



## Chemical characterization and gastroprotective effect of an isolated polysaccharide fraction from *Bletilla striata* against ethanol-induced acute gastric ulcer



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### ARTICLE INFO

#### Keywords:

*Bletilla striata* polysaccharides  
Gastric injury  
Oxidative stress  
MAPK/ NF-κB pathway

### ABSTRACT

The chemical characterization and protective role against ethanol-induced gastric ulcerated rats of a polysaccharide fraction from *Bletilla striata* (BSP) collected by ultrafiltration membrane approach were evaluated. This BSP fraction was consisted of mannose and glucose at a molar ratio of 2.4:1 approximately, with a molecular weight of 146 kDa. FT-IR, NMR and XRD spectra indicated that BSP fraction contained α-Man and β-Glc residues with low overall crystallinity. The polysaccharide exhibited significant scavenging activities of ABTS and FRAP, as well as non-toxicity against human gastric epithelial GES-1 cells. Oral administration with 100 mg/kg of BSP for 3 days continuously could significantly prevent the formation of ethanol-induced gastric mucosal lesion. It could also reduce the levels of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-18, and MPO activity in gastric tissue. Additionally, the BSP fraction exhibited antioxidant activity, increased the content of PEG<sub>2</sub> as a defensive factor, and suppressed MAPK/NF-κB signaling pathway in gastric tissue. These results indicated that the gastroprotective activity of BSP fraction could be attributed to the reduction of pro-inflammatory cytokines and oxidative stress and the inhibition of MAPK/NF-κB pathways. Our results provided substantial evidence that BSP could be a promising phytomedicine for gastric ulcer prevention.

### 1. Introduction

Gastric disorder, including gastritis, peptic ulcer, and gastroesophageal reflux diseases, has become a global health problem (Franke et al., 2005). Among its complex pathogenic factors, excessive alcohol ingestion is widely recognized as the main contributor (Bhattacharyya et al., 2014). As an example of imbalance between gastroprotective and aggressive factors (Lanas and Chan, 2017; Snowden, 2008), alcohol would primarily attack the gastric mucosa directly, resulting in destruction of the mucosal protective layer by depletion of mucus and bicarbonate. Additionally, acetaldehyde is generated via microsomal oxidase during alcohol metabolism, which leads to impaired antioxidant enzyme activity (Alvarez-Suarez et al., 2011; Goodwin et al., 2009; Shin and Kim, 2018). Ethanol-induced lipid peroxidation and oxidative stress have been reported to involve in the pathogenesis of

acute gastric mucosal injury (Hajrezaie et al., 2015). Furthermore, the severe inflammation and irreversible damage to stomach tissue and gastric cells contribute to an increased risk of major upper gastric bleeding, gastric mucosa inflammation, ulcer, or even gastric cancer. Therefore, it is essential for gastric tissue to be protected against injury in order to prevent the development of potential related gastric diseases.

Some chemosynthetic medicines, including proton-pump inhibitors like omeprazole (Zavoshti and Andrews, 2017), antibiotics for *H. pylori* inhibition (Lahner et al., 2018), and gastric acid neutralizers, have been widely adopted as the first-line treatments for potential gastritis and peptic ulcer. However, growing evidence suggests that the long-term intake of these agents may be unsafe, with possibility of various severe side-effects (Cekin et al., 2017; Tran-Duy et al., 2016; Waldum and Fossmark, 2018). Traditional Chinese medicine has played an

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important role in advancing complementary and alternative therapies of gastrointestinal diseases in recent years (Zhang et al., 2016). Natural products from plants and marine lives, as possible gastroprotective alternatives have become popular topics of scientific research (Chen et al., 2018c; Teschke et al., 2015). These natural products are believed to offer effective, affordable, and accessible forms of treatment. Among these natural products, polysaccharides have attracted widespread attention and have been demonstrated to confer significant protective benefits on ethanol-induced gastric injury by ameliorating oxidative stress, inflammation and cellular damage (Choi et al., 2009; Khan et al., 2018; Raish et al., 2018; Zeng et al., 2017b).

*Bletilla striata* (Thunb.) Reichb.f. (Orchidaceae), known as Baiji in Chinese, is an important astringent hemostatic medicinal plant that has been employed to treat hematemesis, hemoptysis, traumatic bleeding, skin and mucosal wounds, as well as gastrointestinal ulcer for thousands of years (He et al., 2017). Owing to its wide repertoire of therapeutic values, the application and market demand of *Bletilla striata* in clinical medication continues to expand, in the form of either single use or combination formula. Previous studies (Song et al., 2017; Wang et al., 2017) mainly focused on the phenols and organic acids contained in *Bletilla striata*, *Bletilla striata* polysaccharides (BSP) have also been demonstrated to contribute to various biological functions, especially its wound healing effect (Chen et al., 2018a; Liao et al., 2019b; Qu et al., 2016). Preliminary investigations (Chen et al., 2018b) have shown that BSP consist of 1,4-linked mannosyl residues and 1,4-linked glucosyl residues, which are demonstrated to modulate inflammatory response and immunological function (Liao et al., 2019a; Wang et al., 2018b). In our previous study, BSP have been found to play a nontrivial role in promoting oral ulcer healing as a component in buccoadhesive wafers (Liao et al., 2019a). Other studies have also indicated that due to the facile ability to form gels at low pH values, polysaccharides could act as a potent anti-gastric ulcer agent (Maeng et al., 2014; Raish et al., 2018). Nevertheless, the potential protective effect of BSP on gastric injury and its related mechanisms remain largely unclear.

In this study, we first isolated the *Bletilla striata* polysaccharide fraction using a molecular weight retention approach and characterized it based on molecular weight distribution, structure, and monosaccharide composition. The protective role of BSP on ethanol-induced gastric mucosal injury and its possible mechanisms were then determined based on promotion of cellular proliferation activity of human gastric epithelial cell strain GES-1 in rat model.

## 2. Materials and methods

### 2.1. Materials, cells and animals

Specific descriptions of chemical materials, cell culture, and animals used in this study are documented in Supplementary Materials.

