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Protective effects of *Banhasasim*-tang, a herbal medicine, against cold restraint stress-induced gastric ulcers

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Abstract: *Banhasasim*-tang (BST), a herbal medicine, has been used for nausea and fever from cold damage. This study aimed to investigate the protective effects of BST in cold restraint stress-induced gastric ulcers. Male Sprague Dawley rats were orally treated with various doses of BST including 0.25, 0.5, 1, 3, 6, 9, 12 and 18g/kg based on the human daily intake dose. After treatment once per day for 3 days, rats were restrained into the cold stress chamber for 12h at 4°C to induce gastric ulcers. Gastric hemorrhagic ulcer area was evaluated and serum adrenocorticotropic hormone (ACTH), corticosterone, epinephrine and dopamine levels were determined. Compared to cold stress-induced gastric ulcer rats, hemorrhage ulcer areas were reduced in BST-treated stomach tissues at all concentrations. Increased serum ACTH, corticosterone and epinephrine levels were significantly decreased by BST treatment in cold stress-induced gastric ulcer rats. Moreover, there were increments of serum dopamine levels in 3 and 6g/kg of BST-treated groups. Taken together, BST positively ameliorated cold restraint stress-induced gastric hemorrhage with decrease in serum stress-related biomarkers such as ACTH, corticosterone, epinephrine and dopamine. The 3-6-fold of human daily intake dose of BST exhibited protective effects as a herbal medicine for gastric ulcers.

Keywords: *Banhasasim*-tang, cold restraint stress, gastric hemorrhage, gastric ulcer, human daily intake dose.

INTRODUCTION

Banhasasim-tang (BST) is a natural herbal medicine composed of *Pinellia ternata*, *Scutellaria baicalensis*, *Panax ginseng*, *Glycyrrhiza uralensis*, *Zingiber officinale*, *Coptis japonica* and *Zizyphus jujube* for human health. According to traditional Korean book (Heo, 2005), it has been used for nausea, fever from cold damage and lower abdominal fullness. BST is prescribed when people feel cold and nauseous and have a stuffy but not very painful epigastrium. Nausea is caused by cramp or stiffness of muscle in the upper digestive tract via inflammation, irritation of dopamine and serotonin receptors in the digestive tract, and abnormal autonomic nervous system (Singh *et al*., 2016). The causes of nausea from cold damage are acute stagnation and stomach cramps by cold qi (Ling *et al*., 2012). Stiffness of upper abdomen with the symptoms including burning sense, soreness of epigastrium and cold sweat is usually associated with the acute inflammation. Therefore, the main indications of BST are the treatment of stiffness of digestive tract smooth muscle, stomach cramps and gastritis, which is caused by cold qi. There may be an improvement in gastritis and gastric acid secretion to treat these problems, but most of all, improving blood flow in the digestive tract and normalizing the rigid digestive tract which was suppressed by cold qi, are expected to be the main treatment mechanism. There were various BST studies on digestive inflammation and smooth muscle motility, but there has been no BST studies related to cold qi. This study was planned to conduct an experiment verifying the effect of BST on gastritis related to cold stimulation.

The technique of perfusion of cold solution to stomach in rats is used to stop emergent hemorrhage during gastrointestinal bleeding. It causes motility reduction and ulceration because of the reduced blood flow of the gastroenteric membrane, therefore the cold irrigation is used as a method of ulcer development by cold shock in a laboratory (Rashad *et al*., 2019). Secondary hypothermia in each organ is caused by weak constitution, circulatory disorder, and problem of thermoregulatory center in pituitary gland, however, it is in contrast to primary hypothermia by exposure in cold environment (Lee *et al*., 1998). Primary hypothermia by exposure of cold-restraint stress leads to hemorrhagic pathological lesions in gastric membrane and histological gastric mucosal injury with inflammation (Wang *et al*., 2015). The environmental cold-restraint model was prepared for studying the effect of BST for improving hormone and neurotransmitter in blood and recovering gastritis.

