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Original Research Paper

Activity of *Brucea javanica* oil emulsion against gastric ulcers in rodents

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ABSTRACT

The present study aims to investigate the gastroprotective effect of *Brucea javanica* oil emulsion (BJOE) in animals. Gastroprotective potential of BJOE was studied on absolute ethanol, aspirin, reserpine and restraint plus water immersion-induced gastric ulcers in mice as well as glacial acetic acid (GAA) and pyloric ligation (PL)-induced gastric ulcers in rats. Except for ulcer scores, total acidity as well as pepsin activity as for the PL-induced gastric ulcer model and ulcer incidence as for the GAA-induced gastric ulcer model were also determined. Histopathological evaluation as for aspirin, reserpine, PL-induced models was conducted. Results showed that BJOE significantly ($P < 0.05$) reduced ulcer index in the mouse and rat models in a dose-dependent manner. It had significant ($P < 0.05$) suppressive effect on total activity of gastric juice as well in PL-induced model. Histopathological examination for the stomach samples confirmed the findings in the aspirin, reserpine or PL-induced gastric lesion models, which showed relatively complete mucosa structure and less inflammation. It is concluded that BJOE could be effective on gastric ulcer in rodents and its gastroprotective activity might be related to antioxidant, anti-inflammatory ability and promote gastric mucus secreted. The results may provide beneficial basis for increasing BJOE's clinical indication in future.

1. Introduction

Peptic ulcer which includes both gastric and duodenal ulcers is one of the most prevalent gastrointestinal tract diseases that affect a wide range of people worldwide [1]. Due to its high morbidity and mortality rates, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery over a century. The pathophysiology of peptic ulcer disease was attributed to the imbalance between the offensive factors (e.g. acid, pepsin,

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Helicobacter infection) and the defensive ones (e.g. bicarbonate, mucin, prostaglandins, nitric oxide and growth factors) [2]. The use of non-steroidal anti-inflammatory drugs (NSAIDs), irregular diet, emotional stress, excessive alcohol use and smoking are all the principal etiological factors associated with the peptic ulcer [3]. Nowadays, the drug treatment of ulcer is commonly focused on the suppression of acid secretion and the enhancement of gastric protection [4]. However, more and more clinical evaluation on the drug treatment showed that tolerance was developed and also incidence of relapses as well as side effects were increased, which made the efficacy of the treatment arguable. Many of the existing medicines have limitations, especially when they were used against the ulcers with complex etiologies [4–6].

Over recent years, abundant work has been accomplished to develop natural products to potentially provide rich sources of new agents with anti-ulcer activity. It is significant to clarify their prevention or management action against gastric ulcer. A few of plant extracts and plant-derived compounds have been found and proved to be safe, effective, relatively less expensive and globally competitive [7,8]. *Brucea javanica* (L.) Merr. seed oil (BJO) which was extracted from the nucleoli of *B. javanica* (L.) Merr. (Simaroubaceae) has been found to be beneficial in clinic. *B. javanica* (L.) Merr. traditional herbal medicine, mainly distributed in tropical and subtropical areas such as Hainan, Guangdong and Yunnan provinces of southern China. BJO has been used in treatment of various ailments including cancer, amoebic dysentery, and malaria. The mechanisms of antitumor activity of BJO include inhibiting DNA polymerase activity, overcoming tumor multidrug resistance, and destructing cancer cell membrane system and autophagy inhibition [9,10]. It is an available anti-tumor drug because of its good therapeutic effect and wide anti-tumor spectra. Interestingly, doctors found that it was, in clinical settings, beneficial for patients with gastric ulcer when it is used as an anti-cancer agent, especially for those with stomach cancer and hepatocellular carcinoma. Based on the clinical findings, anti-gastric ulcer activity of various formulations of BJO in laboratory was studied. It was found that the injection of BJO is effective in treating a few of pathological models of mice with gastric ulcer [11], it also significantly reduces the ulcer scores, total acidity and the incidence of ulcer, and enhances the inhibitory effects on gastric ulcer and gastric acid production in rats [12]. In the present study, the anti-gastric ulcer activity of oral emulsion of BJO was investigated in laboratory animals via oral administration route. The study will further provide experimental basis for a different formulation of the drug to treat gastrointestinal ulcers and provide evidence for increasing BJO's indication in clinic uses.

2. Materials and methods

2.1. Drugs and chemicals

Oral emulsion of BJO (10%, counted by total acid) was provided by Shenyang Yaoda Pharmaceutical Co., Shenyang, China. Cimetidine was purchased from Shanghai Huashi Pharmaceutical Co., Ltd. Normal saline (N.S., 0.9% sodium chloride injection) produced by Shenyang Zhiying Pharmaceutical factory. CMC-Na800-1200 (carboxymethyl cellulose sodium salt), absolute ethanol and phenolphthalein were all products of Tianjin Bodi Chemical Engineering Ltd. Aspirin was product of Shandong Xinhua Pharmaceutical Co., Ltd. Reserpine injection was provided by Tianjin Kingyork Amino Acids Co., Ltd. Glacial acetic acid was produced by Tianjin Baishi Chemical Engineering Ltd. Sodium hydroxide was provided by Shenyang Xinhua chemical reagent factory. All the reagents used in this study were analytical grade.

2.2. Animals

The animal experiments were conducted according to the rules of animal experiment and the guide for the Care and Use of Laboratory Animals of Shenyang Pharmaceutical University (SYPU-IACUC-0415-106). The protocol also followed the rules of the local Animal Ethics Committee. Kunming mice (either sex, 18–22 g) and Wistar rats (either sex, 180–220 g) were obtained from the Animal Center of Shenyang Pharmaceutical University. They were group-housed (6 mice or 5 rats per cage) in standard environmental conditions (22 ± 1 °C, humidity 60% ± 5%, 12 h light-12 h dark cycle) with free access to standard commercial diet and water ad libitum. They were allowed at least one week of acclimatization before use.

