Received: 16 December 2020 | Revised: 3 February 2021 | Accepted: 22 February 2021

DOI: 10.1002/fft2.73

FOOD FRONTIERS Open Access

RESEARCH ARTICLE

Effects of complex extracts of traditional Chinese herbs on gastric mucosal injury in rats and potential underlying mechanism

Erdong Yuan | **Liangyun Liu** | **Min Huang** | **Bo Chang** | **Chunli Qi** | **Na Gou** | **Jiaoyan Ren**

School of Food Science and Technology, South China University of Technology, Wushan Road 381, Guangzhou, Guangdong 510000, China

Correspondence
Jiaoyan Ren, South China University of Technology, Guangzhou, Guangdong 510000, China.
Email: jyren@scut.edu.cn

Funding information
Research and Development Program in Key Areas of Guangdong Province, Grant/Award Number: 2019B020210002; National Key R&D Program of China, Grant/Award Number: 2018YFD0901101

Abstract

Five complex extracts (CEs) of seven Chinese herbs (*Astragalus*, *Poria cocos*, *Alpinia officinarum Hance*, *Radix Puerariae*, *Ginseng*, *Licorice*, *Hericium erinaceus*) were prepared by hot water extraction and evaluated for their effect on gastric ulcer in rats. In rats with acetic acid-induced chronic gastric ulcer, gross and microscopic appearance showed that gastric mucosal injury index and lesion inhibition rate were improved after CEs gavage for 21 days. Pretreatment with CEs for 21 days in rats with acute gastric ulcer could also improve the gastric mucosal injury by ethanol. CE1, CE4, and CE5 showed more obvious effect in two models. The cell experiments results showed that CE1, CE4, and CE5 effectively inhibited Wnt signaling activity. Thus, they could protect gastric mucosa through inhibiting Wnt signaling pathway. These results indicated that CE1, CE4, and CE5 had significant protective effects on gastric mucosal injury by inhibiting Wnt signalling pathway and could be developed into safe functional products.

KEYWORDS
complex extract, gastric ulcer, mechanism, traditional Chinese herbs, Wnt signaling pathway

1 | BACKGROUND

Gastric ulcer is a common type of peptic ulcer with high recurrence rate and is difficult to cure completely, afflicting millions of individuals worldwide (Wang et al., 2018). It is believed to be caused by an imbalance between the offensive factors (such as reactive oxygen species, alcohol consumption, cigarette smoking, *Helicobacter pylori*, etc.) and defensive factors (such as mucosal barrier, mucus secretion, bicarbonate secretion, antioxidants, cell regeneration, etc.) (Graham, 2014; Jayachitra et al., 2018; Klein et al., 2010).

The Wnt/β-catenin signaling pathway is an evolutionarily conserved signaling pathway that plays essential roles in embryonic development and adult tissue homeostasis (Gammons & Bienz, 2018; Song et al., 2015). In addition to the role in early embryogenesis, the canonical Wnt signaling pathway plays a crucial role in regulating proliferation, stem cell maintenance, and homeostasis in normal gastric mucosa (Barker et al., 2010; Chiurillo, 2015). It is associated with gastric epithelial integrity and gastric mucosal repair (Wang et al., 2019).

Currently many synthetic antiulcer drugs are used in the clinic, such as proton pump inhibitors, antacids, histamine H2 receptor

Abbreviations: CEs, Complex extracts; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; SD, Sprague-Dawley; Wnt3a CM, Wnt3a-conditioned media

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Food Frontiers* published by John Wiley & Sons Australia, Ltd and Nanchang University, Northwest University, Jiangsu University, Zhejiang University, Fujian Agriculture and Forestry University

antagonists, and so on. However, these drugs have unacceptably high incidence of adverse effects and linked to ulcer recurrence (Kangwan et al., 2014). Therefore, exploring more effective antigastric ulcer products with little or even without side effects from natural resources has attracted much attention (Awaad et al., 2013; Jain et al., 2010). Now many medicinal resources have been proven to be conducive to improving gastric ulcers in humans and many animal models (Boylan et al., 2014; Gargano et al., 2017).

A variety of traditional Chinese herbs have been proven to be effective on gastric ulcer and gastric cancer. Polysaccharide isolated from the aqueous extract of *Hericium erinaceus* mycelium culture has been identified as an active ingredient in ethanol-induced gastric ulcer mice and cell experiments (Wang et al., 2015). *Astragalus* saponins deceased the invasion ability of gastric cancer BGC-823 cell and induced its apoptosis (Miraj & Kiani, 2016; Wang et al., 2017). PGP2a, a polysaccharide isolated from *Ginseng* roots, has an inhibitory effect on the growth of HGC-27 cells in a dose-dependent manner (Li et al., 2014).

Long-term history of traditional Chinese herb application and recent studies have shown that complex extract of herbs are more effective than single extracts (Bai et al., 2014; You et al., 2019). It was found that complexes of herbs provide greater immune stimulation effects than the sum of the same concentrations of single components (Hong et al., 2018). SiJunZiTang, a classic Traditional Chinese Formula consisting of four Chinese herbs, *Radix Ginseng*, *Atractylodes macrocephala*, *Poria cocos* and *Glycyrrhiza uralensis*, has been demonstrated to show protective effects on intestine and gastric injure (Wang et al., 2013).

In this study, five complex extracts (CEs) of traditional Chinese herbs (*Astragalus*, *Poria cocos*, *Alpinia officinarum Hance*, *Radix Puerariae*, *Ginseng*, *Licorice*, and *Hericium erinaceus*) were prepared and evaluated the effect on chronic and acute gastric ulcer in rats. And the effects of CEs on the Wnt/β-catenin signaling pathway were conducted in cell experiments to discuss the mechanism preliminary.

2 | METHODS

2.1 | Materials and chemicals

Ginseng, *Poria cocos*, *Alpinia officinarum Hance*, *Licorice*, *Glycyrrhiza uralensis FischRadix Puerariae*, *Astragalus membranaceus* and *Hericium erinaceus* mycelium polysaccharide were obtained from Anguo City One Pharmaceutical Co., Ltd. (Hebei, China) Pentobarbital sodium was gained from Sigma-Aldrich (St. Louis, USA). Paraformaldehyde and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Aladdin Industrial Corp. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS, pH 7.4) and penicillin streptomycin were acquired from Gibco Life Technologies (Waltham, MA, USA). Dual Luciferase Reporter Gene Assay Kit was purchased from Promega (Madison, USA). All other chemicals and solvents used in the experiment were of analytical reagent grade.

2.2 | Complex extract preparation

Ginseng, *Poria cocos*, *Alpinia officinarum Hance*, *Licorice*, *Radix Puerariae and Astragalus membranaceus* were mixed at different ratios according to the formula (Table 1) and subjected to hot water at a ratio of 1:10 (w/w), 100 °C for 2 h. After filtration, the residues were subjected to second water extraction at a ratio of 1:8 (w/w), 100°C for 1.5 h and then filtered. The obtained filtrate was mixed, concentrated, and freeze-dried (Wang et al., 2015). At last, the different freeze-dried powder was mixed with *Hericium erinaceus* mycelium polysaccharide to get CE1, CE2, CE3, CE4, and CE5.

2.3 | Animal experiment

2.3.1 | Animals

SPF grade male adult SD rats (weighing 180 ± 20 g), purchased from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. (certificate SCXK (lu) 2014-0007) were applied to acetic acid-induced chronic gastric ulcer animal experiments. Male adult SD rats (weighing 180 ± 20 g) purchased from SPF (Beijing) Biotechnology Co., Ltd. (certificate SCXK (jing) 2016-0002) were used to ethanol-induced acute gastric ulcer animal experiments. The rats were raised under standard laboratory conditions at 25.0 ± 0.5°C, relative humidity 50 ± 5%, 12 h light-dark cycle.

