helicobacter pylori-induced chronic gastric ulcer, and Ziang Wei et al[27] also reported that BHSST improved SOD activities on Helicobacter pylori-induced chronic gastric ulcer. Yoshio et al.[28] presented that BHSST has the effects to suppress ethanol-induced gastric lesions, but the antioxidant effects on HCl/EtOH-induced gastric ulcer in mice of BHSST is not produced yet. Although antioxidant effects of BHSST itself did not reported until now, favorable antioxidant effects of individual 8 types of herbal components, Pinelliae Rhizoma[29], Scutellariae Radix[30], Ginseng Radix Alba[31], Glycyrrhizae Radix[32], Zingiberis Rhizoma Siccus[33], Coptidis Rhizoma[34], Zingiberis

Rhizoma[35] and Zizyphi Fructus[36], have been well-documented, respectively. In the present study, we intended to observe the possible favorable preventive anti-ulcer effects of BHSST on the HCl/EtOH-induced gastric ulcer in mice. Three different dosages of BHSST extract (400, 200 and 100 ㎎/㎏) were once orally administered, and one hour after HCl/EtOH mixture was single orally treated. The changes on the gross hemorrhagic lesion scores, fundic histopathology, gastric nitrate/nitrite contents, lipid peroxidation and antioxidant defense system (catalase and SOD activities) were observed, and compared with that of ranitidine 100 ㎎/㎏[14].

As results of all three different dosages of BHSST extract treatment in the HCl/EtOH-induced gastric ulcer mice, significant and dose-dependent decreased gastric damages - the hemorrhagic gross lesions and histopathological gastric ulcerative lesions were observed as compared with the HCl/EtOH treated control mice, in this study. BHSST extracts also increased gastric nitrate/nitrite contents and strengthened the antioxidant defense systems - decreased the level of gastric lipid peroxidation, but increased the activities of catalase and SOD, respectively. BHSST extracts 200 ㎎/㎏ showed similar anti-ulcerative effect as compared with ranitidine 100 ㎎/㎏, in this experiment. These are considered as direct evidences that BHSST has favorable protective effects against to the HCl/EtOH-induced gastric damages, through the strengthening of the body antioxidant defense systems.

The decreases or inhibition of the gross hemorrhagic lesion areas have been regarded as a valuable indication that test substances have favorable gastric mucosa protective effects based on the previous efficacy studies[37]. Lesser gross lesions mean more favorable protective effects[1]. The decreases of gross lesion areas, detected in BHSST extracts 400, 200 and 100 ㎎/㎏ treated mice as compared with HCl/EtOH control mice in this experiment are considered as direct evidences that BHSST has favorable gastroprotective effects. Our experimental results are also showed that BHSST 200 ㎎/㎏ showed similar inhibitory effects on the gastric gross lesion areas induced by HCl/EtOH treatment as compared with ranitidine 100 ㎎/㎏ as direct evidences that BHSST can be easily adjust to patients suffering from gastric damages.

One of the factors that play a key role in alcohol-induced damage is oxidative stress that results from enhanced generation of ROS. All the doses of BHSST extracts that test in this experiment significantly decreased the MDA (a lipid peroxidation product) content, as compared with HCl/EtOH control group. This increase in MDA content was correlated to the increased tissue damage. BHSST 200 ㎎/㎏ also decreased the MDA content as similar to that of ranitidine 100 ㎎/㎏ in this study. Increasing lipid peroxidation products is an important cause of gastric damages.

The difference between the CAT activity in the stomach tissue of control mice given HCl/EtOH and that of healthy intact mice was statistically significant. CAT activities in the stomach tissue of mice given BHSST 400, 200 and 100 ㎎/㎏ also increased significantly and dose-dependently. A 200 ㎎/㎏ dose of BHSST extracts decreased CAT activities similarly with that of ranitidine 100 ㎎/㎏ in this study. Experimental studies show that CAT activity decreases in the presence of HCl/EtOH-induced stomach damage[15]. The decrease in CAT activity demonstrates that the level of its substrate(H_2O_2) also has decreased in mice that were given HCl/EtOH. The increased CAT activity in mice given BHSST might indicate decreased oxidative stress. So the increased CAT activity in the damaged stomach tissue is important in terms of helping gastric protection.