2.3. Induction of mouse gastric ulcer and pharmacological intervention

2.3.1. Absolute ethanol-induced gastric ulcer

The acute gastric lesion was induced by intragastric application of absolute ethanol according to the method published [13]. The mice were randomly divided into five groups of ten mice. They were given normal saline, cimetidine 200 mg/kg (i.g.), BJOE 0.2, 0.4 and 0.8 ml/kg (i.g.), respectively, once a day for four consecutive days. They were fasted with free access to water after the drug treatment on Day 3 and all the animals received absolute ethanol (0.1 ml/each mouse) by oral route 1 h after the administration on Day 4 to induce gastric ulcer. Thirty minutes later, the mice were sacrificed by diethyl ether and their stomachs were incised along the greater curvature to examine ulcers according to that described in Table 1.

2.3.2. Aspirin-induced gastric ulcer

The experimental procedure was based on that described in a previous publication [14]. The groups and treatment situation are similar to the description in section 2.3.1. All animals received aspirin (200 mg/kg) which was suspended in distilled water with 1% CMC-Na by the oral route to induce gastric

Table 1 – Score of the gastric mucosal lesions.

Gastric mucosal lesion	Points
Normal condition	0
Local congestive redness	1
Local congestive redness and punctate hemorrhage	2
Mild erosion	3
Moderate erosion	4
Severe erosion to perforation	5

The intermediate between the adjacent points was added 0.5.

ulcer 1 h after BJOE or cimetidine treatment on Day 4. Four hours later, the animals were sacrificed and gastric ulcers were scored following the description in Table 1. After the determination, the stomach samples were appropriately kept in the solution with 4% paraformaldehyde for histopathological analysis.

2.3.3. *Reserpine-induced gastric ulcer*

Mice were randomly divided into four groups of ten mice. They were given normal saline, cimetidine 200 mg/kg (i.g.), BJOE 0.4 and 0.8 ml/kg (i.g.), respectively, once a day for four consecutive days. They were fasted with free access to water after the treatment on Day 3 and all the animals received reserpine (10 mg/kg, s.c.) 1 h after the administration on Day 4 to induce gastric ulcer [15]. Six hours later, the mice were sacrificed and the ulcers were scored. After the determination, the tissues were kept in the solution with 4% paraformaldehyde for histopathological analysis as mentioned above.

2.3.4. *Gastric ulcer induced by restraint plus water immersion*

Gastric ulcer induced by restraint plus water immersion was performed according to literature [16,17]. Mice were randomly divided into four groups of ten mice: (Group 1) Model [N.S. 20 ml/kg, i.g.]; (Group 2) cimetidine [200 mg/kg, i.g.]; (Group 3 and 4) BJOE [0.4 and 0.8 ml/kg, i.g.]. They were deprived of food with free access to water for 24 h before the test. Thirty minutes after the first administration of the tested drugs, the mice were immobilized by strapping their fore and hind limbs on a plank and vertically immersed to their xiphoids in water (20 ± 2 °C) for 10 h. In the middle of the 10 h-water immersion, i.e. at the time point of 5 h after the water-immersion stress, they were given the test drugs once again. The mice were killed and the ulcer index was evaluated when the 10 h's stress is over.

2.4. *Induction of rat gastric ulcer and pharmacological intervention*

2.4.1. *Gastric ulcer induced with glacial acetic acid*

Rats were fasted for 15 h with free access to water before experiment, anesthetized and fixed. Abdominal wall was cut along ventrimeson after iodine disinfection and the stomach was gently pulled out. 40 μl of 18% ice acetic acid freshly prepared was injected into the space between the stomach muscularis and serosa layer on the gastric antrum anterior wall. The wounds were sutured before the rats were put back to the cage [18]. All the rats were randomly divided into five groups of eleven or thirteen the next day. They were treated with normal saline, cimetidine 140 mg/kg (i.g.), BJOE 0.25, 0.50 and 1.00 ml/kg (i.g.), respectively. The tested drug or saline was administered to the animals once a day for 5 consecutive days. They were killed at the point of one hour after the fifth administration. Pylorus and cardia ligation were conducted after operation. Four to ten milliliters of 1% formaldehyde solution were injected into the stomachs from the cardia and immersed for 15 min. Thereafter, the stomachs were cut out and opened along the greater curvature. The gastric mucosal lesions were examined.

2.4.2. *Pyloric ligation-induced gastric ulcer*

In the experiment, rats were randomly divided into four groups of nine or ten rats. They were given normal saline, cimetidine 140 mg/kg (i.g.), BJOE 0.50 and 1.00 ml/kg (i.g.), respectively. The animals were administered with the test drugs between 3:00-4:00 p.m. once a day for 5 consecutive days. They were fasted for 48 h with free access to water from Day 3 to Day 5. One hour after the last administration, pylorus ligation surgery was performed [19]. Being deprived of water for fifteen hours after the operation, the rats were killed. The stomachs were removed and opened along the longer curvature after gastric juice was collected. The injured gastric mucosal was examined and ulcers in the glandular portion of the stomach were evaluated. After the determination, the tissues were kept in the solution with 4% paraformaldehyde for histopathological analysis as mentioned above.

