Protective effect of valerian extract capsule (VEC) on ethanol- and indomethacin-induced gastric mucosa injury and ameliorative effect of VEC on gastrointestinal motility disorder

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ABSTRACT
Context: Valerian extract capsule (VEC) is an effective Chinese patent medicine used for gastrointestinal (GI) diseases.
Objective: To investigate the detailed pharmacological activity for VEC clinical effects in GI diseases.
Materials and methods: Sprague-Dawley rats were divided into six groups: control, model, and drug-treated (VEC-L, VEC-M, VEC-H, and teprenone). Rats were orally administered VEC (124, 248, 496 mg/kg) and teprenone (21.43 mg/kg) for 3 consecutive days. After 1 h, the five groups (except the control group) were orally given ethanol (10 mL/kg) for 1 h or indomethacin (80 mg/kg) for 7 h. The spasmylatic activity of VEC (0.01–1 mg/mL) on ACh/BaCl₂-induced New Zealand rabbit smooth muscle contraction was performed. The C57BL/6 mice carbon propelling test evaluated the effects of VEC (248–992 mg/kg) on intestinal motility in normal and neostigmine/adrenaline-induced mice.
Results: Compared with the model group, VEC treatment reduced the gastric lesion index and mucosal damage. Further experiments showed that the pathological ameliorative effect of VEC was accompanied by augmentation of the enzymatic antioxidant system and cytoprotective marker (COX-1, p < 0.01; PGII, p < 0.05), along with the alleviation of the levels of MPO (ethanol: 15.56 ± 0.82 vs. 12.15 ± 2.60, p < 0.01; indomethacin: 9.65 ± 3.06 vs. 6.36 ± 2.43, p < 0.05), MDA (ethanol: 1.66 ± 0.44 vs. 0.81 ± 0.58, p < 0.01; indomethacin: 1.71 ± 0.87 vs. 1.09 ± 0.43, p < 0.05), and inflammatory mediators. VEC decreased the high tone induced by ACh/BaCl₂ and promoted intestinal transit in normal and neostigmine/adrenaline-induced mice.
Discussion and conclusions: VEC showed a potential gastroprotective effect, suggesting that VEC is a promising phytomedicine for the treatment of GI diseases.

Introduction
Gastrointestinal (GI) diseases, such as peptic ulcer, chronic gastritis, and functional dyspepsia, are highly prevalent and generally considered to be a leading cause of the incidence of several other concomitant diseases. GI mucosal as a barrier plays a pivotal role in the protection of digestive organs and the damage of gastric mucosa is considered the early stage of gastric ulcer (Woolf and Rose 2021). Previous studies reported that gastric mucosa damage is attributable to multiple factors including chemical factors (smoking, drinking, and drugs), physical factors (improper diet), inflammation, intestinal bacterium, and physiological stress (Padol et al. 2012; Haj Kheder et al. 2018; Zhou and Zhang 2019; Woolf and Rose 2021). Among the various reasons contributing to gastric injury, non-steroidal anti-inflammatory drugs (NSAIDs) indomethacin can depress the expression of cyclooxygenases (COXs) and increase the free radical formation and excessive generation of inflammatory mediators, thus inducing gastric mucosal damage or gastric ulcer (Abd EI-Rady et al. 2021). In addition, alcohol is another common risk factor for GI disease. Excessive drinking may cause the appearance of bleeding or stomach ulcers by rupturing the gastric mucosa barrier and inducing inflammatory cell infiltration (Ostaff et al. 2015). Recently, the animal models of gastric injuries induced by indomethacin and ethanol have been widely used to investigate the underlying pathophysiological and evaluate the protective effects of herbal medicines against gastric ulcers and gastritis (Dejban et al. 2020).
GI motility is a highly complex physiological process and the disruption of the natural rhythm of GI contractions can also lead to various GI diseases including irritable bowel syndrome (IBS), functional dyspepsia, gastroesophageal reflux, and other symptoms (e.g., diarrhea, vomiting, and abdominal cramps) (Li et al. 2018). Increased GI motility and potential inflammation can change the intestinal environment like the expansion of Enterobacteriaceae and then may contribute to IBS (Pittayanon et al. 2019). Moreover, it has been reported that diabetes patients with GI dysfunction had a slow intestinal propulsion rate (Tian et al. 2017).

Available antulcer drugs and motility-modifying drugs, chemical drugs are common but have various side effects. Thus, it is necessary to develop novel drugs that are safer, effective, and readily accessible in the treatment of gastric ulcers and GI motility disorder. Valeriana officinalis L. (Caprifoliaceae), also called valerian, native to Europe and Asia, is commonly used as a medicinal plant to treat anxiety, sleep disorder, depression, and GI hyperactivity (Houghton 1999; Poyares et al. 2002; Muller et al. 2003; Taavoni et al. 2011; Mineo et al. 2017; Tammadon et al. 2021). Prior work indicates that the extract of *V. officinalis* has antioxidant and antispasmodic effects (Malva et al. 2004; Circosta et al. 2007; Sudati et al. 2009). However, most existing research results associated with valerian or its extracts in treating gastric mucosa damage were reported.