### 2.2. Preparation and physicochemical characterization of *Bletilla striata* polysaccharides

Specific approaches for the preparation and physicochemical characterization of *Bletilla striata* polysaccharides (BSP) are described in Supplementary Materials.

### 2.3. In vitro antioxidant activity analysis of BSP

The radical scavenging activity of the BSP (mg/mL) was measured using an improved ABTS decolorization assay and ferric-reducing antioxidant power (FRAP) assay according to methods described in previous study (Li and Shah, 2016) with some modifications. Fresh Vc solution was used for calibration.

### 2.4. In vitro cell proliferation activity of BSP

A methyl thiazolyl tetrazolium (MTT) assay was employed to evaluate cell proliferation in the presence or absence of various concentrations of BSP (5, 10, 20 and 40  $\mu\text{g}/\text{ml}$ ) in vitro. GES-1 cells were seeded in 96-well plates at a density of  $4 \times 10^4$  cells/well. After 24 h or 48 h incubation, the cells were treated with different concentrations of BSP for another 24 h. Next, the culture medium was replaced with 5 mg/ml MTT solution in fresh medium and cells were incubated for another 4 h. Then, the supernatant was discarded and the formazan was resolved in 100  $\mu\text{l}$  DMSO. Optical density (OD) values were read with a microplate spectrophotometer at 490 nm.

### 2.5. Gastroprotective effects of BSP in rats

All animals were randomly divided into five groups of six animals each: normal control group (distilled water oral gavage), ethanol control group (EtOH group, distilled water oral gavage), BSP group (100 mg/kg BSP oral gavage), Sucralfate control group, and Suc group (100 mg/kg sucralfate oral gavage). All drugs were given once a day for 3 days. After receiving the drug at Day 2, all rats were fasted for 16 h with free access to water. After 24 h, the rats, except for those in the normal control group, were administered absolute ethanol at a dose of 6 ml/kg to induce gastric mucosal injury. Two hours later, the rats were sacrificed with pentobarbital sodium following anesthesia. The stomach was removed, opened and the washed with PBS before photos were taken. Thereafter, each stomach was cut in half. One portion was immersed in 10% formalin for histological observation, while the other portion was stored at  $-80^\circ\text{C}$  for further analysis.

Images of the stomachs were captured with a digital camera (Nikon Inc., Japan). Image-Pro Plus software (Media Cybernetics, USA) was used to calculate the ratio of the hemorrhagic ulcer area to the total area of the gastric mucosa (Zeng et al., 2017c). The stomachs were fixed in 10% formalin and embedded in paraffin. The tissues were then sectioned to 5  $\mu\text{m}$  pieces and stained with hematoxylin and eosin. The glandular portion of the tissues were stained with PAS to visualize the production of mucus and changes in the basic and acidic glycoproteins.

### 2.6. Measurement of MPO, MDA and SOD levels

Stomach tissues were homogenized in cold normal saline and centrifuged at 1200 rpm for 10 min at  $4^\circ\text{C}$ . The supernatant of the homogenate was collected and stored at  $-80^\circ\text{C}$ . The protein content of stomach sample was determined by BCA protein assay. MPO, MDA and SOD levels in stomachs were determined using various test kits (Shaker et al., 2010). All procedures were carried out according to the instruction manuals.

### 2.7. Evaluation of cytokines in gastric tissues

Levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-18 and PGE<sub>2</sub> in the gastric tissues were measured using enzyme-linked immunosorbent assay kits according to the manufacturer's specifications (Li et al., 2013). The absorbance was measured at 450 nm with a microplate spectrophotometer.

### 2.8. Western blot analysis

Proteins were extracted from stomach homogenates with a lysis buffer (RIPA buffer containing protease and phosphatase inhibitors) and subjected to Western blot analyses using anti-phospho p38 MAPK, anti-phospho extracellular signal-regulated kinases (ERK), anti-phospho-c-Jun N-terminal protein kinase (JNK), anti-phospho-NF- $\kappa\text{B}$  p65, anti-phospho-I $\kappa\text{B}\alpha$ , anti-I $\kappa\text{B}\alpha$ , anti-IKK, or anti- $\beta$ -action antibodies (Yan Fu et al., 2018). The signal density corresponding to the protein of interest versus that of  $\beta$ -action was determined as the relative density.

## 2.9. Statistical analysis

All data were expressed as mean  $\pm$  standard deviation (SD) and analyzed with Graphpad Prism 5.0 (GraphPad Software, San Diego, USA). One-way ANOVA with Bonferroni test were used to examine differences among groups. P-value of less than 0.05 was considered as significant difference.

## 3. Results

### 3.1. Preparation and physicochemical characterization of BSP

Ultrafiltration membranes of different molecular weight cut-offs were used successively to collect the main fractions of polysaccharides in *B. striata*. The final yield of BSP fraction, collected by 300 kDa membrane, was 1.23% compared to that of dry raw *B. striata* materials. The total polysaccharide content was estimated to be 82.5% approximately, while the contents of proteins and polyphenols in BSP fraction were lower than 5%. Bradford and Folin-Ciocalteus tests confirmed the absence of proteins and total phenols in BSP. Monosaccharide analysis was performed using reverse-phased HPLC and the resulting chromatogram was shown in Fig. 1A. Monosaccharide analysis revealed that BSP contains mannose (70.59%) and glucose (29.41%) at a molar ratio of 2.4:1. Additionally, the molecular weight of BSP was estimated to be 146 kDa, appearing as a single symmetric peak on the HPGPC chromatogram (Fig. 1B).

XRD was employed to determine the degree of crystallinity of BSP. As shown in Fig. 1C, the XRD pattern of BSP showed a “bun-shaped” curve with only a few small peaks at approximately 14°, 20°, 27°, 32°, and 35° 2 $\theta$  (Fig. 1C). These weak peaks indicated a low overall crystallinity at mainly amorphous regions. This XRD pattern was similar to the reported data in previous study (Kong et al., 2015).