2.5. *Endpoints*

2.5.1. *Determination of ulcer index (UI)*

To evaluate the gastric mucosal injury, the ulcer scores were blindly determined from 0 to 5 points according to Table 1 [13,20]. Data were also transformed into protection percentage using the following equation [21,22]:

$$\text{Protection percentage}\ (\%) = [(UI_{control} - UI_{treated})/UI_{control}] \times 100\%$$

2.5.2. *Determination of the acidity of gastric juice in pyloric ligation (PL)-induced ulcer*

The gastric juice collected was put into tubes, centrifuged (400 g) for 20 min to obtain clear supernatant, which was used to analyze biochemical parameters. The clear supernatant in a volume of 1 ml was diluted by 5 ml distilled water and utilized to determine the concentration of hydrogen ion through acid-base titration reaction with 0.1 mol/l NaOH. Using phenolphthalein dissolved in absolute ethanol (φ = 1%) as indicator, the titration terminal of the total acidity was the point when the solution color turned red and the volume of NaOH exhausted was recorded. Total acidity was calculated with the equation as follows [23]:

$$\text{Total acidity} \times 1\ \text{ml} = 0.1 \times 10^{3}\ \text{mmol/l} \times 10^{-3} V_{NaOH}\ (\text{ml})$$

V_{NaOH} stands for the volume of 0.1 mol/l NaOH consumed.

Gastric acidity was expressed as mmol/l. The inhibitory rate of the total acidity was obtained from the following equation:

$$\text{Inhibitory rate}\ (\%) = \frac{\text{total acidity of the control} - \text{total acidity of the tested group}}{\text{total acidity of the control}} \times 100$$

2.5.3. *Determination of the pepsin activity of gastric juice in pyloric ligation (PL)-induced ulcer*

The determination was made with the Mett method [24]. An appropriate amount of fresh egg white was taken to fill in the glass capillaries (diameter: 0.9–1.1 mm) via siphonage, in which there must be no bubbles. They were placed in the steam of boiling water for solidification, and then taken out to cool down.

They were also sealed with paraffin at both ends and stored under 4 °C. One milliliter gastric juice was put into a bottle of 20 ml volume and added 15 ml hydrochloric acid at the concentration of 0.05 N, which were mixed up. Then two pieces of capillary filled with protein, each of them 2 cm long, were put into the reaction system. The bottle was sealed and put into 37 °C water to make the system hatch for 24 h. The length (mm) of transparent part at both ends of the tubes fraught with protein was measured. The average value of the four terminals was calculated. The activity of pepsin was (U) = mean^2 × 16.

2.5.4. *Histopathological evaluation*

The stomachs of the mice treated with the tested drugs in the aspirin and reserpine models as well as those of the rats in pyloric ligation model were separately fixed in paraformaldehyde, dehydrated using a series of alcohol, cleared in xylene and then treated with paraffin imbedding. Hematoxylin-eosin staining was performed to observe the histopathological changes of these stomach samples under a light microscope.

2.6. *Statistical analysis*

The results from each group were calculated as mean ± standard error of mean (SEM). They were analyzed by one-way analysis of variance (ANOVA), and the statistical evaluation between two groups was determined by LSD on SPSS 20.0. Probability (P) values less than 0.05 were considered significant.

3. Results and discussion

Nowadays, new and significant information has been forthcoming on the pathogenesis of various types of ulcers induced by drugs or operations and great stride has also been achieved in basically understanding the gastro-duodenal physiology. However, mechanisms underlying the gastroprotection have not been well understood yet. This study made a systemic evaluation on the *in vivo* gastroprotective effect of BJOE used in various gastric ulcer animal models. Lower doses of BJOE were applied in the current anti-gastroulcer study than that of BJOE used in the clinical therapy of tumor. The maximal dosage of BJOE used in the present study is 1 ml/kg which is less than the dosage of BJOE as an anti-tumor drug. In Su's study [25], 20 ml was used in human, which is equivalent to 2 ml/kg in rat. Results showed the formulation orally given by 1 ml/kg was effective against rat gastroulcer. The mechanism of the action of BJOE might be involved in its anti-inflammatory ability although the speculation is not completely confirmed yet. Studies found that BJOE effectively alleviated cancer cachexia and treated ulcerative colitis through the anti-inflammatory mechanisms [26,27]. It seems that the anti-inflammation of BJOE is involved in both its anti-cancer and anti-gastric ulcer action.

3.1. *Protecting from the gastric ulcer induced with absolute ethanol in mice*

Ethanol is a chemical irritant and has local and systemic damaging effects. Ethanol-induced injury was characterized, as previously reported [28], by erosive hemorrhagic lesions

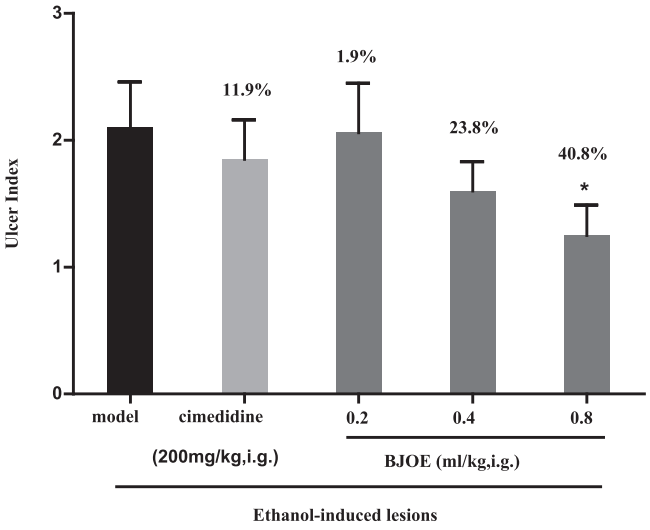

Fig. 1 – BJOE administration reduces dose-dependently ulcer index in mice with gastric ulcers induced by oral ethanol. Data were expressed as mean ± SD (n = 10) and analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The asterisk * stands for $P < 0.05$ compared with model control (gastric ulcer mice treated with normal saline). Superscripts were the protection percentage of gastric ulcer in each dosage group.