Valerian extract capsule (VEC) is made from the root and rhizome of *V. officinalis*, which is an effective Chinese patent medicine for the treatment of stomach pain and bloating caused by GI diseases such as chronic gastritis and functional dyspepsia. VEC is reported to improve clinical symptoms significantly, but there is no compelling evidence of its efficacy for improving GI diseases. This study aims to evaluate the detailed pharmacological activity and efficacy of VEC for the treatment of GI diseases. Therefore, we investigated the protective effects of VEC against ethanol- and indomethacin-induced gastric mucosa injury in rats. In addition, to explore the effect of VEC on GI motility, the spasmyloytic activities of VEC were investigated by ACh or BaCl₂-induced rabbit duodenum and ileum contractions. Meanwhile, the regulatory effects of VEC on small intestinal motility in the normal and neostigmine-induced mice were also done.

### Materials and methods

#### Reagents

The valerian extract capsule was obtained from Wuhan United Pharmaceutical Co. Ltd. (Hubei, China). Teprenone was purchased from Eisai China Inc. (jiangsu, China). Indomethacin was acquired from Sigma (St. Louis, MO, USA). Pinaverium bromide was purchased from Abbott (Chicago, IL, USA). Atropine was purchased from Tianjin Kingyork Pharmaceuticals Co., Ltd. (Tianjin, China). Neostigmine methylsulfate injection was purchased from Shanghai Sine Jinzhu Pharmaceutical Co., Ltd. (Tianjin, China). Neostigmine methylsulfate injection was purchased from Tianjin Kingyork Pharmaceuticals Co., Ltd. (Tianjin, China). Atropine was acquired from Sigma (St. Louis, MO, USA). Pinaverium bromide was purchased from Abbott (Chicago, IL, USA). Teprenone was purchased from Eisai China Inc. (Jiangsu, China). Indomethacin was obtained from Wuhan United Pharmaceutical Co. Ltd. (Hubei, China). The enzyme-linked immunosorbent assay (ELISA) kits measured MPO, MDA, SOD, GSH-PX, PAF, IL-1β, TNF-α, PGE2, PG12, and COX-1 were purchased from Abbkine Scientific Co. Ltd. (Santa Cruz, CA, USA).

#### Characterisation of the VEC by liquid chromatography-coupled with diode array detection and high-resolution mass spectrometry (UPLC-QTOF-MS)

The UPLC-QTOF-MS analysis was performed according to the method described in our previous work (Chen et al. 2019). Ultrapure water with 0.1% formic acid (A) and methanol (B) constituted the mobile phase, and the gradient elution programs for positive are shown in (Table 1). Powder of VEC was dissolved into 70% methanol to 20 mg/mL and then ultrasonic extracted at ambient temperature for 1 h. Data were analysed by MassHunter Workstation software (version B.04.00).

#### Animals

Healthy male C57BL/6 mice weighing 18 ~ 20 g, Sprague-Dawley (SD) rats weighing 200 ~ 220 g, and New Zealand rabbits weighing 2 ~ 2.5 kg were purchased from Guangdong Medical Laboratory Animal Centre and housed in the experimental animal centre of Guangzhou University of Chinese Medicine. All experimental procedures were approved by the committee of animal ethics at Guangzhou University of Chinese Medicine (NO.2016050).

#### Gastric mucosa damage induced by anhydrous ethanol

After 3 days of adaptive feeding, rats were divided into six groups (*n* = 8) randomly. Control group (distilled water), model group (distilled water), VEC-treated groups (124, 248, and 496 mg/kg), and positive control group (teprenone, 21.43 mg/kg) were administered once a day for 3 days by gavage. The dosage of teprenone for the positive control group was the adult (weighed 70 kg) dose in the clinic (take one capsule of 50 mg three times a day). Rats fasted for 24 h before the last treatment. The five groups (except the control group) were intragastrically administered with anhydrous ethanol (10 mL/kg) 1 h after the last medication. After 1 h, rats were narcotised with 10% chloral hydrate and sacrificed for sampling. The levels of MPO, MDA, SOD, and GSH-PX in the mucosa and the levels of PAF, IL-1β, and TNF-α in serum were detected by ELISA.

#### Gastric mucosa damage induced by indomethacin

The grouping and drug administration for this experiment were performed as the method described in the former part. Rats fasted for 24 h before the last treatment. The five groups (except the control group) were orally administered with indomethacin (80 mg/kg, dissolved in 5% NaHCO₃) 1 h after the last medication. After 7 h, rats were narcotised with 10% chloral hydrate and sacrificed for sampling. The levels of MPO, MDA, SOD, and GSH-PX in the mucosa and the content of PG12, PGE2, and COX-1 in serum were measured by ELISA.

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**Table 1. HPLC gradient elution program.**

| Time (min) | A% (0.1% formic acid in H₂O) | B% (methanol) |
|-----------|------------------------------|---------------|
| 0         | 95                           | 5             |
| 30        | 0                            | 100           |
| 30.01     | 0                            | 100           |
| 35        | 0                            | 100           |
| 35.01     | 95                           | 5             |
| 38        | 95                           | 5             |
Table 2. Compounds identified from VEC by UPLC-QTOF-MS.