ABTS cation radicals are generated by the oxidation of ABTS by potassium persulfate (Hu et al., 2017). Result as shown in Fig. 1D demonstrated the noteworthy ABTS scavenging ability of BSP fraction in a concentration dependent manner. Vitamin C, with its well-acknowledged strong antioxidant efficacy, was used as a positive control.

Additionally, reducing power assay was used to evaluate the potential antioxidant activity of BSP and the results showed an obvious dose-dependent relationship. Specifically, 10 mg/ml of BSP could achieve 76% of ABTS scavenging activity, and 46.2% of FRAP ability. These results suggested that BSP exhibited great antioxidant potentials.

The FT-IR spectrum of BSP displayed typical characteristics of polysaccharide profiles. Namely, a major broad stretching peak at 3419  $\text{cm}^{-1}$  for the -OH stretching vibration of polysaccharide and the weak bands at 2925  $\text{cm}^{-1}$  and 2889  $\text{cm}^{-1}$  representing the C-H stretching vibration of -CH<sub>2</sub>-. In addition, the relative strong absorption bands at 1731  $\text{cm}^{-1}$  and 1623  $\text{cm}^{-1}$  were attributed to the -COOH bending vibration and the peaks at 1422  $\text{cm}^{-1}$  and 1380  $\text{cm}^{-1}$  were due to the symmetric stretching of C=O. Furthermore, the absorptions observed at 1030–1140  $\text{cm}^{-1}$  were related to the asymmetric vibration of C-O-C glycosidic rings, indicating the presence of pyranose. The bands at 895  $\text{cm}^{-1}$  and 810  $\text{cm}^{-1}$  were characteristics of  $\beta$ - and  $\alpha$ -pyranoid glucose in the polysaccharides, respectively. Finally, the bands at 875  $\text{cm}^{-1}$  and 810  $\text{cm}^{-1}$  were assigned as the characteristic absorption peaks of mannose (Fig. 2A).

The structural observations of BSP were analyzed by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. The <sup>1</sup>H spectrum and <sup>13</sup>C spectrum exhibited a crowded region ranging from  $\delta_{\text{H}}$  3.0 to  $\delta_{\text{H}}$  4.3 and  $\delta_{\text{C}}$  60.0 to  $\delta_{\text{C}}$  110.0, as contributed by the chemical shift signal of the saccharide ring. It displayed a typical NMR pattern of polysaccharides. Two anomeric proton signals at  $\delta$  5.41 and 4.42 ppm indicated  $\alpha$ -sugar and  $\beta$ -sugar residues, respectively (Suvakanta et al., 2014) (Fig. 2B). The signals observed at around 2.1 ppm were attributed to -O-CO-CH<sub>3</sub>. The <sup>13</sup>C NMR spectrum showed the presence of sugar residues. The chemical shifts at 100.1 and 102.4 ppm provided evidence for the existence of  $\alpha$ -Man and  $\beta$ -Glc residues (Fig. 2C). Meanwhile, the characteristic peaks at 20.2 ppm and 172.9 ppm suggested that BSP contains ethanoyl and uronic acid. These results were in accordance with those from FT-IR analysis.

### 3.2. Effect of BSP on cell proliferation in vitro

MTT cell proliferation assay was employed to detect the cytotoxicity

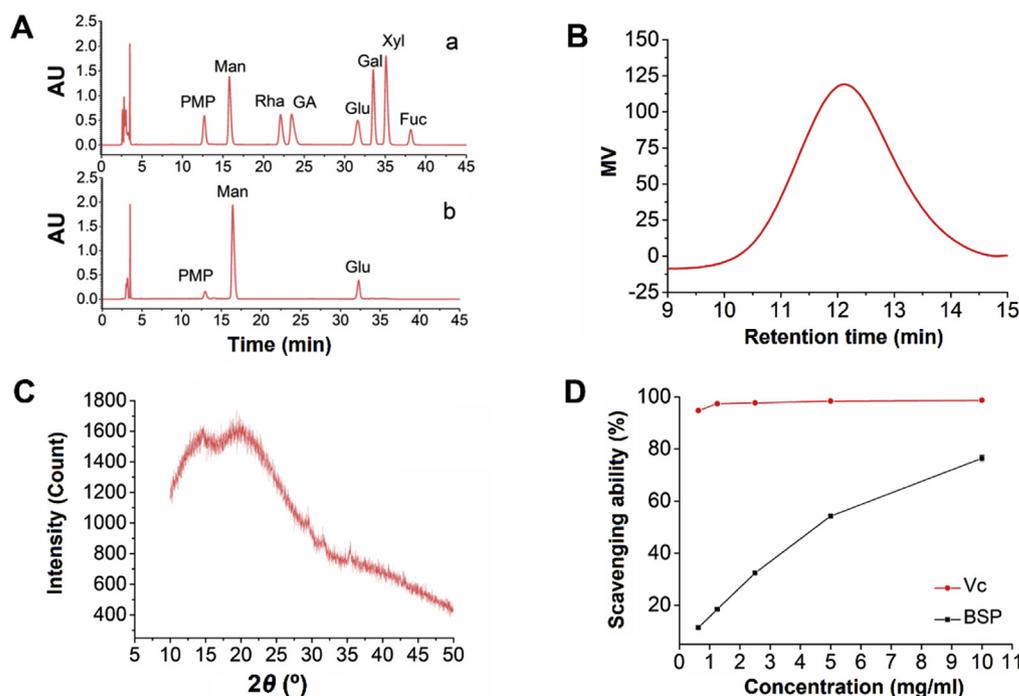


Fig. 1. The preparation of BSP: (A) monosaccharide composition analysis of mixed saccharide standards (a) and BSP (b) by HPLC analysis; (B) Molecular weight determination of BSP by HPGPC; (C) XRD patterns of BSP; (D) Antioxidant activity of BSP by ABTS scavenging assay.