with diffuse coagulative, cell necrosis and multiple superficial erosions, which was marked with vascular congestion and extravasation of erythrocytes. Although the mechanism(s) of ethanol-induced gastric ulcer have not been fully understood yet, it is well documented in the literature that the pathogenesis in animals is multifactorial, involving superficial aggressive cellular necrosis and the release of tissue-derived mediators which act on the gastric microvasculature to trigger a series of events that leads to the damage of mucosal and submucosal tissues [29]. In the present study, results showed that the dose of absolute ethanol administered was sufficient to evoke gastric ulcer (Fig. 1). Compared with the model group, BJOE (0.2, 0.4, or 0.8 ml/kg) or cimetidine (200 mg/kg) reduced the ulcers scores (Fig. 1). Based on the ulcers index, the gastroprotective activity of each treatment was calculated as 11.9% (cimetidine), 1.9% (0.2 ml/kg BJOE), 23.8% (0.4 ml/kg BJOE), and 40.5% (0.8 ml/kg BJOE, $P < 0.05$). The inhibitory rate of the drugs is at least ~12% and the high-dose of BJOE treatment showed a significant inhibition although no statistical significance was seen as for cimetidine. Studies reveal that BJOE can promote wound healing and has obviously curative effect on burn, decubital ulcer, and refractory ulcer [30]. It is indicated that the emulsion directly attaches to the surface of gastric mucosa of mice when it is given by oral route to form a coating to protect the mucus from being further damaged.

3.2. *Protecting from the gastric ulcer induced with aspirin in mice*

In this part, aspirin given at the dosage successfully led to mouse gastric ulcer and the pathological model was stable. Anti-

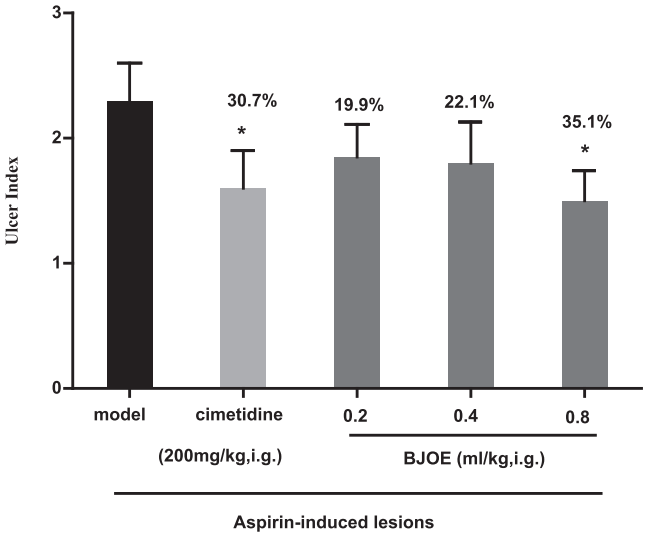

Fig. 2 – BJOE administration reduces dose-dependently ulcer index in mice with gastric ulcers induced by aspirin. Data were expressed as mean ± SD ($n = 10$) and analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The asterisk * stands for $P < 0.05$ compared with model control (gastric ulcer mice treated with normal saline). Superscripts were the protection percentage of gastric ulcer in each dosage group.

ulcer action of BJOE and cimetidine were shown in Fig. 2. BJOE remarkably suppressed the gastric ulcer in a dose-dependent fashion. The protection percentage of drug was 19.9%, 22.1% and 35.1% at the dose of 0.2, 0.4 and 0.8 ml/kg ($P < 0.05$), respectively (Fig. 2). Cimetidine also showed good effect on gastric ulcer and its protection percentage was 30.7% ($P < 0.05$). Histopathological analysis confirmed the result (Fig. 3). Gastric mucosa desquamation and edema could be observed in the stomach of the mice in model group (Fig. 3A). The lesions were not observed in the BJOE-treated (0.8 ml/kg) group as shown in Fig. 3D which expressed a pretty complete gastric mucosa structure. However, local mucosa disruption and edema still could be seen in BJOE-treated (0.2 and 0.4 ml/kg) group (Fig. 3B and Fig. 3C). The pathogenesis of NSAIDs-induced ulcer is multifactorial. The mechanisms are the inhibition of cyclooxygenase (COX), the disturbance of microcirculation and pro-apoptotic signaling [31]. Other studies showed that inhibitory actions on neutrophil influx and antioxidative effects might be the main pathways of the cytoprotection in the NSAIDs-induced ulcer [32,33]. Aspirin, the classic NSAID, is expected to inhibit expression of COX, but it was reported that no statistical change was found in rats with ulcer induced by aspirin [34]. Therefore, there may be a mechanism other than the COX pathway, which is important in NSAIDs-induced gastric mucosal injury. Previous research demonstrated that BJO could inhibit COX-2 expressions to induce T24 bladder cancer cells apoptosis [35]. It inferred that the antioxidative effects may be one of the mechanisms of the gastroprotection exerted by BJOE. Oleic acid and linoleic acid, the main active components of BJOE, which are known to have excellent antioxidant activities. And it is also possible that the other mechanism is involved in the drug's inhibiting COX enzyme.

3.3. *Protecting from the gastric ulcer induced with reserpine in mice*

Reserpine induces gastric mucosal damage through various mechanisms. For instance, it was reported to make vein