| Compd. | Molecular formula | Selected ion | Experimental | Error (ppm) | MS/MS fragmentation | Identification |
|--------|-------------------|--------------|--------------|-------------|---------------------|----------------|
| 1      | C₂₃H₄₂O₁₅         | [M + Na]⁺    | 765.3638     | 3.5         | 683.4139 641.4059 540.3132 467.2863 | Valeriotetrate A |
| 2      | C₂₃H₄₄O₁₄         | [M + Na]⁺    | 701.2793     | –0.8        | 463.2391 277.1312 217.1274 377.2359 | Jatamanvaltrate D |
| 3      | C₂₃H₄₂O₁₄         | [M + H]⁺     | 659.3260     | 1.9         | 301.1736 259.1518 299.1568 134.9958 | 10-Acetoxy-7-[(α-hydroxyisovaleroxy)-5-hydroxy-1-isovaleroxy]-11-[α-(isovaleroxy)isovaleroxy]-5,6-dihydrorvaltrate hydrin |
| 4      | C₂₃H₄₂O₁₂         | [M + H]⁺     | 479.2127     | 0.2         | 419.2114 277.1308 245.1309 203.0655 | Suspensolide F |
| 5      | C₂₃H₄₂O₁₀         | [M + K]⁺     | 535.1964     | –1.8        | 458.2372 363.2546 341.2099 321.2025 | 10-Acetoxy-1-homovaltride hydrin |
| 6/7/8  | C₁₂H₂₀O₉          | [M + NH₄]⁺   | 458.2372     | 1.8         | 363.2546 341.2099 321.2025 421.2278 | “Jatamanvaltrate J/Jatamanvaltrate K/S-hydroxydidrovaltrate” changed to “Jatamanvaltrate J/Jatamanvaltrate K/S-Hydroxydidrovaltrate”. Acevaltrate/1-β-Acevaltrate |
| 9/10   | C₁₂H₂₀O₁₀         | [M + H]⁺     | 481.2062     | 1.2         | 421.2278 377.2347 335.1480 319.1869 | Jatamanvaltrate J/Jatamanvaltrate K/S-Hydroxydidrovaltrate” |
| 11/12  | C₁₂H₂₀O₄          | [M + Na]⁺    | 315.1552     | 2           | 273.1352 235.1068 259.1518 319.1869 | Acetoxyvaleric acid D/Vovalerenal A |

Assessment of gastric mucosal injury index and evaluation of pathological injury

The stomachs were removed and fixed with 10 mL of 4% formaldehyde solution for 10 min after the rats were sacrificed. Each stomach was opened longitudinally, cleaned, and spread for measuring the length of lesions. The scoring system based on previous studies (Cantarella et al. 2007; Sun et al. 2018) was as follows: 0 = no lesions, 1 = lesions < 1.5 mm length, 2 = lesions between 1.5 mm and 3 mm length, 3 = lesions > 3 mm length. The cumulative scores of all lesions in a rat served as the gastric lesion index of the rat.

Rat gastric tissues were collected and fixed with formaldehyde solution. The stomach tissues were paraffin-embedded, sectioned, and stained with hematoxylin-eosin (H&E) in the First Affiliated Hospital of Guangzhou University of Chinese Medicine. The condition of the mucosa and glands and the degree of inflammation and injury were observed in every section.

Examination of biochemical parameters

The rats were anaesthetised with 10% chloral hydrate. Then, peripheral blood was obtained from the abdominal aorta. After standing for 30 min, all blood samples were centrifuged at 3000 rpm for 15 min to collect serum. The serum samples obtained were stored at –80°C before further analysis. The levels of PAF, IL-1β, TNF-α, PGI2, PGE2, and COX-1 in serum were measured by ELISA kits according to the manufacturer’s instructions.

The gastric mucosa tissues were homogenised with phosphate buffer and centrifuged at 5000 rpm for 20 min at 4°C. The supernatants were assessed for MPO, MDA, SOD, and GSH-PX in the mucosa and were performed according to the manufacturer’s instructions.

Preparation of intestinal segments and experimental protocols

The segments of the rabbit duodenum and the ileum were isolated and emptied of their contents. The longitudinal duodenal and ileal segments of 1–1.5 cm in length were prepared. The preparations were suspended in an organ bath containing 20 mL of Tyrode’s solution at a constant temperature (37.0 ± 1°C) and continuously gassed with 95% O₂ and 5% CO₂ for recording mechanical activity. The influence of the VEC on the duodenum and ileum was investigated using acetylcholine (ACh, 0.01 mg/mL) and barium chloride (BaCl₂, 0.1 mg/mL) as agonists. Atropine (0.01 mg/mL) and pinaverium bromide (0.1 mg/mL) were used as positive control. The inhibitory rates of VEC (0.01, 0.1, and 1 mg/mL) on ACh- or BaCl₂-induced smooth muscle contractility were measured.

Intestinal propulsion rate testing in normal mice

After 3 days of adaptive feeding, mice were divided into five groups (n = 20) randomly. The control group (distilled water) and VEC-treated groups (248, 496, and 992 mg/kg) were orally administered at a volume of 20 mL/kg once a day for 3 days. The positive control group was given atropine (50 mg/mL) by subcutaneous injection in a volume of 15 mL/kg. Small intestinal transit rates in 12 h fasted mice were measured using the carbon propelling test. A test meal containing carbonic ink (0.05 mg/mL) was intragastrically administered in a volume of 10 mL/kg to mice 30 min after the last administration. Mice were sacrificed by cervical dislocation after 20 min. Immediately, the small intestine was removed. The lengths of the whole intestinal tract and the distance of ink propulsion were measured. The percentage of blackened intestinal tracts was calculated: intestinal propulsion rate (%) = pushing length/total length × 100%.
Intestinal propulsion rate testing in neostigmine-induced mice