2.3.2 | Acetic acid-induced chronic gastric ulcer and treatment

The animals were randomly divided into seven groups with nine rats in each group: control group, model group, and five experimental groups (CE1, CE2, CE3, CE4, and CE5). Chronic gastric ulcer was induced by acetic acid treatment in rats of model group and experimental groups (Almasaudi et al., 2017; Okabe & Pfeiffer, 1972). Animals were fasted for 12 h before the operation and were given free access to water. Under anesthesia, the stomach was exposed by a longitudinal incision below the xiphoid process and then was injected with 0.05 mL (v/v) of 2.5% acetic acid into the subserosal layer in the glandular part of the anterior wall. After that, the abdomen was closed to allow the rats to feed normally. From the second day after ulcer induction, the animals were treated with water (control group and model group), or CE1, CE2, CE3, CE4, CE5 (experimental groups) by gavage once daily for 21 day (Table 1). At last, with a 12 h fast and free access to water, the animals were sacrificed and the stomachs were removed for examination.

2.3.3 | Ethanol-induced acute gastric ulcer and treatment

Protective effects of CEs on acute gastric ulcer were evaluated using an ethanol-induced gastric mucosal lesion model (Al-Qarawi et al.,

TABLE 1 CEs composition and gavage amount

Group	Daily usage of Chinese herb raw materials (adult, g/day)	Extract lyophilized powder Daily intake (adult, g/day)	HMP Daily intake (adult, g/day)	Gavage amount (rat, mg/kg·day)
CE1	*Ginseng* (0.5), *Poria cocos* (1.5), *Alpinia officinarum Hance* (0.5) (1:3:1)	0.15	0.22	33.70
CE2	*Ginseng* (0.5), *Licorice* (0.5), *Radix Puerariae* (1.5)	0.34	0.22	50.8
CE3	*Ginseng* (0.5), *Poria cocos* (1.5), *Radix Puerariae* (1.5) (1:3:3)	0.48	0.22	63.6
CE4	*Astragalus membranaceus* (1.5), *Poria cocos* (1.5), *Alpinia officinarum Hance* (0.5) (3:3:1)	0.47	0.22	62.4
CE5	*Astragalus membranaceus* (1.0), *Poria cocos* (1.0), *Radix Puerariae* (1.0) (1:1:1)	0.38	0.22	54.9

2005). The animals were randomly divided into seven groups with six rats in each group: control group, model group, and five experimental groups (CE1, CE2, CE3, CE4, and CE5). Control group and model group received water, and experimental groups received CE1, CE2, CE3, CE4, or CE5 by gavage once daily for 21 day. After the last administration, rats were fasted and given free access to water for 12 h, and then received 1.0 mL of absolute ethanol by gavage except the rats in the control group. One hour later, rats were sacrificed and the stomachs were removed for examination.

2.3.4 | Stomach tissue sample for examination

After experiment, the rats were killed and abdominal cavity was opened immediately. After ligation at the cardia and pylorus, the stomach was removed, opened along the greater curvature and then rinsed with saline. The stomach tissue was spread out for observation and photographed for macroscopic assessment of gastric mucosal injury. Next, part of the gastric tissue was fixed in 4% paraformaldehyde solution for histopathological analysis.

2.3.5 | Macroscopic assessment

Ulcer occurrence in the gastric mucosal layer was carefully observed. Meanwhile, number of hemorrhagic spots was recorded and the length and width of ulcer stripes were measured with a vernier caliper. Gastric ulcer index was calculated through Guth method (Park et al., 2015), based on the following scoring criteria: Normally 0 points; 1 point for spot smashing; 2 points for smashing length <1 mm; 3 points for 1–2 mm; 4 points for 3–4 mm; 5 points for > 4 mm. The lesion inhibition rate was calculated using Equation (1) (Zhang et al., 2018):

$$\text{Lesion inhibition rate } (\%) = \frac{A0 - A1}{A0} \times 100, \quad (1)$$

Where A0 is the gastric ulcer index of model group, A1 is the gastric ulcer index of experimental groups.

2.3.6 | Histological analysis

Hematoxylin and eosin staining method were used for histopathological observation on general microstructure of gastric mucosa. The most serious parts of ulcer region were chosen to produce paraffin wax tissue sections (4 μm). The sections were stained in hematoxylin and eosin stain, and then observed under a light microscopy. Gastric ulcer index was calculated according to Ateufack et al. (2015) as following: No abnormalities 0 points; 1 point for Lesion involvement range <1/3; 2 point for 1/3–2/3; 3 point for 2/3–Mucosal full layer; 4 point for submucosal. The lesion inhibition rate was calculated using Equation (1).

2.4 | Cell experiment

2.4.1 | Cell line and culture

The RKO-reporter cell was generous gifts from Professor Jieqiong Tan from XiangYa School of Medicine, CSU. RKO-reporter cells were cultured in high-glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at a density of 2.0×10^4 cells/ well and incubated at 37 °C in 5% CO_2 environment for 12 h. Wnt3a-secreting L cells were cultured according to ATCC instructions for producing Wnt3a-conditioned media (Wnt3a CM) and control media, respectively.

2.4.2 | MTT assay

RKO-reporter cell viability was determined by a standard assay using MTT assay (Wang et al., 2017). Cells were plated at a density of

1.0×10^4 cells/well in 96-well plates and incubated with DMEM for 24 h. Then, the medium was replaced with a new medium containing different concentrations of CEs from 0.56 to 9.6 mg/mL. After incubation for 24 h, 20 μL MTT (5.0 mg/mL) dissolved in PBS was added and incubated for 4h at 37 °C. Remove the medium and dissolve the formazans with DMSO (150 μL), then the absorbance at 490 nm was measured using microplate reader.

2.4.3 | Luciferase reporter assays for Wnt/β-catenin signaling pathway

Wnt3a CM was prepared as following: Wnt3a-secreting L cells were cultured in DMEM with 10% FBS for 4 days. The medium was harvested and sterilized using a 0.22 μm filter. After addition of fresh medium, the cells were cultured for another 3 days, and the medium was collected and combined with the previous medium.

RKO-reporter cells contain two reporter plasmids, TOPflash (containing TCF binding site) and FOPflash (containing mutated TCF binding sites). RKO reporter cells were seeded in 96-well plates at a density of 2.0×10^4 cells/ well and incubated at 37 °C in 5% CO_2 environment for 12 h. Then, the cells were treated with Wnt activator and 4.5 mg /mL CE1 and CE5 or 4.8 mg /mL CE4 for another 24 h. Luciferase activity was measured with the dual Luciferase Reporter Gene Assay Kit (Promega) according to the manufacturer's instructions. Wnt signaling activity was calculated as fold change (TOP/FOP Luciferase activity) (Shim et al., 2015).

2.5 | Statistical analysis

All values were represented as mean ± SEM. Statistical analysis was carried out using SPSS 22.0 software. One-way analysis of variance (ANOVA) was adopted to compare the significant differences among all of groups using Tukey's analysis. Differences were considered to be significant at $p < 0.05$.

3 | RESULTS

3.1 | Repairing effect of CEs on acetic acid-induced chronic gastric ulcer in rats

3.1.1 | Effect of CEs on body weight and histological morphology of liver in acetic acid-induced chronic gastric ulcer rats

The body weights of rats all gradually increased during the experimental period. There was no significant difference in weight gain and clinical/behavior signs among all groups during the experiments. The morphological changes of liver were evaluated by histological examinations to analyze the hepatotoxicity of the CEs. The liver tissue of each group was similar in shape and there was no significant difference. These results suggested that these CEs are relatively safe.