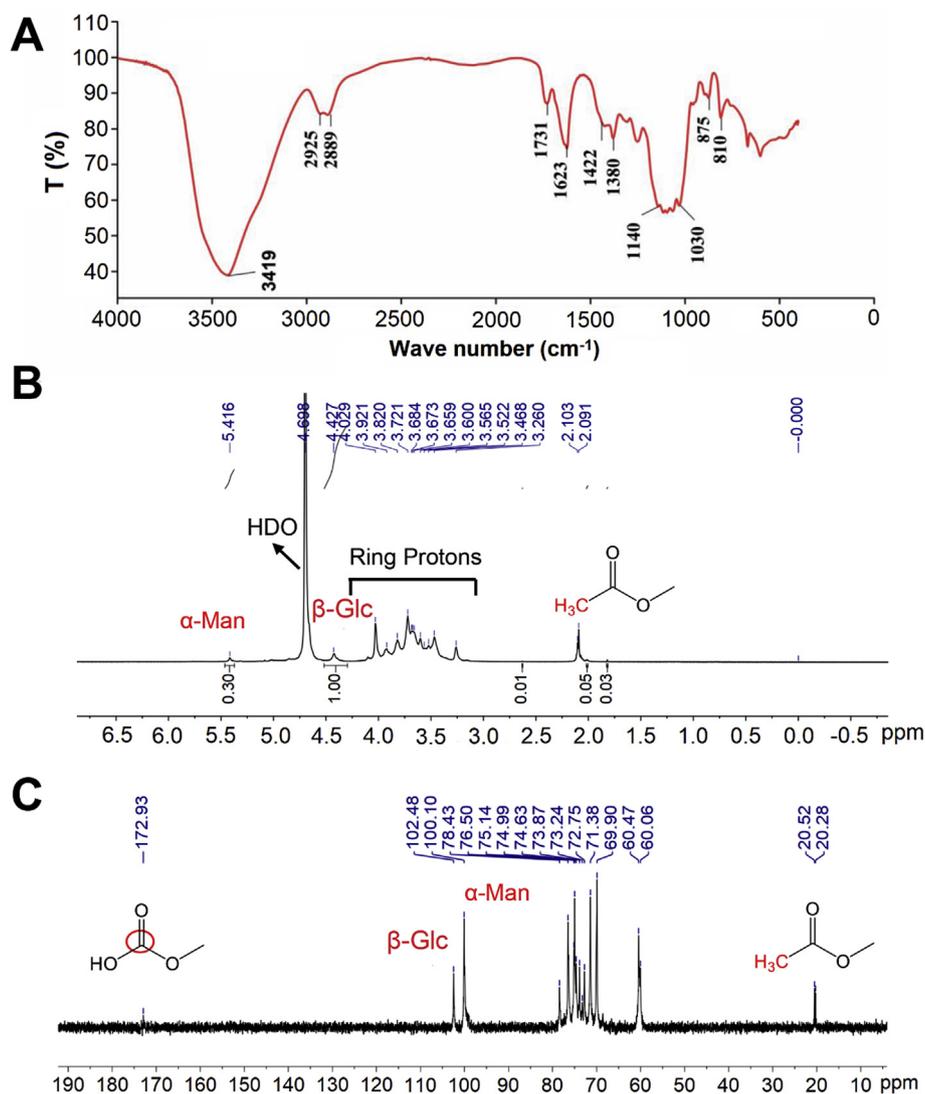


Fig. 2. Spectra of FT-IR (A),  $^1\text{H}$  NMR (B) and  $^{13}\text{C}$  NMR (C) of BSP.

of BSP on GES-1 cells. Keeping in mind that the high concentrations of polysaccharides in culture medium could potentially increase permeability of cell membrane, we asked whether  $5\ \mu\text{g}/\text{ml} \sim 200\ \mu\text{g}/\text{ml}$  of BSP could affect cell viability of GES-1 cells. As illustrated in Fig. 3, even at  $200\ \mu\text{g}/\text{ml}$ , BSP did not significantly affect cell viability of GES-1 cells after 24 h or 48 h treatment. Notably,  $5\ \mu\text{g}/\text{ml}$  and  $10\ \mu\text{g}/\text{ml}$  of BSP could slightly promote cell growth, compared with control group. These results demonstrated the non-cytotoxicity of BSP on gastric mucosa epithelial cell.

### 3.3. Effect of BSP on ethanol induced gastric lesions

Oral administration of excessive ethanol could lead to acute gastric injury, as characterized by mucosal edema, glandular area hyperemia, and linear hemorrhage necrosis. As shown in Fig. 4, extensive elongated thick, dark red and black bands of hemorrhagic gastric lesions were observed in the ethanol-stimulated rats, compared with control rats. Treatment with BSP or Sucralfate, however, could significantly mitigate the area of mucosal injury induced by ethanol.

Based on images of the stomach tissues, gastric ulceration induced by oral administration of ethanol was characterized based on the ulcer area. The gastric injury model group without other treatments exhibited an average ulcer area of 26%. After pretreatment with BSP, the ulcer area was found to have decreased by almost 80% decrease as compared

to the ulcer model group ( $p < 0.05$ ). Meanwhile, as a well-known gastric mucosa protectant, Suc treatment also led to a 92% reduction in ulcer area. These results indicated that BSP exhibited a comparative protective efficacy as Suc on ethanol-induced gastric injuries.

### 3.4. Histological evaluation of gastric lesions

Histological examination revealed comprehensive stomach mucosal damage induced by ethanol and the protective bioactivity of BSP. Compared to the non-disruption of surface epithelium in normal rats, necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer were found in rats subjected to excessive ethanol administration by H&E staining (Fig. 5). Conversely, animals pre-fed with BSP or Suc presented relatively enhanced protection on their stomach mucosa, with mild disruption of the surface epithelium. Meanwhile, rats treated with Suc also exhibited mild edema and leucocyte infiltration of the submucosal layer. Furthermore, Periodic acid-Schiff (PAS) staining was performed to examine production of total glycoproteins, including mucins, in the gastric epithelium. Mucus secretion in the gastrointestinal tract is an important mucosal defensive factor during gastric injury, the acid mucopolysaccharide in mucus serves as the main protective layer on gastric epithelium. Herein, PAS staining was employed to examine the quantity of acid mucopolysaccharide on the surface of