After 3 days of adaptive feeding, mice were divided into six groups ($n = 20$) randomly. The control group (distilled water), model group (distilled water), and VEC-treated groups (248, 496, and 992 mg/kg) were orally administered at a volume of 20 mL/kg once a day for 3 days. The positive control group was given atropine (50 mg/mL) by subcutaneous injection in a volume of 15 mL/kg. Mice fasted for 12 h before the last treatment. The five groups (except the control group) were given neostigmine (0.12 mg/kg) by subcutaneous injection in a volume of 15 mL/kg 30 min after the last medication. After 15 min, carbonic ink (10 mL/kg) was intragastrically administered. After 20 min, the mice were sacrificed. The small intestinal propulsion rate was calculated according to the method described in the previous section.

Intestinal propulsion rate testing in adrenaline-induced mice

After 3 days of adaptive feeding, mice were divided into six groups ($n = 20$) randomly. The control group (distilled water), model group (distilled water), VEC-treated groups (248, 496, and 992 mg/kg), and positive control group (mosapride, 3.86 mg/kg) were orally administered in a volume of 20 mL/kg once a day for 3 days. Mice fasted for 12 h before the last treatment. The five groups (except the control group) were given adrenaline (0.5 mg/kg) by subcutaneous injection in a volume of 10 mL/kg 10 min after the last medication. Carbonic ink (10 mL/kg) was simultaneously intragastrically administered. After 30 min, the mice were sacrificed. The small intestinal propulsion rate was calculated according to the method described in the previous section.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) and evaluated with Stata 12.0. The significance of differences among the experimental groups was tested by one-way analysis of variance (ANOVA) test with post hoc contrasts by Student-Newman-Keuls test. Differences were considered statistically at $p < 0.05$. 

Figure 1. Base peak chromatogram and structure of identified compounds. (A) Base peak chromatogram using the positive ionisation mode of VEC by UPLC-QTOF-MS. (B) Structures of compounds identified from VEC by UPLC-QTOF-MS. Compounds are numbered according to Table 2. VEC: valerian extract capsule.
Results

Chemical analysis of VEC

UPLC-QTOF-MS analysis was performed to analyse and identify the chemical constituents of VEC. The base peak chromatogram of VEC using the positive ionisation mode and the various chemical contents analysed by UPLC-DAD-MS and -MS/MS from VEC were shown in Table 2 and Figure 1. The characterisation of VEC was based on the generation of molecular formula generated by accurate mass measurements, the comparison of MS/MS fragmentation rules from the literature, and information retrieved from chemical databases. As a result, 12 compounds consisting of 10 iridoids and 2 germacrane-type sesquiterpenoids were identified in our tentative to characterise the VEC.

VEC alleviates anhydrous ethanol-induced gastric mucosal injury

To determine the role of VEC in the protection of gastric mucosal injury, anhydrous ethanol was used to induce mucosal damage. Herein, teprenone was used as a positive control. The gastric mucosal lesion index is presented in Figure 2A. The gastric lesion index (GLI) in the ethanol-treated group was 81.33 ± 40.31, whereas that in rats pre-treated with VEC or teprenone was 61.08 ± 34.45 (VEC-L), 75.83 ± 37.26 (VEC-M), 53.50 ± 16.78 (VEC-H), and 76.33 ± 14.49 (teprenone), respectively. Compared with the model group, the GLI of the VEC-H group was significantly decreased (p < 0.05). Furthermore, H&E staining results showed that congested blood vessels and atrophied glands were observed in the ethanol-treated group. In contrast, the gastric mucosal damage induced by ethanol was mitigated in VEC-treated groups (Figure 2B).

Myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) were used as biochemical parameters to measure the level of oxidative stress and the degree of tissue injury in gastric mucosa. As shown in Figure 3, compared with the control, MPO and MDA activities were observably increased, but the content of SOD and GSH-PX showed a significant decrease in the model group (MPO and MDA, p < 0.01; GSH-PX and SOD, p < 0.05). In contrast, the levels of MPO, MDA, and GSH-PX changed by the ethanol were significantly reversed in the group treated with VEC-H or teprenone (p < 0.01, p < 0.05), while VEC had little effect on the level of SOD (p > 0.05).

Tumour necrosis factor α (TNF-α), interleukin-1β (IL-1β), and platelet-activating factor (PAF), as key inflammatory cytokines or mediators, were closely related to gastric mucosal injury (Amirshahrokhi and Khalili 2016; Wang et al. 2017). Ethanol could significantly increase the levels of TNF-α and IL-1β (both p < 0.05), whereas the TNF-α and IL-1β levels in rats treated with VEC were dramatically decreased except for the level of IL-1β treated by VEC-H (both p < 0.05). However, there was no obvious difference in the level of PAF (Figure 4), which may be attributed to the differential expression of inflammatory factors in serum and local tissue.

Taken together, these results suggested that VEC reduced ethanol-induced gastric injury, and VEC pre-treatment at a high dose more effectively alleviated the damage of gastric mucosa by ethanol than in other dose groups.