3.1.2 | Repairing effect of CEs on acetic acid-induced chronic gastric injury

Figure 1 shows the gross appearance and microscopic images of the representative stomach of each group. In the control group, the gastric mucosa was smooth with no gastric glandular structure loss, disorganized glandular structure, hemorrhage, and submucosal edema, whereas these phenomena were observed in the model group, which indicated that the chronic gastric ulcer was successful generated. In experimental groups, these symptoms were alleviated. There were fewer glandular structure losses and disordered glandular structures in the CE2 and CE3 groups. Remarkably, the gastric mucosa of rats in CE1, CE4, and CE5 groups were almost smooth, with no obvious disordered glandular structure.

The gastric ulcer index and lesion inhibition rate by histological morphology were consistent with those by macroscopic observations (Figure 2). Compared with the model group, gastric ulcer index in the experimental groups decreased in different degree, mostly in CE1, CE4, and CE5 groups. There was no significant difference in gastric ulcer index and efficacy among the experimental groups. However, the CE1, CE4, and CE5 appeared apparent decreases in gastric ulcer index and increases in lesion inhibition rate in comparison with the model group. Lesion inhibition rate of CE1, CE4, and CE5 were the greatest in the experimental groups, although there were no obvious differences.

It was suggested that CEs could accelerate the healing of chronic gastric ulcer of rats and CE1, CE4, and CE5 were more effective in repairing acetic acid-induced gastric mucosal injury.

3.2 | Preventive effect of CEs on ethanol-induced acute gastric ulcer in rats

3.2.1 | Effect of CEs pretreatment on the body weight of ethanol-induced acute gastric ulcer rats

During the experiment, the body weight of all rats gradually increased. There was no significant difference in weight gain, clinical/behavioral signs and adverse events among all groups.

3.2.2 | Preventive effect of CEs on ethanol-induced acute gastric injury in rats

Compared with normal appearance of gastric mucosal epithelium and folding in the control group, it was found a large area of gastric mucosa hyperemia, ulcers, or perforations in model group (Figure 3). The gastric ulcer index was also highest in the model group (Figure 4), which indicated the most serious lesion. So, ethanol produced serious acute gastric ulcer in rats.

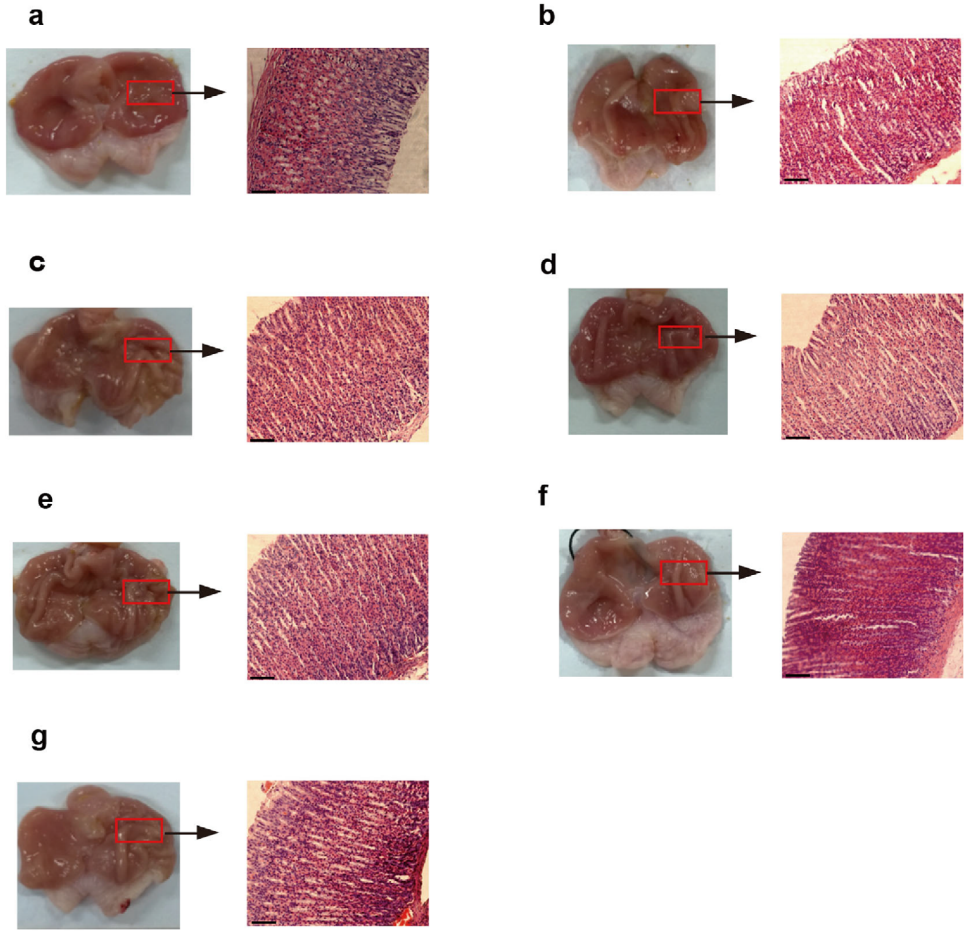


FIGURE 1 Gross appearance and microscopic images of acetic acid-induced chronic gastric ulcer in rats (magnification 200X), (a) control group; (b) model group; (c) CE1; (d) CE2; (e) CE3; (f) CE4; (g) CE5

In the experimental groups, it was observed fewer glandular structure losses and disordered glandular structures than in the model group. And gastric ulcer index of rats in experimental groups were less than model group. Especially, the gastric mucosa of rats in CE1, CE4, and CE5 groups were almost smooth with no obvious glandular structure disorder. Meanwhile, there was significant difference in gastric ulcer index between model groups and CE1, CE4, or CE5 groups. And CE1, CE4, and CE5 have the greatest lesion inhibition rate, although there was no significant difference in gastric index and lesion inhibition rate among experimental groups.

The results implied that CEs exerted preventive effects against ethanol-induced acute gastric mucosal injury, especially CE1, CE4, and CE5.

3.3 | Interaction of bioactive ingredients may affect their bioactivity

The effect of complex extract may be due to the interaction of bioactive ingredients. As is shown in Table 2, the CEs had different concentrations of polysaccharide and polyphenol.

TABLE 2 Total polysaccharide and total polyphenol contents of the CEs

Sample	Total polysaccharide (%)	Total polyphenol (%)
CE1	60.27 ± 0.136^{b}	5.23 ± 0.245^{b}
CE2	53.56 ± 0.215^{d}	4.01 ± 0.187^{c}
CE3	50.18 ± 0.089^{e}	3.89 ± 0.301^{c}
CE4	59.32 ± 0.278^{c}	5.45 ± 0.085^{a}
CE5	61.11 ± 0.053^{a}	3.96 ± 0.146^{c}

3.4 | Inhibitory effect of CEs on the Wnt/β-catenin signaling pathway

To investigate the possible mechanisms, a mechanistic study of CE1, CE4 and CE5 on Wnt/β-catenin signaling pathways was conducted.