MATERIALS AND METHODS

Animals

All experiments were conducted in accordance to the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

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The experiments were approved by the Institutional Animal Care and Use Committee of NIKOM, Jangheung, Republic of Korea (TKM-2016-005). Male Sprague Dawley rats weighing 250-280 g were purchased from RAON Bio (Youngin, Korea). All animals were housed in plastic cage at 24±1°C and 50±5% humidity. After 1 week of adaptation, vehicle and sample were orally treated for continuous 3 days. The treatment doses of BST were calculated based on the human daily intake dose using human equivalent dose formula. 10g/60 kg for human daily dose was conversed to 1 g/kg for rat (1×). 0.25g/kg (1/4×), 0.5g/kg (1/2×), 3g/kg (3×), 6g/kg (6×), 9g/kg (9×), 12g/kg (12×) and 18g/kg (18×) of BST were also orally administrated once per day for 3 days. In addition, 1.03 mg/kg of Buscopan Compositum (Buscopan Compositum-A®, anticholinergics, 10mg/60kg for human BCP) and 41.11mg/kg of cimetidine hydrochloride (histamine-2 receptor antagonists 400mg/60kg for human; C-HCl) were given to positive control groups 3 times per day and 2 times per day, respectively. All samples were suspended in distilled water. Rats except normal group were fasted for 12 h and restrained into the cold stress chamber for 12h at 4-5°C. Blood sample was collected in heparin tube and rats were sacrificed in CO_2 chamber.

Gastric hemorrhagic ulcer area determination

Stomach was dissected and photographed by a digital camera (Sony, Japan). Lesions of gastric hemorrhage were quantified by a computerized densitometry system Image J (NIH, Bethesda, MD, USA). The ulcer index was expressed as of the ratio of gastric hemorrhagic ulcer area and total gastric area.

Serum analysis

The collected blood was centrifuged. Serum adrenocorticotropic hormone (ACTH), corticosterone, epinephrine and dopamine levels were measured by each commercial enzyme-linked immunosorbent assay kits (BD Biosciences, CA, USA).

STATISTICAL ANALYSIS

Significance was determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests using SPSS Data Analysis Version 17.0 (SPSS Inc, Chicago, IL). In all analyses, $p<0.05$ was taken to indicate statistical significance.

RESULTS

Effects of BST on morphological changes of stomach in cold stress-induced gastric ulcer

Restraint cold stress induced severe gastric hemorrhagic injuries. The increase rate of gastric hemorrhage was 6.93 in the cold stress group compared to normal group. There was red appearance in cold stress-induced stomach tissue. Treatment with various concentrations of BST ameliorated the degree of gastric hemorrhage severity (fig. 1). The ratio of hemorrhage ulcer area and total area was dose-dependently decreased by BST administration. There were noticeable improvement of ulcer index in all BST treatment group in cold stress-affected rats without adverse effects. Additionally, treatment with 3g/kg (3×), 6g/kg (6×), 9g/kg (9×), 12g/kg (12×) and 18g/kg (18×) of BST also decreased the gastric hemorrhage ulcer area same as Buscopan and Cimetidine hydrochloride-treated groups.

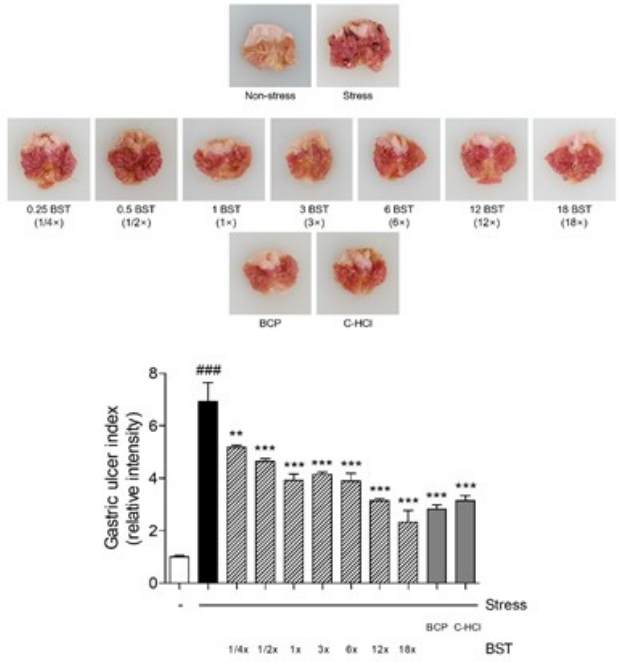

Fig. 1: Gastric mucosal lesions in no cold restraint stress or cold restraint stress-induced gastric ulcer rats. Representative images of stomach and ulcer index expressed as the ratio of gastric hemorrhagic ulcer area and total gastric area. Results are presented as mean ± standard error of the mean. $^{###}p<0.001$ vs. Non-stress group; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. cold restraint stress group.