VEC mitigates indomethacin-induced gastric mucosal injury

We investigated the effects of VEC on the indomethacin-induced gastric injury. The GLI in the indomethacin-treated group was 43.73 ± 15.83, whereas that in rats pre-treated with VEC or teprenone was 42.00 ± 19.57 (VEC-L), 35.15 ± 20.49 (VEC-M), 30.77 ± 13.82 (VEC-H), and 30.92 ± 13.07 (teprenone), respectively. As presented in Figure 5A, the gastric mucosal damage index showed that the VEC-H group and teprenone could significantly decrease the damage caused by indomethacin (p < 0.05). Histological analysis of the gastric mucosa of animals in the model group demonstrated that the mucosa epithelial cells were infiltrated with many inflammatory cells. Furthermore, atrophied glands and congested blood vessels could be observed.
in the lamina propria of the mucosa. VEC ameliorated the injury caused by indomethacin administration (Figure 5B).

MPO, MDA, SOD, and GSH-PX were also used to measure the level of oxidative stress and the degree of tissue injury in indomethacin-induced gastric injury. As shown in Figure 6, when gastric tissues were exposed to indomethacin, the contents of MPO and MDA were significantly increased \(p < 0.05\), but there were no significant differences in SOD and GSH-PX activities. By contrast, the levels of MPO, MDA, and GSH-PX in the VEC-H-treated groups were observably reversed compared with the model \(p < 0.05\).

To further confirm the protective effects of VEC in gastric mucosal, we detected the expression levels of prostaglandin I2 (PGI2), prostaglandin E2 (PGE2), and cyclooxygenase 1 (COX-1) which played a pivotal role in maintaining mucosal integrity (Koji and Kikuko 2018). As presented in Figure 7, the results
Figure 5. Effect of VEC on the indomethacin-induced gastric injury. (A) Gastric mucosal lesion index of VEC administration at different concentrations in indomethacin-treated rats (VEC-L, 124 mg/kg; VEC-M, 248 mg/kg; VEC-H, 496 mg/kg; teprenone, 21.43 mg/kg). Values are means ± SEM (n = 8). *p < 0.05 compared with the Indo group. (B) HE staining (100×) of gastric mucosa of rats described in panel (A): (a) Control; (b) Indo; (c) Indo + teprenone; (d) Indo + VEC-L; (e) Indo + VEC-M; (f) Indo + VEC-H. Indo: indomethacin; VEC: valerian extract capsule; VEC-L: low dose of VEC; VEC-M: medium dose of VEC; VEC-H: high dose of VEC.

Figure 6. Effect of VEC on the level of MPO, MDA, SOD, and GSH-PX induced by indomethacin in gastric mucosa of rats. (A) MPO; (B) MDA; (C) SOD; (D) GSH-PX. Values are means ± SEM (n = 8). #p < 0.05 compared with the control group; *p < 0.05 compared with Indo group. Indo: indomethacin; VEC: valerian extract capsule; VEC-L: low dose of VEC; VEC-M: medium dose of VEC; VEC-H: high dose of VEC; MPO, myeloperoxidase; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase.
showed that indomethacin decreased the levels of PGE2, PGI2, and COX-1 ($p < 0.05$). Both VEC-M and teprenone could partially reverse the inhibition of PGE2, PGI2, and COX-1 caused by indomethacin ($p < 0.01$ or $p < 0.05$) with the increased levels of PGI2 and COX-1 in the VEC-H group ($p < 0.01$ or $p < 0.05$).

**Effects of VEC on the ACh- or BaCl2-induced contraction of isolated duodenum and ileum in rabbits**

We investigated the spasmolytic activity of VEC on duodenum and ileum contraction, ACh and BaCl2 were used to induce muscle contraction. As shown in Supplemental data (Figures S1 and S2), when acetylcholine (ACh, 0.01 mg/mL) or barium chloride (BaCl2, 0.1 g/mL) was added to the preparations of duodenum and ileum, the spontaneous contraction was remarkably potentiated. VEC markedly inhibited the potentiated spontaneous contraction, while atropine or pinaverium bromide had a more significant effect on reducing ACh- and BaCl2-induced contraction. Further quantitative results were shown in Table 3. The inhibitory rates on ACh or BaCl2-induced smooth muscle contractility in the VEC (0.1 mg/mL) group in the duodenum (ACh: 61.09 ± 22.12%; BaCl2: 64.15 ± 21.83%) and ileum (ACh: 63.76 ± 18.02%; BaCl2: 60.51 ± 18.23%) was significative higher (ACh: 61.09 ± 22.12%; BaCl2: 60.51 ± 18.23%) than solvent control in the duodenum (ACh: 5.34 ± 1.84%; BaCl2: 60.51 ± 18.23%) and ileum (ACh: 7.62 ± 4.65%; BaCl2: 12.52 ± 7.65%). These results suggested that VEC can function as a treatment for intestinal spasm.

**Effects of VEC on small intestinal transit in normal and neostigmine/adrenaline-induced mice**

*In vivo* study, the intestinal propulsion rate was 48.24 ± 10.47% in normal conditions (Table 4). After treatment with different doses of VEC (248, 496, and 992 mg/kg) and atropine, the intestinal propulsion rate increased significantly in the VEC and atropine groups. Intestinal transit by 496 and 992 mg/kg VEC was 56.52 ± 12.02% and 58.29 ± 11.34%, respectively. In neostigmine-treated mice, the intestinal transit was significantly promoted by neostigmine in normal mice (normal, 47.35 ± 9.10%; neostigmine, 68.28 ± 13.99%). Administration of VEC further increased the intestinal propulsion rate induced by neostigmine, especially in the dose of 992 mg/kg (85.75 ± 13.42%, $p < 0.01$, compared with the neostigmine group). Moreover, the percent intestinal transit was decreased significantly with adrenaline but increased observably by VEC. Intestinal transit in the adenalin group was 34.78 ± 10.93%, while the groups of 248 and 992 mg/kg VEC were 46.34 ± 12.82% and 47.84 ± 16.15%, respectively. Intestinal transit results indicated that VEC could improve intestinal motility.