SOD is one of the antioxidant enzymes that contribute to enzymatic defense mechanisms. Our results support previous findings that HCl/EtOH mixtures decrease SOD activity in mouse stomach tissues[15]. In our study, HCl/EtOH inhibited SOD activity, but all doses of BHSST extracts and ranitidine 100 ㎎/㎏ increased SOD activity. These results indicate that SOD plays an important role in eliminating gastric damage by partially preventing oxidative damage. In tissue in which ROS has been produced, SOD and other antioxidants are known to protect against oxidative stress-induced damage[38]. BHSST 200 ㎎/㎏ showed similar inhibitory effects against HCl/EtOH-induced SOD activity inhibitions as compared with ranitidine 100 ㎎/㎏ in this experiment.

Nitric oxide (NO) also appears to be a key mediator of gastrointestinal mucosal defense[24]. NO releasing drugs protect against ethanol-induced gastric lesions, and conversely, inhibition of NO synthesis increases the susceptibility of the stomach to ethanol injury[39]. Our findings on nitrate/nitrite levels in the gastric tissue further confirm this view. Total nitrate/nitrite, a marker of endogenous nitric oxide[15] that was markedly reduced by ethanol treatment was found to be significantly

elevated in mice pretreated with all three different dosages of BHSST, and also in ranitidine 100 ㎎/㎏ treated mice. Constantly, BHSST 200 ㎎/㎏ also showed similar elevation effects on gastric nitrate/nitrite levels as compared with ranitidine 100 ㎎/㎏ in the present study. NO is considered an effective chain breaking antioxidant in free radical-mediated lipid peroxidation[40]. Moreover, it interacts with prostanoids and sensory neuropeptides in the regulation of gastric mucosal blood flow and thus improves microcirculation.

Histopathologically, severe focal extensive superficial epithelial damage, desquamation of focal epithelium, focal hemorrhages/congestions, inflammatory cell infiltrations and necrosis of gastric glands, the ulcerative lesions have been detected on the fundus after treatment of HCl/EtOH, and also in this experiment. Changes on histopathological images have been used as a valuable criteria index to confirm the gastroprotective effects of various candidates including medicinal herbs[1,23]. In our results, HCl/EtOH associated microscopic ulcerative lesions were markedly inhibited by pre-treatment of ranitidine 100 ㎎/㎏, and also dose-dependently by treatment of all three different dosages of BHSST, respectively. BHSST 200 ㎎/㎏ showed similar histopathological effects as compared with ranitidine 100 ㎎/㎏ in the present study well correspond to other results in gross observation, gastric nitrate/nitrite levels and also antioxidant defense systems. These effective changes were also re-confirmed by semiquantative histological scores. Semiquantative histological scores were marked increased by treatment of HCl/EtOH, but they were dose-dependently and favorably normalized by treatment of all three different dosages of BHSST and also after single oral administration of ranitidine 100 ㎎/㎏, respectively.

The results obtained in this study suggest that BHSST has favorable protective effects against to the HCl/EtOH-induced gastric damages, through the strengthening of the body antioxidant defense systems. Our experimental results are also showed that BHSST 200 ㎎/㎏ showed similar inhibitory effects against HCl/EtOH-induced gastric ulcer as compared with ranitidine 100 ㎎/㎏ as direct evidences that BHSST can be easily adjust to patients suffering from gastric damages. These gastroprotective effects of BHSST against HCl/EtOH-induced gastric ulcer considered as results of complicated synergic effects of their 8 kinds of herbal components, but exact synergic or individual herbal effects are difficult to discuss in this study. Therefore, more detail synergic effects between 8 kinds of individual herbal components of BHSST should be tested with screening of active anti-inflammatory chemical ingredients, in future.

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