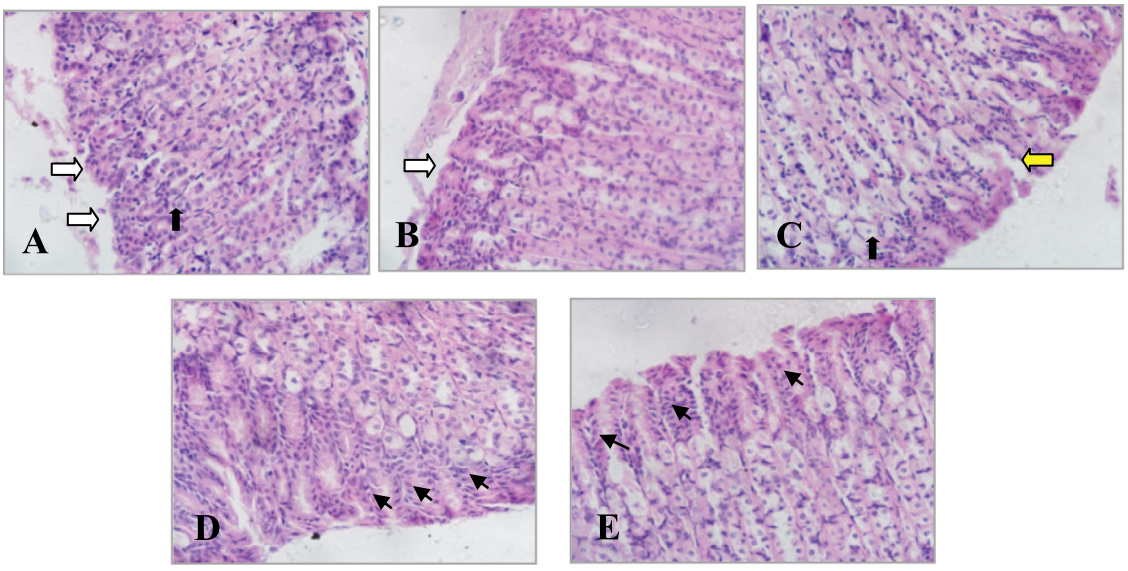

Fig. 3 – Slices of the mouse stomach mucus which is subjected to the gastric ulcer induced by aspirin. Samples are from the mice with the gastric ulcer treated with saline (A), BJOE 0.2, 0.4 and 0.8 ml/kg (B, C, D) and cimetidine 200 mg/kg (E), respectively. The slices are stained with hematoxylin and eosine and then examined under optical microscope (40×). Mucosa desquamation (white arrows); Edematous mucosa (black arrows); Local disruption of gastric mucosa (yellow arrows); Undamaged gastric mucosal architecture (arrowheads).

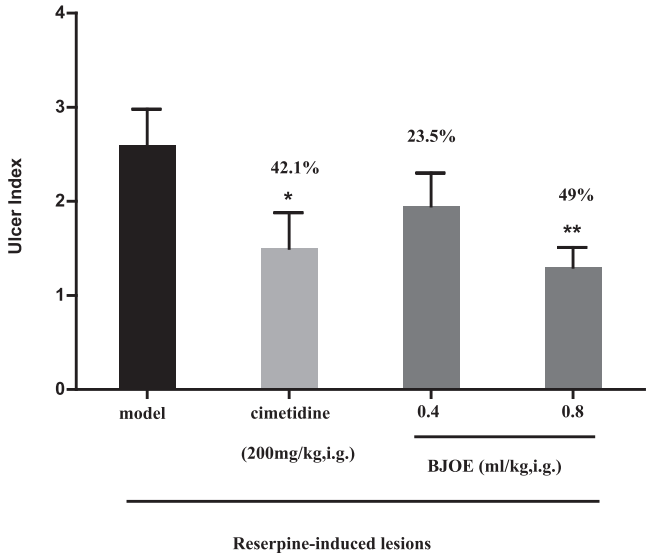

Fig. 4 – BJOE administration reduces dose-dependently ulcer index in mice with gastric ulcers induced by reserpine. Data were expressed as mean ± SD (n = 10) and analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The single asterisk * stands for $P < 0.05$ and the double asterisks **$P < 0.01$ compared with model control (gastric ulcer mice treated with normal saline). Superscripts were the protection percentage of gastric ulcer in each dosage group.

constrict in the middle layer as well as in the muscularis mucosa and produce congestion, which often was accompanied with ischemia in the gastric mucosa and followed by gastric hypermotility [15]. The agent reduces sympathetic tone and increases cholinergic tone, which leads to excessive acid secretion [36]. Recent studies also proved that cyclooxygenase-1 (COX-1) and COX-2 were implicated in the maintenance of mucosal integrity and inflammatory reactions, which was originally suggested to be implicated in gastric ulcerogenesis [37]. It is also clearly established the important contributions of COX-2 to mucosal defense [38,39]. Related study showed the increased COX-2 expression in gastric mucosa of reserpine-induced ulcer rats and the expressions were significantly downregulated after drug treatment (insect tea, Larimichthys crocea swim bladder or ranitidine) [40,41]. In the present study, it was observed in Fig. 4 that the reserpine induced gastric ulcer in mice. BJOE and cimetidine treatment attenuated the gastric lesions, especially 0.8 ml/kg of BJOE had a remarkable therapeutic effect with the 49.0% protection percentage ($P < 0.05$, Fig. 4). Histopathological data showed that the two doses of 0.4 and 0.8 ml/kg of BJOE (Fig. 5B and Fig. 5C) could effectively suppress the damage of gastric mucus induced with reserpine and the effect of 0.8 ml/kg was better than that of 0.4 ml/kg. The histopathological examination of the mice stomachs obtained a commendable finding. Lou et al. indicated that BJO inhibited COX-2 expressions [35]. It is supposed that the gastroprotective activity of BJOE in the reserpine-induced ulcer is possible associated with the inhibition of COX-2 expressions.