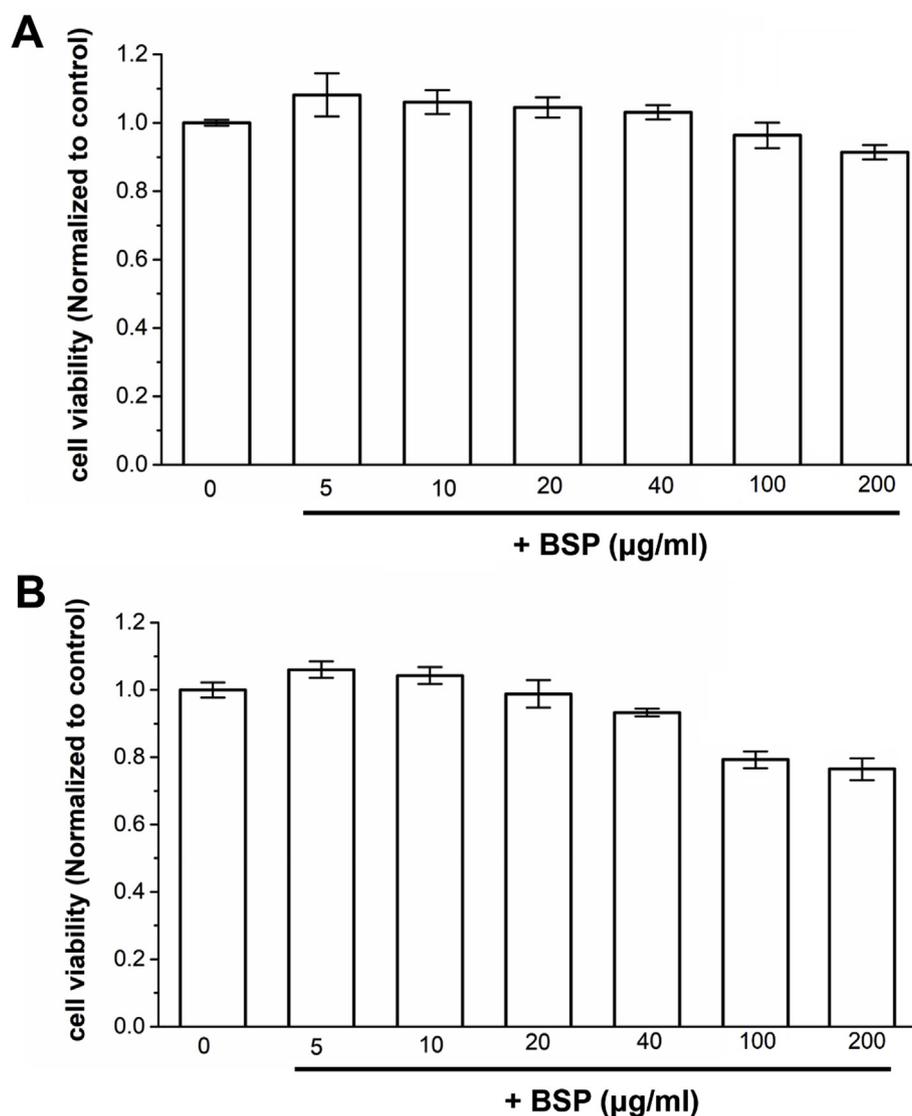


Fig. 3. Effects of various concentrations of BSP on cell proliferation of GES-1 cells after 24 h (A) and 48 h (B) treatment.

gastric mucosa as illustrated by the number of blue violet spots. As shown in Fig. 5, the ulcer model group exhibited a remarkably gradual decrease in PAS staining intensity, compared to the normal group. The gastric mucosa in the pre-treatment BSP and Suc groups exhibited a significant increase in PAS positive staining intensity. These results were supportive of the gastroprotective activity of BSP on gastric mucosa against ethanol damage.

### 3.5. Effects of BSP on pro-inflammatory cytokine production

To further evaluate the role of inflammation in gastric mucosa, the levels of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-18, in the gastric tissues were determined by ELISA kits. The gastric mucosal cytokine levels were significantly enhanced in ethanol-induced rats. The levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-18 in ethanol-induced rats increased by approximately 9.34-fold, 71.61-fold, 6.86-fold, and 3.88-fold of normal rats, respectively ( $P < 0.05$ ). Rats administrated with Suc pretreatment displayed a dramatic drop in inflammatory cytokines as compared to ethanol-induced ulcerated rats without any treatment. Interestingly, BSP pretreatment also suppressed the increase in TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-18 concentrations in stomach tissue by 46.24%, 67.58%, 35.85%, and 43.57%, respectively. Although the decreases induced by BSP were milder than those resulted from Suc pretreatment,

these results provided clear evidence that BSP pretreatment significantly restricted the production of pro-inflammatory cytokines and suppressed inflammation of stomach tissues (Fig. 6).