Effects of BST on serum stress-related marker levels in cold stress-induced gastric ulcer

The levels of stress-related serum markers such as ACTH, corticosterone and epinephrine were significantly increased in cold restraint stress-induced gastric ulcer group. Serum ACTH concentration in cold stress-induced gastric ulcer rats was 3.25 time higher than non-stress group. In addition, cold restraint stress increased the serum corticosterone (~50.98 fold) and epinephrine (~1.8 fold). Treatment of BST markedly decreased serum stress-related marker levels. Serum ACTH concentrations were diminished in all concentrations of BST-treated groups, as in serum corticosterone. In rats pre-treated with 3 g/kg (3×) of BST, serum epinephrine was significantly decreased compared to stress-induced rats. Dose-dependent effect on serum epinephrine was observed by

treatment with 3g/kg (3×), 6g/kg (6×), 9g/kg (9×), 12g/kg (12×) and 18g/kg (18×) of BST in cold stress-induced rats (fig. 2).

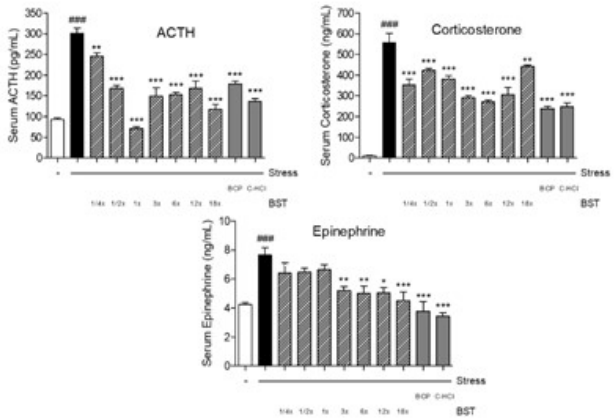

Fig. 2: Serum ACTH, corticosterone and epinephrine levels in no cold restraint stress or cold restraint stress-induced gastric ulcer rats. Results are presented as mean ± standard error of the mean. $^{###}p<0.001$ vs. Non-stress group; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. cold restraint stress group.

Effects of BST on serum dopamine levels in cold stress-induced gastric ulcer

Serum dopamine level in cold restraint stress-induced rat was significantly 54.5% lower than normal rat. There were notable increases of dopamine levels at concentrations of 3/kg (3×), 6g/kg (6×), 12g/kg (12×) and 18g/kg (18×) of BST in stress-induced ulcer rats (fig. 3).

DISCUSSION

In human studies, BST has been used for chronic gastritis (Xia, 2004), functional dyspepsia (Yoon *et al*., 2003), peptic ulcer (Park *et al*., 2012), reflux laryngopharyngitis (Xu, 2006) and postoperative pharyngolaryngitis (Kuwamura *et al*., 2015). The level of somatostatin, motilin, and gastrin in the plasma is increased when the BST is given to healthy subjects (Naito *et al*., 2002) and diarrhea was prevented during afatinib therapy in cancer patients (Yamaguchi *et al*., 2015). In mice studies, BST improved reflux esophagitis (Jang and Lim, 2013), and dextran sulfate sodium-induced chronic ulcerative colitis (Chen *et al*., 2015). BST also prevented cardiac, hepatic and splenic toxicity induced by doxorubicin (Hwang *et al*., 2002; Shin *et al*., 2002). *S. baicalensis*, *C. japonica*, *G.uralensis*, and *P. ginseng* were involved in the antidiarrheal action (Kase *et al*., 1999) and *S. baicalensis*, *C. japonica*, and *G.uralensis* were involved in decreasing cyclooxygenase-2 and prostaglandin E2 (Kase *et al*., 1998a). In rats studies, BST improved reflux esophagitis (Shi *et al*., 2008), stress-induced gastric ulcer (Li *et al*., 1998), acute duodenitis (Han *et al*., 2002), and colitis (Kawashima *et al*., 2004). It also improved electrogastric dysrhythmia (Li *et al*., 2006) and cholinergic or serotoninergic gastric dysmotility (Lee *et al*., 2006). Administration of BST with cimetidine inhibited gastric ulcer (Lee *et al*., 2002). BST treated gastric ulcer through energy production by up egulating leptin and vascular expansion by upregulating endothelin-1 (Liang *et al*., 2012). It also showed antidiarrheal effects via suppressing small intestinal fluid secretion (Kase *et al*., 1998b), repressing prostaglandin E2 increase and promoting water absorption (Kase *et al*., 1997) and inhibiting contractile activity of circular smooth muscle (Kito and Teramoto, 2012). Overall, BST has the effect of improving digestive issues such as gastritis, ulceration, reflux disease, diarrhea and dysmotility.