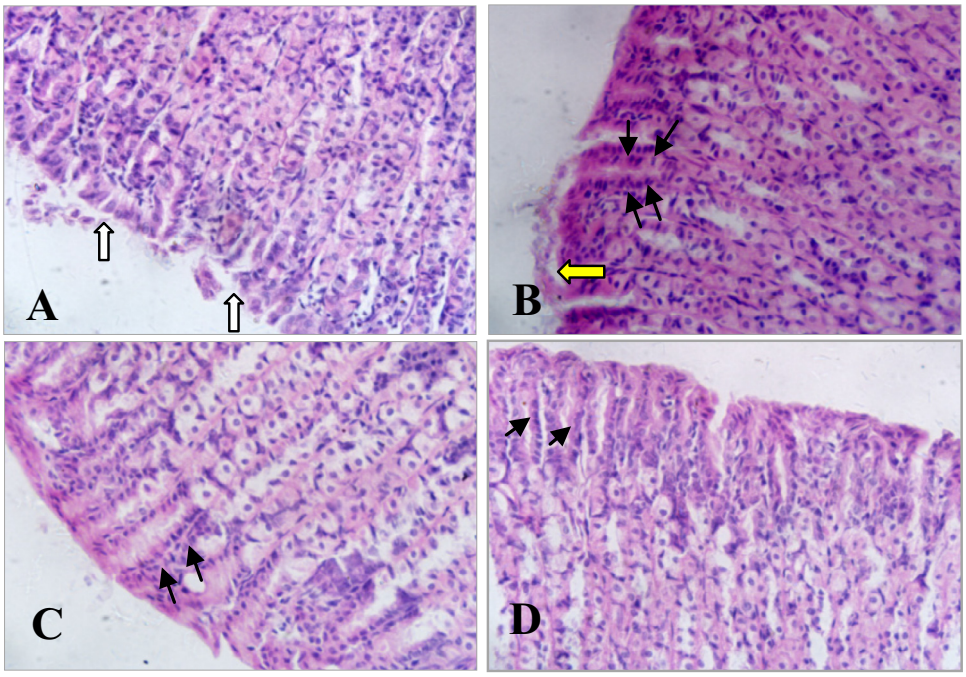

Fig. 5 – Slices of the mouse stomach mucus which is subjected to the gastric ulcer induced by reserpine. Samples are from the mice with the gastric ulcer treated with saline (A), BJOE 0.4 and 0.8 ml/kg (B and C) and cimetidine 200 mg/kg (D), respectively. The slices are stained with hematoxylin and eosine and then examined under optical microscope (40×). Gastric mucosal desquamation or atrophy (white arrows); Disruption in the region of the gastric mucosa with epithelial cell loss (yellow arrows); Well-organized glandular structures (arrowheads).

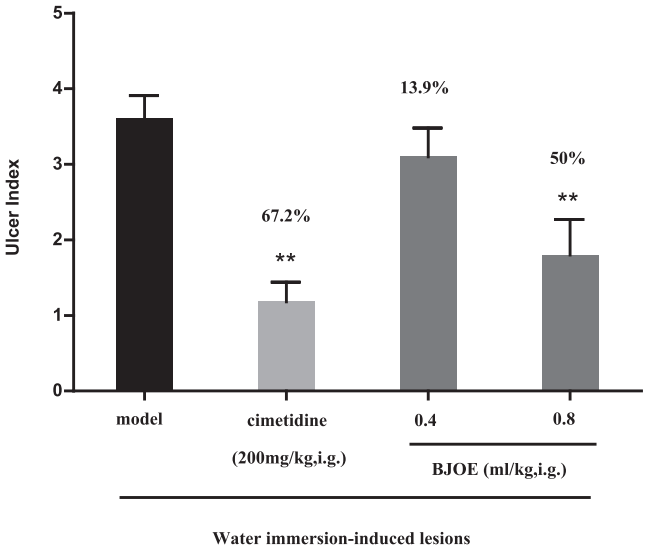

Fig. 6 – BJOE administration reduces dose-dependently ulcer index in mice with gastric ulcers induced by restraint plus water immersion. Data were expressed as mean ± SD (n = 10) and analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The double asterisks ** stands for $P < 0.01$ compared with model control (gastric ulcer mice treated with normal saline). Superscripts were the protection percentage of gastric ulcer in each dosage group.

3.4. *Protecting from the gastric ulcer induced with restraint plus water immersion in mice*

After making mice fasted for 24 h, in cold bath with bondage as well, and keeping them in the stressed status for 10 h, the gastric ulcer was then induced by the restraint plus water immersion. Water-immersion stress is widely used as an experimental model to induce acute stress ulcers in rats because of its reliable reproducibility. It also can mimic clinical acute gastric lesions which may appear in the gastric mucosa as a consequence of major trauma, surgery or sepsis. Changes in gastric secretion, abnormal gastric motility and disturbance of gastric mucosal microcirculation have been implicated in underlying pathogenetic mechanisms [42]. It is well known that the stress can provoke acute inflammation in gastric mucosa which accompanied to increasing the count of white blood cells (WBC). The present results showed that pretreatment with both BJOE and cimetidine had anti-ulcer activity on the gastric lesion in mice subjected to the stress of restraint plus water immersion (Fig. 6). Cimetidine decreased the ulcer scores to 1.18 ± 0.26 and the protection percentage was 67.2% ($P < 0.01$). The scores in BJOE group also declined. The dose of 0.8 ml/kg had an obvious effect than that of 0.4 ml/kg and its ulcer scores was 1.80 ± 0.47 and the protection percentage was 50% ($P < 0.01$, Fig. 6). It indicated that the drug was greatly against the ulcer in mice. Recent study revealed that BJOE inhibited the increase of WBC in tumor-bearing mice, which was perhaps due to its anti-inflammatory property [43]. Whether the action of BJOE against the stress-induced gastric ulcer is involved in its anti-inflammatory property, however, still needs to be confirmed.