The cytotoxic effect of CE1, CE4, and CE5 on macrophages was determined by MTT assay. As shown in Figure 5, no significant changes in the cell viability rate were found after treatment with CE1, CE4, or CE5 at the concentration of 0.56–9.60 mg/mL, and the cell morphology was good under the microscope. Therefore, the 0.56–9.60 mg/mL

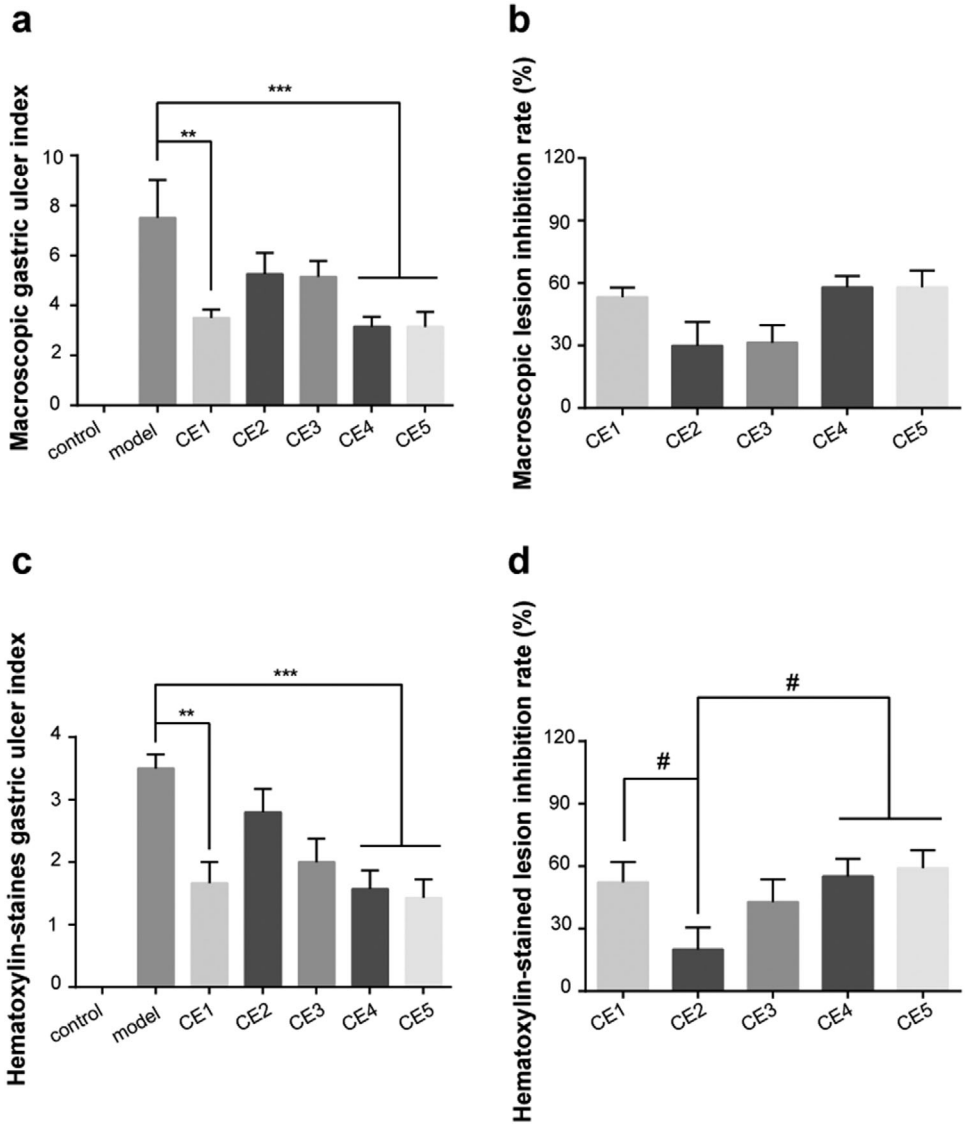


FIGURE 2 Effect of complex extracts on the gastric ulcer in acetic acid-induced chronic gastric ulcer rats. Values represent the mean ± SEM ($n = 9$), $^{**}p < 0.01$ versus model group; $^{***}p < 0.005$ versus model group; $\#p < 0.05$ versus CE2

served as safe concentrations were selected for the luciferase reporter assays.

To verify that the effect of CEs on gastric mucosal lesions is related to Wnt pathway, RKO-reporter cells were treated with CEs for 24 h. It was found that Wnt3a levels were greater when cells grew in Wnt3a-CM than in Wnt3a medium. However, the inhibitory effects of CEs on Wnt3a were much better, which indicated that this Wnt3a suppression was more dramatic in cells treated with these CEs. Combined with the results of effect on gastric mucosal injury, these results showed that the greater Wnt inhibitory activity of CEs, the more significant effect on gastric mucosa. It was confirmed that CEs could protect gastric mucosa by inhibiting Wnt signaling pathway.

4 | DISCUSSION

Gastric ulcers produced by acetic acid become chronic within 2-_3 days after ulcer begins, and the model is very similar to human peptic ulcer disease in terms of pathological features and the healing process (Bonamin et al., 2011). Acetic acid-induced gastric ulcers are caused by a variety of factors, including prostaglandins, growth factors, nitric oxide, and cytokine levels, as well as mucus adhesion patterns and microcirculation (Kobayashi et al., 2001). It has been previously reported that acetic acid can induce ulceration by penetrating the gastric mucosa as well as submucous layers and the muscular layer (Najm, 2011; Okabe & Amagase, 2005). Current treatments for gastric ulcers are based on the use of antisecretory drugs, including type 2 histamine receptor antagonists (H2RA) and proton pump inhibitors (PPIs). Although these drugs are effective, they have been used for a long time, especially PPI which has many side effects associated with gastric repair process (Yu et al., 2017). These limitations made people recognize that the treatment of gastric ulcers requires more effective or auxiliary therapeutic agents. In addition, Chinese herbal medicines have a therapeutic effect on gastric ulcer with fewer side effects and lower cost which is only one-sixth of western medicine.