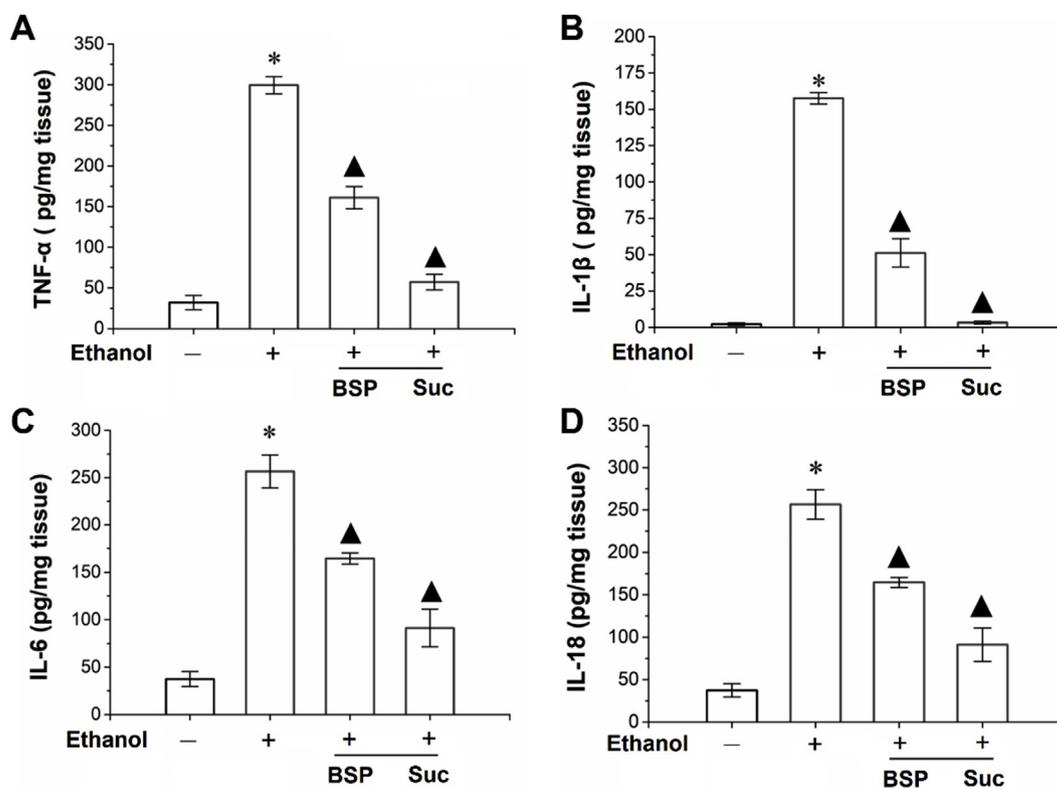
### 3.6. Effects of BSP on the production of PGE<sub>2</sub>, MPO, MDA and SOD

PGE<sub>2</sub> was also implicated to play an important cytoprotective role in the gastric mucosa. Depletion of PGE<sub>2</sub> was contributive to ethanol-induced mucosal damage. The mucosal level of PGE<sub>2</sub> in ethanol-induced ulcer rats was markedly suppressed by 56.13%, compared to normal control ( $P < 0.05$ ) (Fig. 7A). Whereas, pretreatment with BSP up-regulated the mucosal PGE<sub>2</sub> level by 76.19%, compared to ulcerated rats.

MPO is a key marker for assessing degree of neutrophil infiltration into gastric mucosa in gastric injury model (Hamaguchi et al., 2001). As shown in Fig. 7B, the MPO level was considerably increased by 3.78-fold in ulcerated rats, compared to their normal counterparts ( $P < 0.05$ ). This observation suggested an increased neutrophil influx into the gastric mucosa in ethanol-treated rats. However, the increased MPO level was distinctly mitigated by pretreatment of BSP or Suc, as demonstrated by a significant decrease of 31.36% and 17.31% in MPO level, respectively, compared with that in ulcerated rats.

Oxidative stress is a critical pathogenic factor during gastric mucosa injury. MDA and SOD are critical indexes of lipid peroxidation (Girotti,



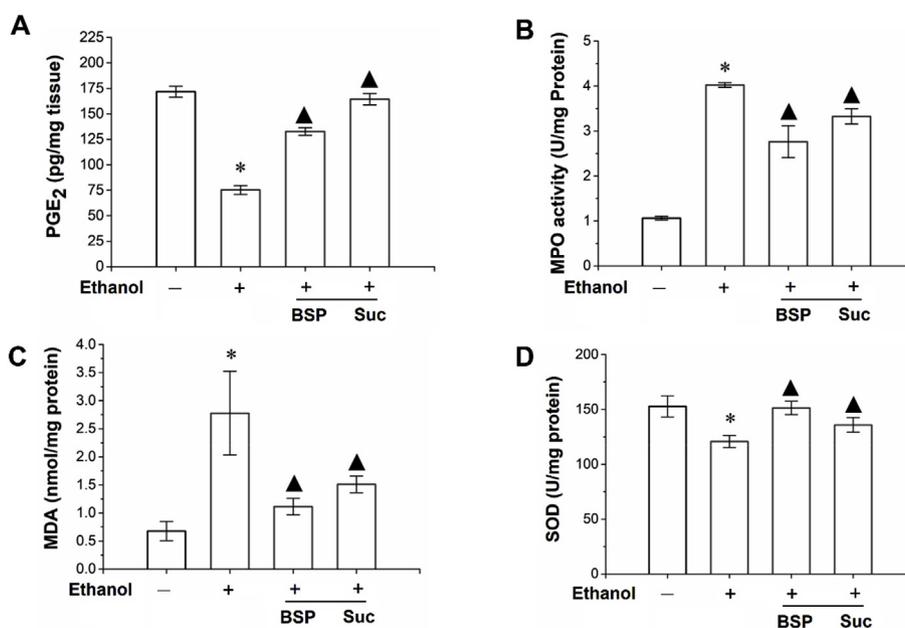


**Fig. 6.** Effects of BSP pretreatment on gastric mucosal inflammatory cytokines in ethanol-treated ulcerate rats, including TNF- $\alpha$  (A), IL-1 $\beta$  (B), IL-6 (C), and IL-18 (D), by ELISA measurement (n = 6). Note: \*P < 0.05 vs. normal control group, ▲P < 0.05 vs. ethanol-induced ulcer model group.