**Discussion**

The current investigation evaluated the ameliorative effect of VEC against ethanol and indomethacin-induced gastric injury. Our data about gastric mucosal damage index and pathological sections of the gastric tissue indicated that VEC could reduce the degree of mucosal impairment. Further experimental investigations on the detection of oxidant/antioxidant parameters and pro-inflammatory cytokines suggested that VEC has potential antioxidant and anti-inflammatory activities, which is in accordance with previous studies (Sudati et al. 2009; Neamati et al. 2014). Inflammation and oxygen free radicals are two important systems to maintain homeostasis in healthy individuals and many studies have demonstrated that both inflammation and oxidation can promote GI tract damage (Mitobe et al. 2000; Bartchewsky et al. 2009; Pavan et al. 2021). In ethanol-induced gastric injury, we measured the levels of classical pro-inflammatory cytokines (TNF-α, IL-1β, and PAF) while expression of PGI2, PGE2, and COX-1 was detected in indomethacin-induce gastric injury because of the COX-dependent mechanisms in the pathogenesis of mucosal damage caused by indomethacin (Guo et al. 2005). It has been reported that the gastric ulcerogenic properties of NSAIDs are due to the inhibition of COX-1 and the PGs (PGI2 and PGE2) deficiency as observed herein and elsewhere (Takeuchi 2012). Oxidation is closely connected with inflammation in GI tract damage (Verma and Kumar 2016). Radicals are constantly generated from normal metabolic processes but excessive secretions of free radicals under pathological conditions such as increased neutrophil secretion may induce gastric mucosa injury by aggravating the degree of inflammatory cell infiltration and denaturing the membrane of epithelial cells (Jones et al. 2011). The levels of MDA and MPO were enhanced in ethanol- and indomethacin-induced gastric injury as observed in the current study and associated with increased tissue damage and neutrophil secretion in stomach lesions, respectively (Jung et al. 2012; Moussa et al. 2019; Lim et al. 2019).

Subsequently, we examined the levels of SOD and GSH-PX which as members of the cellular antioxidant defense system usually altered by free radicals. Ethanol significantly decreased the gastric levels of SOD and GSH-PX as observed herein and
motility of the normal and neostigmine/adrenaline-induced mice (Table 4). The contradictory result of VEC on the intestinal propulsion rates of VEC in mice (n = 20).

| Name          | c (mg/mL) | Duodenum | Ileum | Duodenum | Ileum |
|---------------|-----------|----------|-------|----------|-------|
| Control       | 48.24 ± 10.47 | 53.74 ± 11.07 | 68.28 ± 13.99 | 62.65 ± 13.73 | 60.51 ± 18.23 |
| VEC 248 mg/kg | 53.74 ± 11.07 | 68.28 ± 13.99 | 62.65 ± 13.73 | 60.51 ± 18.23 | 60.51 ± 18.23 |
| VEC 496 mg/kg | 56.52 ± 12.02 | 62.65 ± 13.73 | 62.65 ± 13.73 | 60.51 ± 18.23 | 60.51 ± 18.23 |
| VEC 992 mg/kg | 58.29 ± 11.34 | 62.65 ± 13.73 | 62.65 ± 13.73 | 60.51 ± 18.23 | 60.51 ± 18.23 |
| Atropine³     | 0.05 mg/mL  | 40.94 ± 8.32 | 56.33 ± 14.69 | 3.86 mg/kg | 35.77 ± 10.43 |

³: Positive control. Values are the means ± SEM (n = 20). *p < 0.05, **p < 0.01, ***p < 0.001 compared with control group; **p < 0.01 compared with neostigmine/adrenaline group. VEC: valerian extract capsule.

elsewhere (Mousa et al. 2019) but the levels were not changed remarkably in indomethacin-treated gastric damage, which might be caused by the compensatory mechanism of the organism and the SOD and GSH-PX levels depended on the sampling time point (Widmaier 2006). The present study showed that the pathological ameliorating effect of VEC was accompanied by the augmentation of the enzymatic antioxidant system (GSH-Px) and the cytoprotective marker (COX-1, PGE2, and PGI2), and alleviation of the levels of MPO, MDA, and inflammatory mediators.

Motility disorders of GI tracts are one of the causes of GI disorders and may increase the severity of the intestinal injury. It has been reported that the effects of intestinal inflammation induced by TNBS were directly related to changes in colon contractility (Calabresi et al. 2019). Additionally, abnormal contraction of intestinal smooth muscle was a potentially important factor in producing the main symptoms of IBS (Wei et al. 2013). It is well-recognized that mental health and GI disorders co-occur at remarkably high rates, such as anxiety/depression and IBS (Simpson et al. 2020). Similarly, chronic unpredictable mild stress (CUMS) is a promising rodent model of depression, and Wang et al. (2020) reported that the CUMS rats exhibited significant GI motility insufficiency although the relationship between depression and GI disorders is not still clear. In our results, VEC extracted from V. officinalis which was widely used to treat anxiety/depression could alleviate the intestinal motility changes by ACh or BaCl₂. To some extent, the result implies a potential possibility that VEC can be used as a drug treatment for individuals with both anxiety/depression and GI disorders. However, this finding needs to be further clarified by longitudinal and case-control studies.