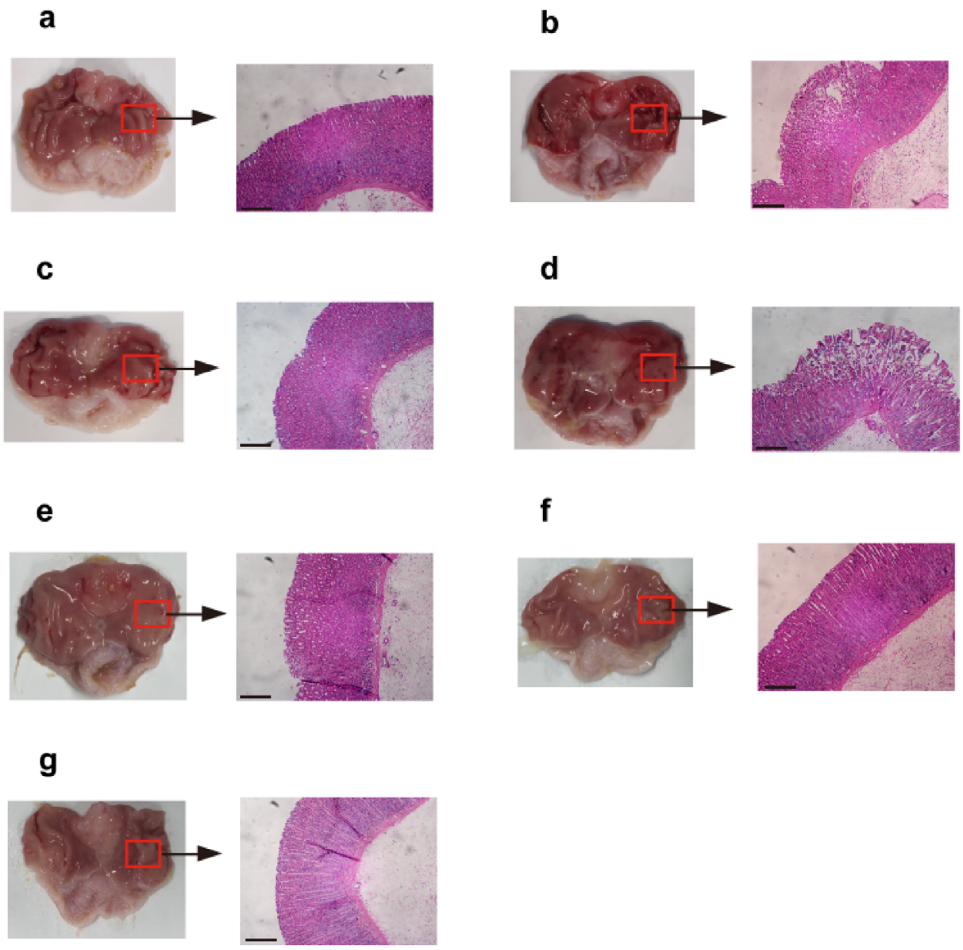


FIGURE 3 Gross appearance and microscopic images of ethanol-induced acute gastric ulcer in rats (magnification 200X), (a) control group; (b) model group; (c) CE1; (d) CE2; (e) CE3; (f) CE4; (g) CE5

Current study was designed to evaluate the effects of different prescriptions on acetic acid-induced gastric ulcer repair. The results obtained from this work showed that CE1, CE3, and CE4 are able to reduce the gastric ulcer index induced by this model. This speculate that the mechanism is to inhibit the Wnt pathway which can repair the damaged gastric mucosa, for which CE1, CE3, and CE4 can achieve it well.

The Wnt/β-catenin signaling pathway is a highly conserved pathway in the evolution of organisms and regulates many biological processes (Wang et al., 2011). In this way, extracellular signals are delivered to the cells by activating the intracellular domain of the cell surface receptor. Wnt/β-catenin signaling pathway is an important signaling pathway in gastric cancer, and it is often activated abnormally. Luciferase reporter assay is a method of confirming the function of the β-catenin/TCF complexes by using a reporter containing multimeric TCF recognition sequences (TOPFLASH). It is well known that Wnt signaling directly leads to heterogenous expression of related factors of epithelial–mesenchymal transition (EMT), such as N-cadherin and E-cadherin. Epithelial cells express high levels of E-cadherin, whereas mesenchymal cells express high levels of N-cadherin and lose E-cadherin expression. The development of gastric cancer involves the expression or absence of E-cadherin function. Downregulation of E-cadherin reduces the intensity of cell adhesion in tissues, leading to an increase in cellular motility, which in turn allows cancer cells to invade surrounding tissues through the basement membrane (Huang et al., 2014; Park et al., 2018; Su et al., 2015; Zhang et al., 2019). When acetic acid is used to induce gastric mucosal injury, Wnt signaling pathway is activated in gastric mucosal epithelial cells, resulting in decreased expression of E-cadherin, which leads to the development of gastric ulcer. This study found that CEs can effectively inhibit the Wnt signaling pathway, which increases the expression of E-cadherin, thus accelerating the repair of gastric mucosal injury caused by acetic acid.

Combined with animal experiments and cell experiments, it can be seen that CE3 has the best repair effect of gastric mucosal injury and inhibits the Wnt signaling pathway. In particular, CE3 contains both *Astragalus membranaceus* and *Alpinia officinarum Hance*. The main active ingredient in *Astragalus membranaceus* is *Astragalus* polysaccharide and the main active ingredient in *Alpinia officinarum Hance* is *galangin* which belongs to flavonoids.

Previous studies have shown that the antiulcer effects of polysaccharides were mainly achieved in one or several pathways (Ali Khan et al., 2018; Cantu-Jungles et al., 2014, Raish et al., 2018): binding

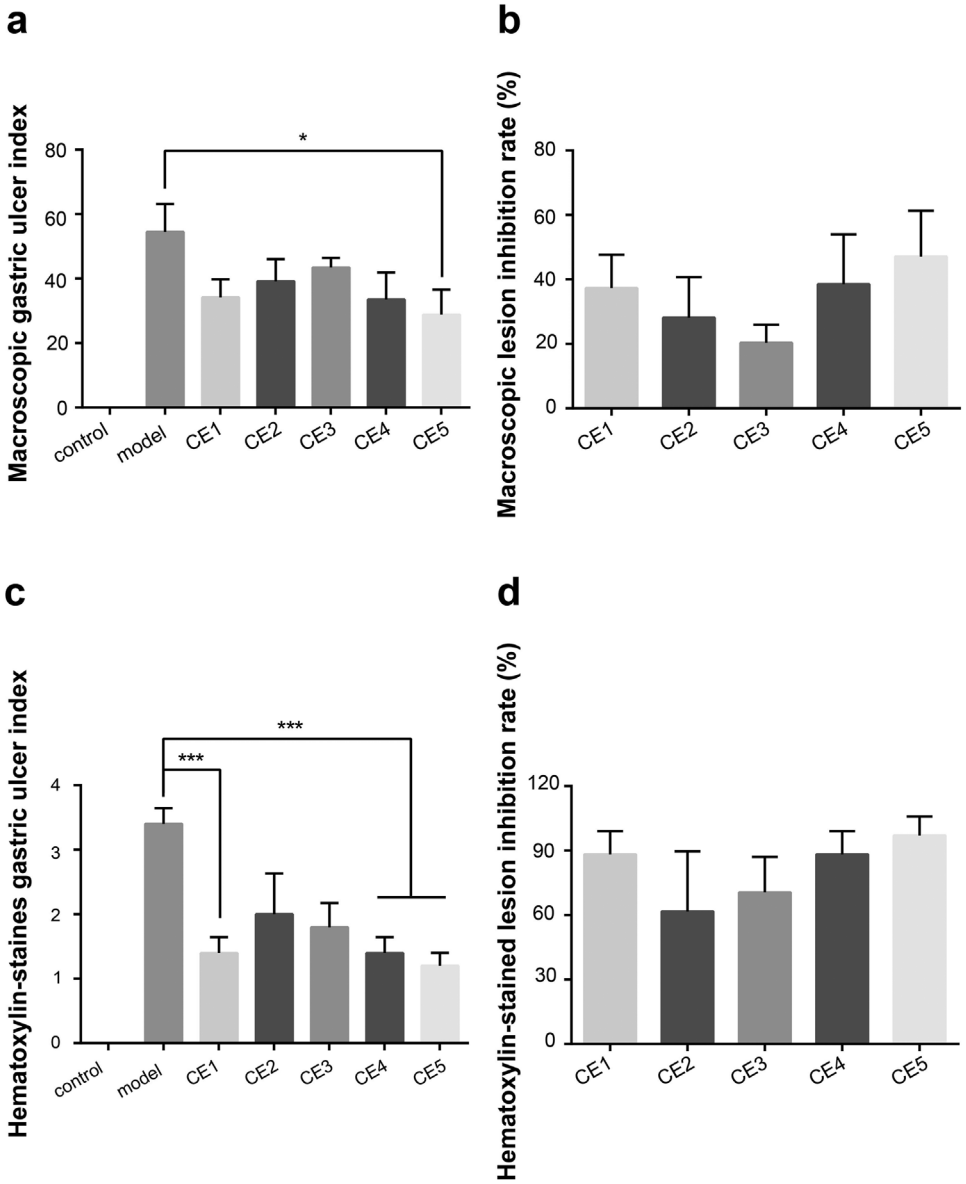


FIGURE 4 Effect of complex extracts on the gastric ulcer in acute ethanol-induced gastric ulcer rats. Values represent the mean ± SEM ($n = 9$), $^{*}p < 0.05$ versus model group; $^{**}p < 0.01$ versus model group; $^{***}p < 0.005$ versus model group

to the mucosa surface to provide a protective coating, reducing the secretions of gastric acid and pepsin, protecting the mucus barrier or reducing oxidative stress or inflammatory response of gastric mucosa. The hypothesis that flavonoids can accelerate gastric healing has been demonstrated, and treatment with this compound in a dose-dependent manner can significantly reduce the extent of the gastric injury induced by acetic acid (Balan et al., 2015; Li et al., 2015; Vasconcelos et al., 2010).