3.5. *Inhibition on the incidence of gastric ulcer induced with glacial acetic acid in rats*

A high concentration of acetic acid can directly damage gastric wall and then lead to gastric ulcer. The model can easily and successfully be prepared in rats, having higher occurred rate. By being injected into the site between stomach muscularis and serosa layer, excess acetic acid damages epithelial cell and submucosal vessels so as to cause mucosal inflammation. Therefore, mucosal barrier was deteriorated. This gastric ulcer model, established 40 years ago, has been widely used to investigate

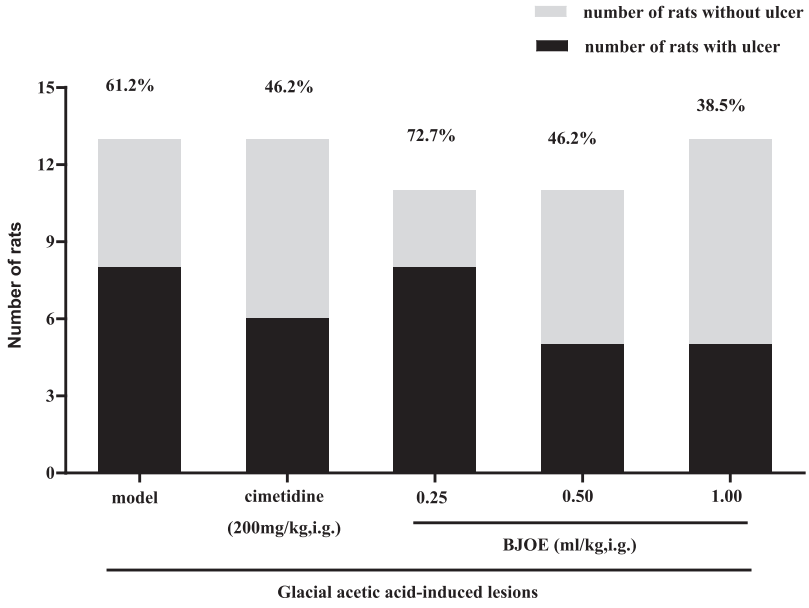

Fig. 7 – Inhibition of BJOE on the incidence of gastric ulcer in rats with lesion induced by glacial acetic acid (n = 11–13). Incidence rate of gastric ulcer in each group was showed at the upside.

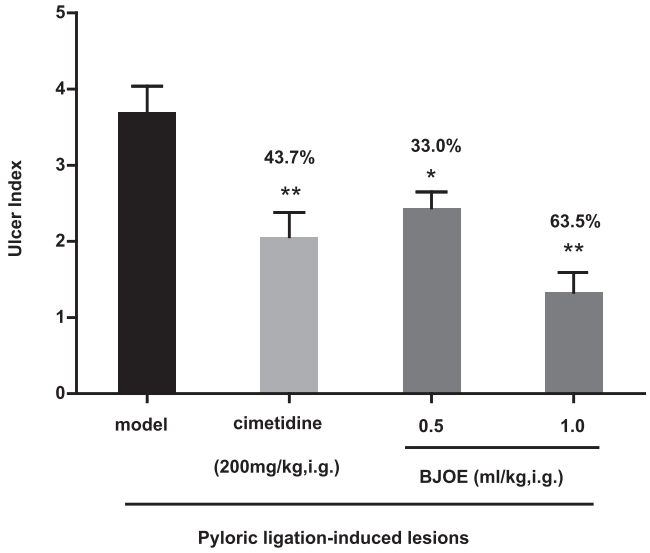

Fig. 8 – BJOE administration reduces dose-dependently ulcer index in rats with gastric ulcers induced by pylorus ligation. Data were expressed as mean ± SD (n = 9–10) and analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The single asterisk * stands for $P < 0.05$ and the double asterisks **$P < 0.01$ compared with model control (gastric ulcer rats treated with normal saline). Superscripts were the protection percentage of gastric ulcer in each dosage group.

the effect and mechanism of drugs in improving ulcer healing. It is reliable and repeatable and the ulcer is highly resemblant to that in human [44]. Results showed that the incidence of gastric ulcer induced with glacial acetic acid was 61.2% (Fig. 7). Data obtained from BJOE (0.25 ml/kg) was even higher than that of the control group (Fig. 7), which indicated that the BJOE (0.25 ml/kg) didn't have any influence on this ulcer. However, high dose of BJOE (0.50 ml/kg and 1.00 ml/kg) and cimetidine, at a dose of 200 mg/kg, provided gastric protection and significantly decreased the levels of the incidence of the gastric ulcer caused by glacial acetic acid (Fig. 7). It is demonstrated that BJOE given before of the acetic acid injection could dose-dependently decrease the incident probability of the GAA-induced gastric ulcer although the mechanism is not clear yet. Considering the characteristics of the animal ulcer model in terms of the site occurred, the severity and chronicity, it is clear that administration of BJOE was partially effective on this ulcer.

3.6. *Protecting from the gastric ulcer induced by pyloric ligation in rats*

Gastric ulcer model prepared with pylorus ligation is also used in rat mostly and it is generally used to investigate the effect of tested drugs on gastric secretions. The ligation of the pyloric end of stomach causes the accumulation of gastric secretion, enhances the secretion of pepsin which leads to the auto-digestion of gastric mucosa, breaks down the gastric mucosal barrier and finally results in gastric wall injury [45,46]. Pretreatment with BJOE (0.5 ml/kg and 1.0 ml/kg) and cimetidine showed protective effect on the gastric ulcer. The protection percentage of the high dose of BJOE was 63.5% ($P < 0.01$, Fig. 8). The results of total acidity presented in Table 2 confirmed the data, which clearly showed the inhibitory effects of the drug on gastric acid. The inhibitory rate of BJOE (1.0 ml/kg) was 33.9% ($P < 0.05$, Table 2). However, there were no obvious changes in the pepsin activity among all the groups ($P > 0.05$, Table 2). Data from histopathological examination showed that the mucosa epithelium of gastric tissues in the rats with the ulcer desquamated and inflammatory cell infiltration was observed (Fig. 9A). Pretreatment with 1.0 ml/kg of BJOE had a certain protection effect on the lesions and mucosa epithelium looked almost normal (Fig. 9C). The effect of 0.5 ml/kg of BJOE was not obvious as that of 1.0 ml/kg for some disruption of the mucosa also could be seen in Fig. 9B. The possible mechanism of the anti-ulcer action of BJOE may be relevant to the characteristics of the drug. When it is given before the ligation surgery, it may be absorbed into the bloodstream to be in a ready status for regulating the intrinsic factors ahead of the occurrence of gastric ulcer. In addition, the advantage of BJO emulsion, when it is orally applied in the therapy of gastric ulcer, is that a colloidal protective layer can be formed in advance which covers the inner surface of stomach to resist aggressive factors produced after the stimulation of pylorus ligation. The data about pepsin activity, however, did not show any significant effect. It might be suggested that the suppressive effect of BJOE on the gastric ulcer mainly come from its strengthening of the protective facet such as gastric mucus secreted rather than decreasing of the erosive facet like pepsin.