VEC treating GI disorders may be through multicomponent, multiple targets, multiple pathways synergy. The contraction induced by ACh and BaCl₂ was relaxed by VEC, suggesting that VEC has an antispasmodic effect on motility disorders of GI disorders (Table 3). Most notably, VEC could also promote small intestinal motility of the normal and neostigmine/adrenaline-induced mice (Table 4). The contradictory result of VEC on the regulation of intestinal motility may be attributed to the complex chemical constituents of VEC. Essential oils (sesquiterpenes) and iridoids (valepotriates) are two major types of constituents isolated from V. officinalis and have shown spasmytotytic activity in guinea-pig ileum through direct effects on smooth muscle (Hazelhoff et al. 1982; Circosta et al. 2007). But which components are the effective ones for curing GI motility disorder remains to be elucidated.

Furthermore, the findings of this study are considered direct evidence that VEC had promising gastroprotective effects by strengthening the anti-inflammatory effects and the innate antioxidant defense system. It has been reported that valepotriates, as a typical constituent of the genus Valeriana, possess strong antioxidant activities (Wang et al. 2017). The Valeriana genus including V. officinalis showed potential anti-inflammatory properties and valepotriates prevent lipopolysaccharide-induced sickness and depressive-like behaviour by inhibiting an inflammatory pathway (Müller et al. 2015). Thus, the valepotriates contents of VEC may be responsible for the anti-inflammatory, antioxidant, and gastroprotective effects of VEC. There is also an emergent need for further evaluation of the main components in valerian to determine the definite bioactive component responsible for the GI therapeutic activity.

Conclusions
The present study demonstrates that VEC decreased the ethanol- and indomethacin-induced gastric mucosa injury. This ameliorative effect of VEC is mediated by alleviating oxidative stress and inflammation. VEC also played a dual role in regulating GI tract motility by relieving spasms and promoting intestinal peristalsis. Thus, VEC has further development value for the treatment of GI diseases including peptic ulcer diseases and GI motility disorders. However, further research should be performed to further explore the underlying mechanisms of action.

Disclosure statement
The authors declare that there is no conflict of interest.
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References

Abd El-Rady NM, Dahy Ma, Ahmed A, Elgaml DA, Hadiya S, Ahmed MAM, Sayed ZE-AA, Abdelwab A, Abdelmohsen AS, Farrag AAM, et al. 2021. Interplay of biochemical, genetic, and immunohistochemical factors in the etio-pathogenesis of gastric ulcer in rats: a comparative study of the effect of pomegranate loaded nanoparticles versus pomegranate peel extract. Front Physiol. 12:1095.

Amirshahrorkhi K, Khalili AR. 2016. Gastroprotective effect of 2-mercapto-ethane sulfonate against acute gastric mucosal damage induced by ethanol. Int Immunopharmacol. 34:183–188.

Bartchewsky W, Martini MR, Masiero M, Squassoni AC, Alvarez MC, Ladeira MS, Salvatore D, Trevisan M, Pedrazzoli J, Ribeiro ML. 2009. Effect of Helicobacter pylori infection on IL-8, IL-1beta and COX-2 expression in patients with chronic gastritis and gastric cancer. Scand J Gastroenterol. 44(2):153–161.

Calabresi MFF, Tanimoto A, Prospero AG, Mello FFP, Soares G, Di Stasi LC, Miranda JRA. 2019. Changes in colonic contractility in response to inflammatory bowel disease: long-term assessment in a model of TNBS-induced inflammation in rats. Life Sci. 236:116833.

Cantarella G, Martinez G, Di Benedetto G, Loreto C, Musumeci G, Prato A, Lempereur L, Matera M, Amico-Roxas M, Bernardino R, et al. 2007. Protective effects of amylin on reserpine-induced gastric damage in the rat. Pharmacol Res. 56(1):27–34.

Chen G, Han YQ, Feng Y, Wang A, Li X, Deng S, Zhang L, Xiao J, Li Y, Li N. 2019. Effect of Ilex rotundata Thunb alleviates experimental colitis-ass ociated cancer via suppressing inflammation-induced miR-31–3p/YAP overexpression. Phytomedicine. 62:152941.

Circosta C, Pasquale RD, Samperi S, Pino A, Occhiuto F. 2007. Biological and analytical characterization of two extracts from Valeriana officinalis. J Ethnopharmacol. 112(2):361–367.

Dejan P, Edami F, Rahimi F, Khosravi N, Jazaniyeh M, Dehpour AR. 2020. Involution of nitric oxide pathway in the anti-inflammatory effect of modafinil on indomethacin-, stress-, and ethanol-induced gastric mucosal injury in rat. Eur J Pharm Pharmacol. 887:137539.

Guo JS, Chau FL, Cho CH, Koo MW. 2005. Worsening effect of partial sleep deprivation on indomethacin-induced gastric mucosal damage. Pharmacol Biochem Behav. 82(3):515–521.