Many studies have shown that flavonoids could react with polysaccharide, form different complex microstructures, and may affect the physicochemical properties and biological activities of polysaccharides and flavonoids. Flavonoids can significantly improve the activities of polysaccharides, including antioxidant, antimicrobial, antiulcer, antitumor, and enzyme-inhibiting activities (Fu et al., 2014; Kolodziejczyk-Czepas et al., 2015; Ni et al., 2010). Among the above seven Chinese herbs, the main bioactive ingredients are polysaccharides or flavonioids. The main bioactive ingredients of *Astragalus membranaceus*, *Hericium erinaceus*, *Ginseng*, *Poria cocos*, *Licorice* are polysaccharides. The main bioactive ingredients of *Alpinia officinarum Hance* and *Radix Puerariae* are flavonoid (galangin, puerarin). It may be speculated that the interaction between polysaccharide and flavonoid may gain more significant effects on gastric mucosal damage. The effect of complex extract may be due to the interaction of bioactive ingredients. Many studies have shown that flavonoids could react with polysaccharide, form different complex microstructures, and may affect the physicochemical properties and biological activities of

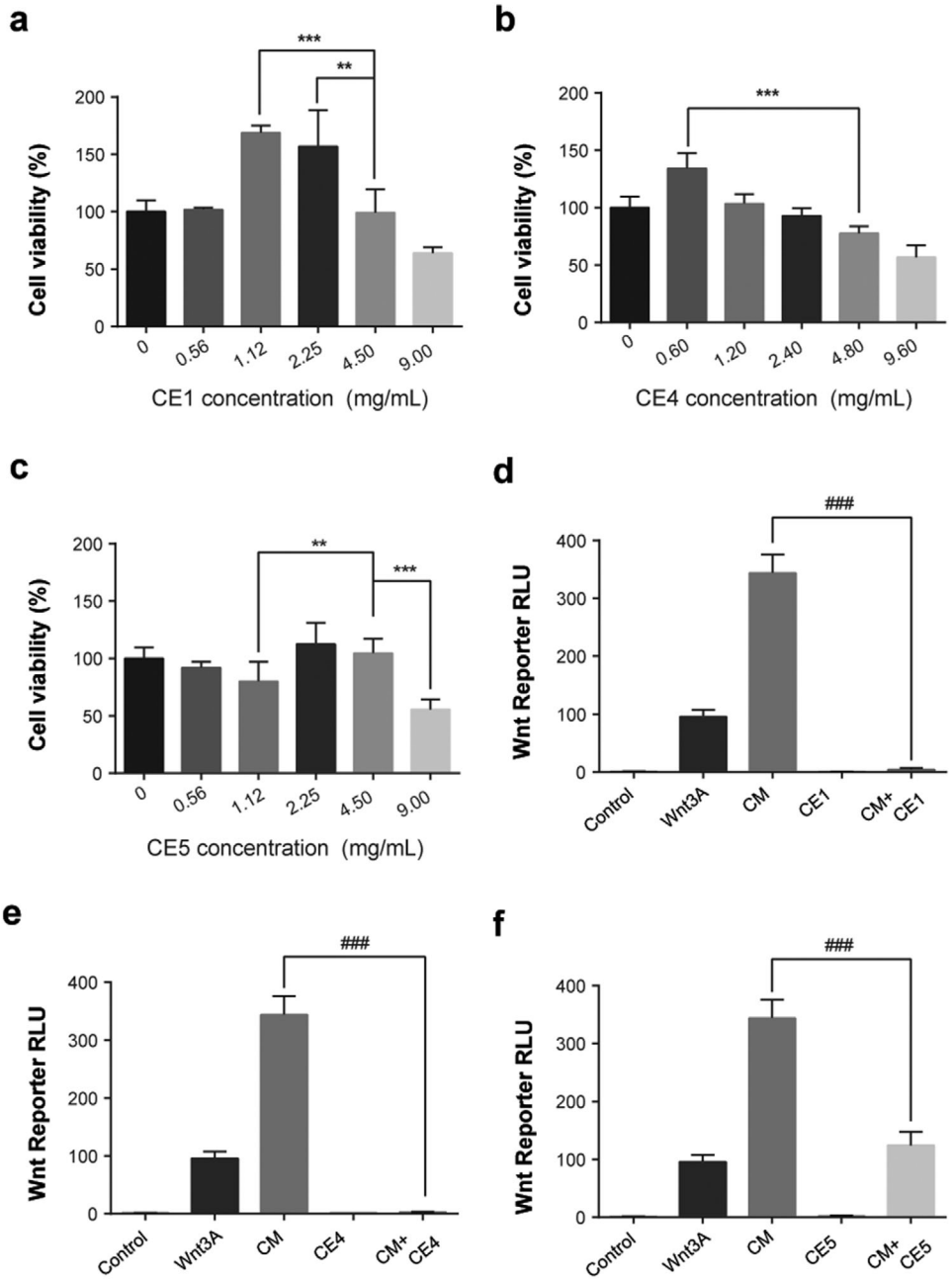


FIGURE 5 Effect of CE on RKO-reporter cells. (a), (b), (c): Cell viability of RKO-reporter cells; (d), (e), (f): Wnt Reporter RLU. Values represent the mean ± SEM ($n = 3$), versus 4.50 mg/mL or 4.80 mg/mL, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$; versus CM, $^{\#\#\#}p < 0.005$

polysaccharides and flavonoids. Flavonoids can significantly improve the activities of polysaccharides, including antioxidant, antimicrobial, antiulcer, antitumor, and enzyme-inhibiting activities (Fu et al., 2014; Kolodziejczyk-Czepas et al., 2015; Ni et al., 2010). Among the above seven Chinese herbs, the main bioactive ingredients are polysaccharides or flavonioids. The main bioactive ingredients of *Astragalus membranaceus*, *Hericium erinaceus*, *Ginseng*, *Poria cocos*, *Licorice* are polysaccharides. The main bioactive ingredients of *Alpinia officinarum Hance* and *Radix Puerariae* are flavonoid (galangin, puerarin). It may be speculated that the interaction between polysaccharide and flavonoid may gain more significant effects on gastric mucosal damage.

5 | CONCLUSION

CEs have repairing effect on chronic gastric mucosal injury induced by acetic acid and preventive effect on acute gastric mucosal injury induced by ethanol in rats. Among them, CE1, CE4, and CE5 have greater effects. They could protect gastric mucosa by inhibiting Wnt signaling pathway. They may be new safe products on the treatment or prevention of gastric ulcer.