It is previously showed that BJO injection has gastroprotective action in mice, but its effect on mucosal damage induced with high dose of ethanol is limited [11]. This study, however, showed that ORAL emulsion of BJO reduced acute and chronic gastric lesions in experimental animals. The drug significantly reduces ulcer index and increases

Table 2 – Effects of BJOE on pepsin activity and total acidity in rats with gastric lesions induced by pylorus ligation.

Treatment		n	Total acidity (mM) (mean ± SD)	Inhibitory rate of total acidity (%)	Activity of pepsin (U) (mean ± SD)
Normal saline		10	71.4 ± 11.0	–	386.9 ± 61.5
Cimetidine (140 mg/kg)		9	60.7 ± 9.77	15.0	338.8 ± 88.2
BJOE (ml/kg)	0.50	9	62.3 ± 6.05	12.7	351.1 ± 43.1
	1.00	9	47.2 ± 6.67*	33.9	350.0 ± 56.2

Data were analyzed with one-way ANOVA followed by LSD with SPSS (Version 20.0). The single asterisk * stands for $P < 0.05$ compared with model control (gastric ulcer rats treated with normal saline).

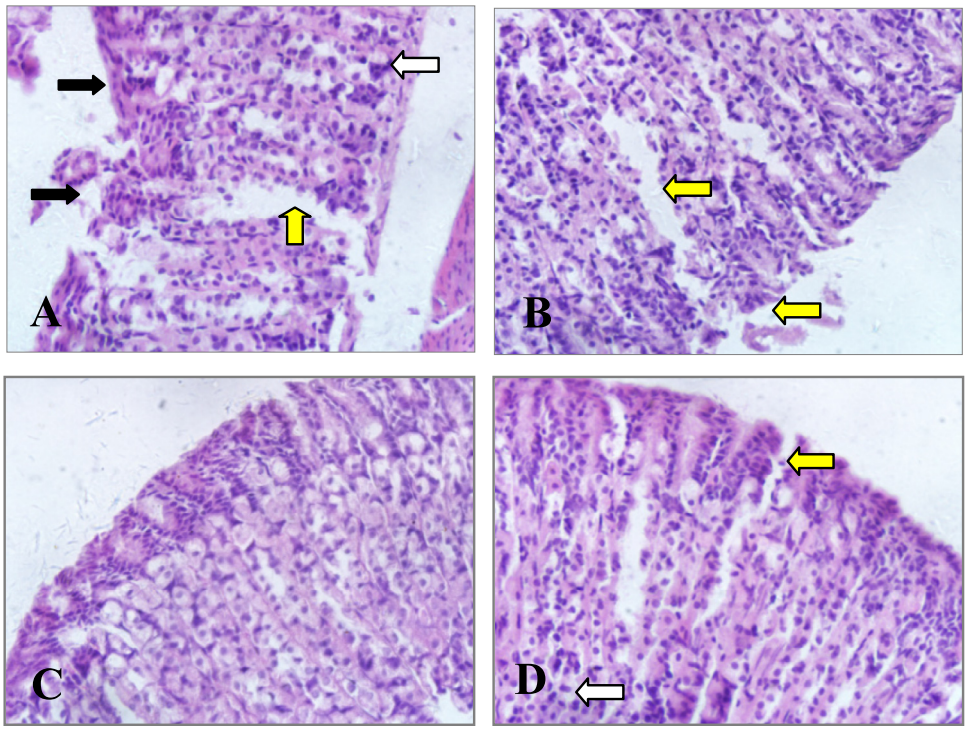

Fig. 9 – Slices of the rat stomach which is subjected to the gastric ulcer induced by pyloric ligation. Samples are from rats with gastric ulcer treated with saline (A), BJOE 0.5 and 1.0 ml/kg (B, C) and cimetidine 140 mg/kg (D), respectively. The slices are stained with hematoxylin and eosine and then examined under optical microscope (40×). Inflammation and polymorphonuclear infiltrate (white arrows); Gastric mucosal desquamation (black arrows); Local disruption of the gastric mucosa (yellow arrows).

protection percentage of mouse as well as rat gastric ulcer and it can also reduce total acidity in rat stomach. The better effect of BJOE than BJO injection might be related to the different route of administration and the different formulation of the drug. BJOE can form a colloidal protective layer in advance resisting aggressive factors when it is given by oral route. Further studies, however, are needed to investigate accurate mechanisms of its action, and more importantly, it may thus be used to protect from gastric mucosal damage in human.

4. Conclusion

The present findings conclude that BJOE has significant antiulcer activity as it exhibited protective effect on gastric ulcer in mice and rats. It is speculated that the mechanism of this gastroprotective activity is likely to be related to its emulsion formulation that covers the ulcer surface protecting from aggressive factors and being helpful for promoting gastric mucus secreted, although the precise mechanisms still need to be further studied.

Conflict of interest

The authors declare that there are no conflicts of interest.

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