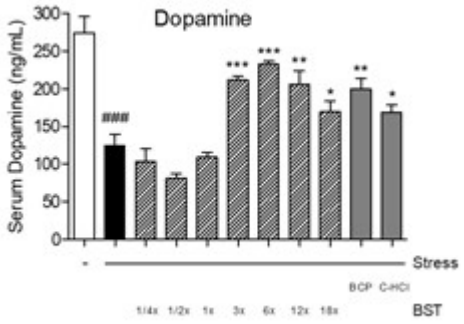

Fig. 3: Serum dopamine levels in no cold restraint stress or cold restraint stress-induced gastric ulcer rats. Results are presented as mean ± standard error of the mean. $^{###}p<0.001$ vs. Non-stress group; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. cold restraint stress group.

In this study, BST suppressed the gastric hemorrhage and shrinkage of membrane in cold-restraint stress-caused gastritis, similar to previous studies. An acute cold exposure induced increase in stress hormones and neurotransmitter levels in serum (Camerino *et al*., 2018). Corticosterone improved by more than 50%, epinephrine improved by more than 70% and dopamine improved by more than 60% at 6 folds of human equivalent daily intake dose of BST. BST has been mainly prescribed for fever, nausea, acute stagnation and stomach cramps against cold damage as a traditional medicine as well as a health insurance medicine. Because seven herbs including *P. ternata*, *S. baicalensis*, *P. ginseng*, *G. uralensis*, *Z. officinale*, *C. japonica* and *Z. jujube* are composed of BST, we assumed that a lot of active components may contribute to effects of BST on stress hormone release derived from cold stress. Previous studies reported the inhibitory effects and underlying mechanism of the constituents of BST against stress. Ginseoside Rd and Rb1, the active ingredients in ginseng, attenuated the corticosterone secretion and dopamine release, respectively (Jin *et al*., 2020; Wang *et al*., 2017). Baicalin, a main compound of *S. baicalensis*, has ameliorative effects on depression-like behavior with increase of dopamine level in restraint stressed rats (Lee *et al*., 2013). In addition, increment of corticosterone of flavonoids

from *G. uralensis* and Zingerone from *Z. officinale* has been reported in the previous literatures (Fan *et al*., 2012; Banji *et al*., 2014). Furthermore, it is well known that *Z. jujube* exerts anti-depressant and sedative activities (Wado *et al*., 2020; Shen *et al*., 2020). These references suggest that various constituents derived from herbs consisting of BST might be responsible for regulation of stress hormone and further effectiveness on cold restraint stress-induced gastric ulcers.

In summary, ACTH reduction was the most at human equivalent daily intake dose of BST, while dopamine increase was significant at 3- and 6-fold of general daily intake dose of BST. BST is thought to be used with 3–6-fold of general daily intake dose.

Dysregulation of acetylcholine, gastrin, and histamine can induce stress gastritis (Megha and Lopez, 2019). BST did not affect contraction of guinea pig ileum in response to acetylcholine and histamine (Kase *et al*., 1996) but raised gastrin in healthy subjects (Naito *et al*., 2002). Gastrin, which is mainly secreted from the G cells in the antrum of the stomach, activates hydrogen/potassium ATPase for acid secretion (Megha and Lopez, 2019). BST effects for gastrin in gastritis subjects needs to be verified in further studies.

CONCLUSION

In conclusion, BST decreased serum stress-related marker and the gastric hemorrhage ulcer area in cold-restraint stress-induced gastritis model. The 3-6-fold of general daily intake dose of BST would be the most effective for treating gastritis, demonstrating the therapeutic properties of BST for human health as an herbal formula.

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