DECLARATIONS

Ethics approval and consent to participate

All procedures performed in studies involving animals were handled humanely according to the guidelines of the National Institutes of Health guide (NIH Publications No. 8023, revised 1978) for animal care and use were followed, and the animal protocol was approved by the animal ethics committee of Jinan University.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

RJY contributed to the conception of the study. YED helped perform the analysis with constructive discussions and revise manuscript. LLY contributed to in vitro studies and manuscript preparation. HM performed the animal experiment and wrote the manuscript. CB helped perform the animal experiment. QCL helped build animal models. GN performed assays for Wnt/-catenin signaling pathway. All authors read and approved the final manuscript.

ORCID

Jiaoyan Ren https://orcid.org/0000-0002-2063-601X

REFERENCES

Ali Khan, M. S., Khumdmiri, S. U. K., Khudmiri, S. R., Al-Sanea, M. M., & Pooi Ling, M. (2018). Fruit-derived polysaccharides and terpenoids: Recent update on the gastroprotective effects and mechanisms. *Frontiers in Pharmacology*, *9*, 569.

Almasaudi, S. B., Abbas, A. T., Al-Hindi, R. R., El-Shitany, N. A., Abdel-dayem, U. A., Ali, S. S., Saleh, R. M., Al Jaouni, S. K., Kamal, M. A., & Harakeh, S. M. (2017). Manuka honey exerts antioxidant and anti-inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–12.

Al-Qarawi, A., Abdel-Rahman, H., Ali, B., Mousa, H., & El-Mougy, S. (2005). The ameliorative effect of dates (Phoenix dactylifera L.) on ethanol-induced gastric ulcer in rats. *Journal of Ethnopharmacology*, *98*(3), 313–317.

Ateufack, G., Domgnim Mokam, E. C., Mbiantcha, M., Dongmo Feudjio, R. B., David, N., & Kamanyi, A. (2015). Gastroprotective and ulcer healing effects of piptadeniastrum Africanum on experimentally induced gastric ulcers in rats. *BMC Complementary and Alternative Medicine*, *15*(1).

Awaad, A. S., El-Meligy, R. M., & Soliman, G. A. (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. *Journal of Saudi Chemical Society*. *17*(1), 101–124.

Bai, H., Wang, Z., Cui, J., Yun, K., Zhang, H., Liu, R. H., Fan, Z., & Cheng, C. (2014). Synergistic radiation protective effect of purified Auricularia auricular-judae polysaccharide (AAP IV) with grape seed procyanidins. *Molecules (Basel, Switzerland)*, *19*(12), 20675–20694.

Balan, T., Sani, M. H. M., Ahmad, S. H. M., Suppaiah, V., Mohtarrudin, N., & Zakaria, Z. A. (2015). Antioxidant and anti-inflammatory activities contribute to the prophylactic effect of semi-purified fractions obtained from the crude methanol extract of Muntingia calabura leaves against gastric ulceration in rats. *Journal of Ethnopharmacology*, *164*, 1–15.

Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H. J., van Es, J. H., Sato, T., Stange, D. E., Begthel, H., van den Born, M., Danenberg, E., van den Brink, S., Korving, J., Abo, A., Peters, P. J., Wright, N., Poulsom, R., & Clevers, H. (2010). Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell*, *6*(1), 25–36.

Bonamin, F., Moraes, T. M., Kushima, H., Silva, M. A., Rozza, A. L., Pellizzon, C. H., Bauab, T. M., Rocha, L. R. M., Vilegas, W., & Hiruma-Lima, C. A. (2011). Can a Strychnos species be used as antiulcer agent? Ulcer healing action from alkaloid fraction of Strychnos pseudoquina St. Hil.(Loganiaceae). *Journal of Ethnopharmacology*, *138*(1), 47–52.

Boylan, M. R., Khalili, H., Huang, E. S., Michaud, D. S., Izard, J., Joshipura, K. J., & Chan, A. T. (2014). A prospective study of periodontal disease and risk of gastric and duodenal ulcer in male health professionals. *Clinical and Translational Gastroenterology*, *5*(2), e49.

Cantu-Jungles, T. M., Maria-Ferreira, D., da Silva, L. M., Baggio, C. H., de Paula Werner, M. F., Iacomini, M., Cipriani, T. R., & Cordeiro, L. M. C. (2014). Polysaccharides from prunes: Gastroprotective activity and structural elucidation of bioactive pectins. *Food Chemistry*, *146*, 492–499.

Chiurillo, M. A. (2015). Role of the Wnt/β-catenin pathway in gastric cancer: An in-depth literature review. *World Journal of Experimental Medicine*, *5*(2), 84.

Fu, J., Wang, Z., Huang, L., Zheng, S., Wang, D., Chen, S., Zhang, H., & Yang, S. (2014). Review of the botanical characteristics, phytochemistry, and pharmacology of Astragalus membranaceus (Huangqi). *Phytotherapy Research*, *28*(9), 1275–1283.

Gammons, M., & Bienz, M. (2018). Multiprotein complexes governing Wnt signal transduction. *Current Opinion in Cell Biology*, *51*, 42–49.

Gargano, M. L., van Griensven, L., Isikhuemhen, O. S., Lindequist, U., Venturella, G., Wasser, S. P., & Zervakis, G. I. (2017). Medicinal mushrooms: Valuable biological resources of high exploitation potential. *Plant Biosystems*, *151*(3), 548–565.

Graham, D. Y. (2014). History of Helicobacter pylori, duodenal ulcer, gastric ulcer and gastric cancer. *World Journal of Gastroenterology*, *20*(18), 5191–5204.

Hong, H.-D., Kim, J.-C., Lim, T.-G., Song, Y.-R., Cho, C.-W., & Jang, M. (2018). Mixing ratio optimization for functional complex extracts of Rhodiola crenulata, Panax quinquefolius, and Astragalus membranaceus using mixture design and verification of immune functional efficacy in animal models. *Journal of Functional Foods*, *40*, 447–454.

Huang, J., Xiao, D., Li, G., Ma, J., Chen, P., Yuan, W., Hou, F., Ge, J., Zhong, M., Tang, Y., Xia, X., & Chen, Z. (2014). EphA2 promotes epithelial–mesenchymal transition through the Wnt/β-catenin pathway in gastric cancer cells. *Oncogene*, *33*(21), 2737.

Jain, N. K., Singh, N., Kannojiya, P., Garud, N., Garud, A., & Tonpay, S. (2010). Pharmacological screening of antiulcer agents: A review. *International Journal of Pharmaceutical Sciences and Research*, *1*, 29–37.

Jayachitra, C., Jamuna, S., Ali, M. A., Paulsamy, S., & Al-Hemaid, F. M. A. (2018). Evaluation of traditional medicinal plant, Cissus setosa Roxb. (Vitaceae) for antiulcer property. *Saudi Journal of Biological Sciences*, *25*(2), 293–297.

Kangwan, N., Park, J.-M., Kim, E.-H., & Hahm, K. B. (2014). Quality of healing of gastric ulcers: natural products beyond acid suppression. *World Journal of Gastrointestinal Pathophysiology*, *5*(1), 40.

Klein, L. C., Gandolfi, R. B., Santin, J. R., Lemos, M., Cechinel, V., & de Andrade, S. F. (2010). Antiulcerogenic activity of extract, fractions, and some compounds obtained from Polygala cyparissias St. Hillaire & Moquin (Polygalaceae). *Naunyn-Schmiedeberg's Archives of Pharmacology*, *381*(2), 121–126.

Kobayashi, T., Ohta, Y., Yoshino, J., & Nakazawa, S. (2001). Teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats by inhibiting neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues. *Pharmacological Research*, *43*(1), 23–30.