Haj Kheder S, Heller J, Bar JK, Wutzler A, Menge BA, Junkel G. 2018. Autonomic dysfunction of gastric motility in major depression. J Affect Disord. 226:196–202.

Hazelhoff B, Malingrè TM, Meijer DK. 1982. Antispasmodic effects of Valeriana officinalis extract against different neurotoxic agents. Neurochem Res. 7(5):583–590.

Jones MK, Zhu E, Sarino EV, Padilla OR, Takahashi T, Shimizu T, Shirasawa SM, McDonald KL, Miranda JRA. 2009. Changes in colonic contractility in response to chronic heavy alcohol use is associated with upregulated paneth cell antimicrobials in gastric mucosa. Clin Trans Gastroenterol. 6:e103.

Koj T, Kikuko A. 2018. Roles of cyclooxygenase, prostaglandin E2, and EP receptors in mucosal protection and ulcer healing in the gastrointestinal tract. Curr Pharm Des. 24:2002–2011.

Li H, Qu Y, Zhang J, Zhang J, Gao W. 2018. Spasmolytic activity of Aqulariae Lignum Resinatum extract on gastrointestinal motility involves muscarinic receptors, calcium channels and NO release. Pharm Biol. 56(6):559–566.

Lim JM, Song CH, Park SJ, Park DC, Jung GW, Cho HR, Bashir KMI, Ku SK, Choi JS. 2019. Protective effects of triple fermented barley extract (FBE) on indomethacin-induced gastric mucosal damage in rats. BMC Complement Altern M. 19:49.

Malva JO, Santos S, Macedo T. 2004. Neuroprotective properties of Valeriana officinalis extracts. Neurotox Res. 6(2):131–140.

Mousa AM, El-Sammad NM, Hassan SK, Madboli AENA, Hashim AN, Moustafa ES, Bakry SM, Elsayed EA. 2019. Antiulcerogenic effect of Cuphea ignea extract against ethanol-induced gastric ulcer in rats. BMC Complement Altern M. 19:345.

Muller D, Pfeil T, Von Den Driesch V. 2003. Treating depression comorbid with anxiety-results of an open, practice-oriented study with St. John’s wort WS 5572 and valerian extract in high doses. Phytomedicine. 10 Suppl 4:225–230.

Sudati JH, Fachinetto R, Pereira RP, Boligon AA, Athayde ML, Soares FA, de Vargas Barbosa NB, Rocha JB. 2009. In vitro antioxidant activity of Valeriana officinalis against different neurotoxic agents. Neurochem Res. 34(8):1372–1379.

Sun H, Zhao P, Liu W, Li L, Ai H, Ma X. 2018. Ventromedial hypothalamic nucleus in regulation of stress-induced gastric mucosal injury in rats. Sci Rep. 8(1):10170.

Taovani S, Ebbatani N, Kashiyanian M, Haghani H. 2011. Effect of valerian on sleep quality in postmenopausal women: a randomized placebo-controlled clinical trial. Menopause. 18(9):951–955.

Takeuchi K. 2012. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. World J Gastroenterol. 18(18):2147–2160.

Tammador MD, Nobahar M, Hydarinia-Naieni Z, Ebrahimian A, Ghorbani R, Vafaei AA. 2021. The effects of valerian on sleep quality, depression, and state anxiety in hemodialysis patients: a randomized, double-blind, crossover clinical trial. Oman Med J. 36(2):e225–e225.

Tian J, Li M, Zhao J, Li J, Liu G, Zhen Z, Cao Y, Gregersen H, Tong X. 2017. Research on the traditional Chinese medicine treating gastrointestinal motility in diabetic rats by improving biomechanical remodeling and neuroendocrine regulations. Am J Transl Res. 9(5):229–233.

Verma S, Kumar VL. 2016. Attenuation of gastric mucosal damage by artemunate in rat: modulation of oxidative stress and NFkappaB mediated signaling. Chem Biol Interact. 257:46–53.

Wang F, Zhang T, Wu S, He Y, Dai Z, Ma S, Liu B. 2017. Studies of the structure-antioxidant activity relationships and antioxidant activity mechanism of arnoid valopatiretates and their degradation products. PLoS One. 12(12):e0189198.

Wang L, Wang X, Zhang SL, Zhu XM, Liu YQ, Song ZJ, Du WJ, Ji J, Cui CL, He X, et al. 2017. Gastroprotective effect of palmatine against acetic acid-induced gastric ulcers in rats. J Nat Med. 71(1):257–264.

Wang X, Yan YF, Yang L, Huang YZ, Duan XH, Su KH, Liu WL. 2020. Effects of Mojin pill on depression by improving gastrointestinal function in rats with chronic unpredictable mild stress: role of the brain-gut axis. J Ethnopharmacol. 254:112173.
Wei YY, Sun LL, Fu ST. 2013. HEF-19-induced relaxation of colonic smooth muscles and the underlying mechanisms. World J Gastroenterol. 19(32):5314–5319.

Widmaier EP, Raff H, Strang KT. 2006. Vander’s human physiology: the mechanisms of body function. (10th ed.). Boston (BSN): McGraw-Hill Companies.

Woolf A, Rose R. 2021. Gastric ulcer. In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Zhou JC, Zhang XW. 2019. Akkermansia muciniphila: a promising target for the therapy of metabolic syndrome and related diseases. Chin J Nat Med. 17(11):835–841.