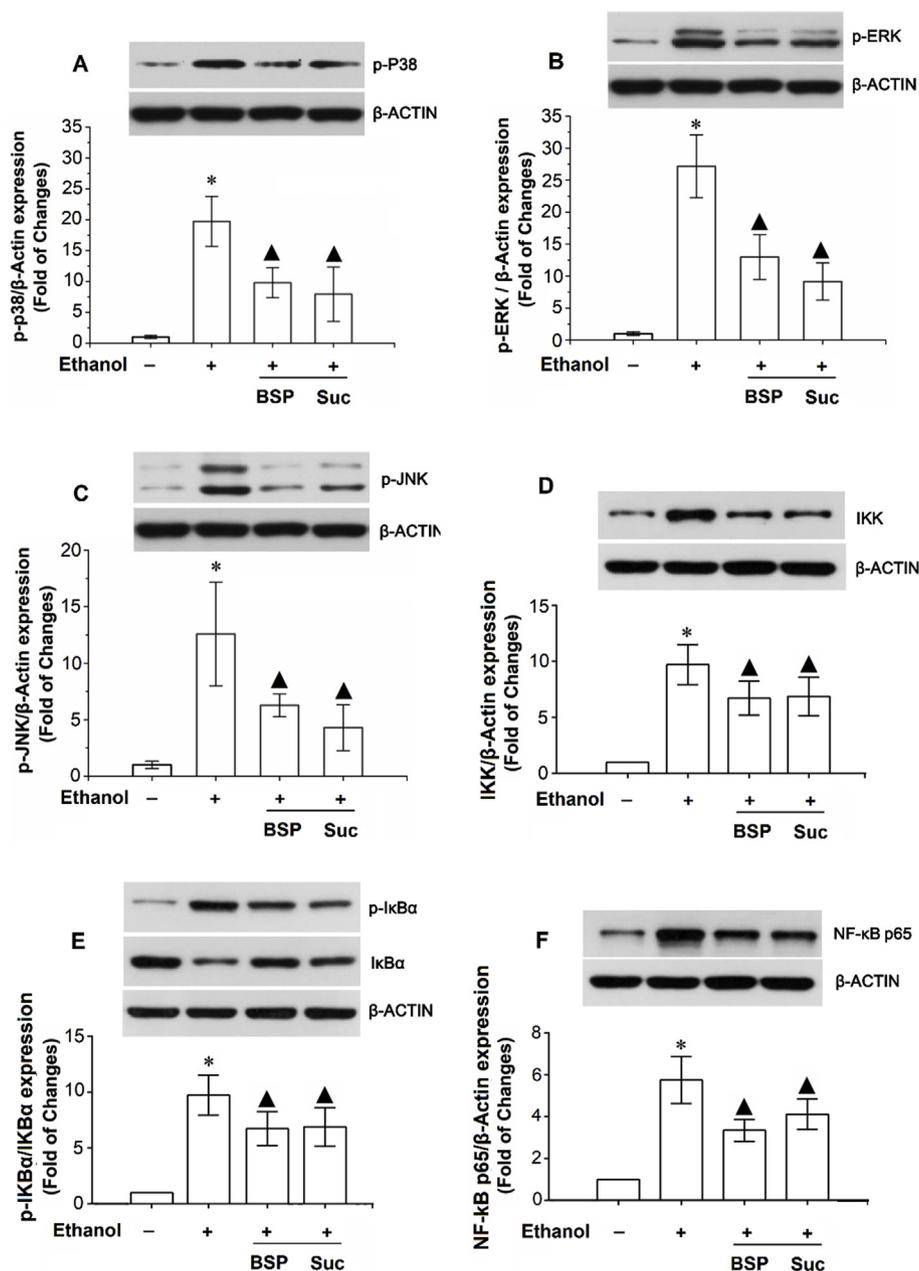
1985). We found that excessive ethanol could increase MDA level and decrease SOD content, compared to those in normal control group (Fig. 7C and D). BSP pretreatment successfully reduced MDA production and elevated SOD bioactivity. These results corroborated to indicate that BSP could relieve the oxidative stress and neutrophil influx induced by excessive ethanol administration.

3.7. BSP blocked ethanol-induced activation of MAPK and NF- $\kappa$ B pathways

In view of the oxidative stress and inflammation of ethanol-induced injury as mentioned above, we elucidated an underlying mechanism of BSP effect via the MAPK and NF- $\kappa$ B signal pathways, which are commonly involved in inflammatory signaling cascades (Fu and Wu, 2018; Kaminska, 2005). The activation of three major subgroups in MAPK family, i.e. ERK1/2, JNK, and p38, by phosphorylation was evaluated.



**Fig. 7.** Effects of BSP pretreatment on mucosal levels of PGE<sub>2</sub> (A), MPO (B), MDA (C) and SOD activity (D) in ethanol-induced ulcerated rats (n = 6). Note: \*P < 0.05 vs. normal control group, ▲P < 0.05 vs. ethanol-induced ulcer model group.



**Fig. 8.** Effects of BSP on MAPK pathway phosphorylation/activation and NF- $\kappa$ B related signaling pathway. All tests were performed for three times independently. Note: \*P < 0.05 vs. normal control group, ▲P < 0.05 vs. ethanol-induced ulcer model group.

As shown in Fig. 8, expression of p-p38, p-JNK, and p-ERK1/2 in the ulcerated group was significantly up-regulated, compared with normal control group. As expected, pretreatment of BSP or Suc decreased expression of MAPK family proteins (Fig. 8A–C). Moreover, ethanol treatment also activated the NF- $\kappa$ B signaling pathway in gastric tissue, with increased expression of IKK, I $\kappa$ B $\alpha$ , and NF- $\kappa$ B p65. Likewise, BSP pretreatment could significantly decrease activation of NF- $\kappa$ B signaling (Fig. 8D–F). These results indicated that BSP exert its gastro-protective effects by means of blocking the MAPK and NF- $\kappa$ B signaling networks.

#### 4. Discussion

Excessive ethanol consumption can lead to serious health problems, such as liver disease, acute gastric mucosal injury and so on (Williams et al., 2014). After ingestion, ethanol diffuses into the gastric mucosa and triggers oxidative stress and inflammatory signaling pathways (Bagchi et al., 1998; Zeng et al., 2017a), resulting in stomach damage

conclusively. Reports demonstrated that high concentration of ethanol exposure would induce high levels of ROS in the gastric tissues and aggravates lipid peroxidation. Polysaccharides widely existed in natural plants or herbs have been reported to exhibit immunomodulatory, antitumor, antioxidant and hypoglycemic effects. Especially, natural polysaccharides possess protective activity against oxidative injury including gastric disorders like ulcer and gastritis in numerous studies. For example, Zeng et al. (2017b) reported that the protective effects and underlying mechanism of polysaccharides from *Dendrobium officinale* Kimura & Migo (Tie Pi Shi Hu) on gastric mucosal injury, through inhibiting oxidative stress-induced apoptosis by via suppression of NF- $\kappa$ B activation and downregulating Bax/Bcl2 ratio in gastric mucosa. Moreover, prophylactic administration of polysaccharides from *Momordica charantia* reduced ethanol-induced gastric injury in rats through the suppression of gastric inflammation and oxidative stress, predominantly via NF- $\kappa$ B inhibition (Raish et al., 2018). In present study, we revealed that ethanol exposure causes typical

macroscopic changes in the gastric mucosa of rats, including adenoidal hyperemia, mucosal edema, point and line hemorrhage. Interestingly, BSP pretreatment significantly mitigated macroscopic and microscopic damage caused by ethanol challenge.

Potential anti-ulcer drugs can exert their protective effects against mucosal damage in ulcerative gastric tissue by inhibiting neutrophils infiltration, as neutrophil accumulation in the gastric mucosa has been shown to induce abnormal microcirculation. Permeation and accumulation of leukocytes in the gastric mucosa are usually assessed based on MPO activity (Chen et al., 2016), which has been widely utilized as a standard index for assessing the level of neutrophil infiltration in gastric injuries and human gastric ulcer. In our study, MPO activity of gastric tissue in the BSP pre-treatment group was lower than that in the ethanol induced ulcer group, confirming an effect of BSP on the reduction of inflammatory response. Additionally, our results also revealed a key role of oxidative stress in the pathogenesis of ethanol induced gastric ulcer (Bhattacharyya et al., 2014). SOD has been shown to participate in various physiological activities, including anti-inflammatory and antioxidant properties (Tu et al., 2017). As the polyunsaturated fatty acid composition of the final product, MDA is used to assess lipid peroxidation in the experimental design of reactions (Kwiecien et al., 2014). Our results showed that BSP could increase SOD activity and decrease the levels of MDA and MPO in gastric tissue. Taken together, our study demonstrated that BSP exhibits gastric protective properties that could potentially alleviate neutrophil hyperplasia and lipid peroxidation induced by ROS levels through the antioxidant system.

One of the pathogenic mechanisms of gastric ulcers involves imbalanced factors such as gastric acid and protective factors, including cytokines (Glavin and Szabo, 1992; Playford and Ghosh, 2005). Different cytokine networks contain a variety of cell ulcerative factors, which ultimately lead to enhanced development of gastric mucosal injury. TNF- $\alpha$ , for example, is known to stimulate neutrophil infiltration, IL-1 $\beta$  production and epithelial cell apoptosis, reduces microcirculation around the ulcer area and delays healing of gastric ulcer (Odashima et al., 2006). Moreover, over-production of IL-6 could activate the inflammatory sites of neutrophils, which trigger oxidative stress and lysosomal enzymes that are responsible for tissue damage in peptic ulcer disease (Wang et al., 2018a). The pro-inflammatory factor, IL-18, could increase the secretion of other cytokines and regulate the expression levels of adhesion molecules in inflammatory cells (Arend et al., 2008). Our results indicated that BSP could inhibit inflammatory cytokine levels (TNF- $\alpha$ , IL-1, IL-6 and IL-18), which implicated the anti-inflammatory effects of BSP in ethanol-induced gastric ulcers.

As an endogenous gastric protective factor, PGE<sub>2</sub> are responsible for modulating the integrity of gastric mucosa and regulating gastric pH and mucus secretion (Kim and Ho, 2010). The inhibitory effect of ethanol on PGE<sub>2</sub> levels in gastric mucosa is due to the presence of oxidative damage leading to the conversion of prostaglandins to oxidation products (Li et al., 2016). However, pre-treatment with BSP markedly increased PGE<sub>2</sub> level. Our results revealed that BSP might reverse gastric mucosal integrity, along with gastric pH and mucus secretion regulation.

MAPK, the common pathway of intracellular information transmission, is involved in extracellular signal transduction from the surface to the interior of cells (Li et al., 2017; Pearson et al., 2001). The three major subgroups of MAPK, ERK, JNK and p38 MAPK, require activation by upstream kinase phosphorylation to perform their biological functions. The activation of major subgroups in the MAPK family would regulate the expression of pro-inflammatory mediators, a phenomenon that has been reported in ethanol-induced gastric ulcers (Zhang et al., 2018). Furthermore, it is well known that activation of MAPK cascade and NF- $\kappa$ B transcriptional pathways is necessary in many inflammatory and immunomodulatory diseases. Ethanol, as a well-recognized irritating agent, has been demonstrated to induce inflammation in the gastric mucosal epithelial cells and activate NF- $\kappa$ B pathway. In this study, we found that BSP pre-treatment could prevent

the activating phosphorylation of ERK, JNK and p38 MAPK, as well as IKK/I $\kappa$ B $\alpha$ /NF- $\kappa$ B p65, after ethanol stimulation. This study also indicated that BSP could suppress the production of inflammatory cytokines induced by ethanol, and that the mechanism could be related to its inhibition of the MAPK/NF- $\kappa$ B pathway.

## 5. Conclusion

In conclusion, this study corroborated, for the first time, that polysaccharide fraction isolated from *Bletilla striata* exhibited a strong gastroprotective effect against ethanol-induced acute gastric lesions in rats. The gastroprotective mechanisms of BSP were primarily related to mitigation of oxidative stress, neutrophil infiltration, and inflammatory cytokines accumulation. Specifically, the inhibition of MAPK/NF- $\kappa$ B signaling pathway activation was mediated by BSP. Moreover, the pretreatment with BSP also promoted the production of acid mucus and up-regulated endogenous PGE<sub>2</sub> production, which protected the gastric mucosa from damage induced by ethanol. Therefore, BSP fraction could be a promising source for novel therapeutic agents for the prevention of gastric ulcer. However, further studies are required to elucidate the exact chemical structure and gastroprotective mechanisms of BSP.

## 6. Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC, no. 81373987, no. 81603309), Young Elite Scientists Sponsorship Program by CAST (2018QNR001) and Program for Excellent Talents in Chengdu University of Traditional Chinese Medicine.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2019.05.047>.

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