Kolodziejczyk-Czepas, J., Bijak, M., Saluk, J., Ponczek, M. B., Zbikowska, H. M., Nowak, P., Tsirigotis-Maniecka, M., & Pawlaczyk, I. (2015). Radical scavenging and antioxidant effects of Matricaria chamomilla polyphenolic-polysaccharide conjugates. *International Journal of Biological Macromolecules*, *72*, 1152–1158.

Li, C., Tian, Z.-N., Cai, J.-P., Chen, K.-X., Zhang, B., Feng, M.-Y., Shi, Q.-T., Li, R., Qin, Y., & Geng, J.-S. (2014). Panax ginseng polysaccharide induces apoptosis by targeting Twist/AKR1C2/NF-1 pathway in human gastric cancer. *Carbohydrate Polymers*, *102*, 103–109.

Li, H., Song, Z.-J., Dai, Y.-P., Zhang, S.-L., He, X., Guo, C.-R., Zhang, W.-J., Wang, J.-Y., Zhang, C.-F., Wang, C.-Z., & Yuan, C.-S. (2015). Antioxidative activity of flavonoids from Abrus cantoniensis against ethanol-induced gastric ulcer in mice. *Planta Medica*, *81*(10), 784–790.

Miraj, S., & Kiani, S. (2016). Astragalus membranaceus: A review study of its anti-carcinoma activities. *Der Pharmacia Lettre*, *8*(6), 59–65.

Najm, W. I. (2011). Peptic ulcer disease. *Primary Care: Clinics in Office Practice*, *38*(3), 383–394.

Ni, W. H., Zhang, X., Wang, B., Chen, Y., Han, H., Fan, Y. Y., Zhou, Y., & Tai, G. (2010). Antitumor activities and immunomodulatory effects of ginseng neutral polysaccharides in combination with 5-fluorouracil. *Journal of Medicinal Food*, *13*(2), 270–277.

Okabe, S., & Amagase, K. (2005). An overview of acetic acid ulcer models—the history and state of the art of peptic ulcer research. *Biological and Pharmaceutical Bulletin*, *28*(8), 1321–1341.

Okabe, S., & Pfeiffer, C. J. (1972). Chronicity of acetic acid ulcer in the rat stomach. *The American Journal of Digestive Diseases*, *17*(7), 619–629.

Park, J. W., Kim, M. S., Voon, D. C., Kim, S. J., Bae, J., Mun, D. G., Ko, S.-I., Kim, H. K., Lee, S.-W., & Kim, D.-Y. (2018). Multi-omics analysis identifies pathways and genes involved in diffuse-type gastric carcinogenesis induced by E-cadherin, p53, and Smad4 loss in mice. *Molecular Carcinogenesis*, *57*(7), 947–954.

Park, J.-H., Jang, K.-J., Kim, C.-H., Kim, J.-H., Kim, Y.-K., & Yoon, H.-M. (2015). Ganoderma lucidum pharmacopuncture for teating ethanol-induced chronic gastric ulcers in rats. *Journal of Pharmacopuncture*, *18*(1), 72.

Raish, M., Ahmad, A., Ansari, M. A., Alkharfy, K. M., Aljenoobi, F. I., Jan, B. L., Al-Mohizea, A. M., Khan, A., & Ali, N. (2018). Momordica charantia polysaccharides ameliorate oxidative stress, inflammation, and apoptosis in ethanol-induced gastritis in mucosa through NF-kB signaling pathway inhibition. *International Journal of Biological Macromolecules*, *111*, 193–199.

Shim, A.-R., Dong, G.-Z., Lee, H. J., & Ryu, J.-H. (2015). Atractylochromene is a repressor of Wnt/β-catenin signaling in colon cancer cells. *Biomolecules & Therapeutics*, *23*(1), 26.

Song, X., Xin, N., Wang, W., & Zhao, C. (2015). Wnt/β-catenin, an oncogenic pathway targeted by H. pylori in gastric carcinogenesis. *Oncotarget*, *6*(34), 35579.

Su, Y., Chang, Y., Lin, W., Liang, C., & Lee, J. (2015). An aberrant nuclear localization of E-cadherin is a potent inhibitor of Wnt/β-catenin-elicited promotion of the cancer stem cell phenotype. *Oncogenesis*, *4*(6), e157.

Vasconcelos, P., Andreo, M., Vilegas, W., Hiruma-Lima, C. A., & Pellizzon, C. (2010). Effect of Mouriri pusa tannins and flavonoids on prevention and treatment against experimental gastric ulcer. *Journal of Ethnopharmacology*, *131*(1), 146–153.

Wang, F., Zhu, W., Yang, R., Xie, W., & Wang, D. (2019). LncRNA ZEB2-AS1 contributes to the tumorigenesis of gastric cancer via activating the Wnt/β-catenin pathway. *Molecular and Cellular Biochemistry*, *456*, 1–11.

Wang, M. X., Kanako, N., Zhang, Y. Q., Xiao, X. L., Gao, Q. P., & Tetsuya, K. (2017). A unique polysaccharide purified from Hericium erinaceus mycelium prevents oxidative stress induced by H_2O_2 in human gastric mucosa epithelium cell. *Plos One*, *12*(7), e0181546.

Wang, M. X., Konishi, T., Gao, Y., Xu, D. D., & Gao, Q. P. (2015). Anti-gastric ulcer activity of polysaccharide fraction isolated from mycelium culture of lion's mane medicinal mushroom, hericium erinaceus (higher basidiomycetes). *International Journal of Medicinal Mushrooms*, *17*(11), 1055–1060.

Wang, T., Xuan, X. Y., Li, M., Gao, P., Zheng, Y. L., Zang, W. Q., & Zhao, G. (2017). Astragalus saponins affect proliferation, invasion and apoptosis of gastric cancer BGC-823 cells (Retraction of Vol 8, Pg 170, 2013). *Diagnostic Pathology*, *12*(1), 67.

Wang, W., Liu, H., Wang, S., Hao, X., & Li, L. (2011). A diterpenoid derivative 15-oxospiramilactone inhibits Wnt/β-catenin signaling and colon cancer cell tumorigenesis. *Cell Research*, *21*(5), 730.

Wang, X. Y., Yin, J. Y., Zhao, M. M., Liu, S. Y., Nie, S. P., & Xie, M. Y. (2018). Gastroprotective activity of polysaccharide from Hericium erinaceus against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer, and its antioxidant activities. *Carbohydrate Polymers*, *186*, 100–109.

Wang, Y. Y., He, S., Cheng, X. C., Lu, Y. X., Zou, Y. P., & Zhang, Q. L. (2013). UPLC-Q-TOF-MS/MS fingerprinting of traditional chinese formula SiJunZiTang. *Journal of Pharmaceutical and Biomedical*, *80*, 24–33.

Wang, Y., Huang, M., Sun, R., & Pan, L. (2015). Extraction, characterization of a Ginseng fruits polysaccharide and its immune modulating activities in rats with Lewis lung carcinoma. *Carbohydrate Polymers*, *127*, 215–221.

You, J., Chang, Y., Zhao, D., Zhuang, J., & Zhuang, W. (2019). A mixture of functional complex extracts from lycium barbarum and grape seed enhances immunity synergistically in vitro and in vivo. *Journal of Food Science*, *84*(6), 1577–1585.

Yu, L. Y., Sun, L. N., Zhang, X. H., Li, Y. Q., Yu, L., Yuan, Z. Q. Y., Meng, L., Zhang, H.-W., & Wang, Y.-Q. (2017). A review of the novel application and potential adverse effects of proton pump inhibitors. *Advances in Therapy*, *34*(5), 1070–1086.

Zhang, X., Peng, Y., Huang, Y., Yang, M., Yan, R., Zhao, Y., Cheng, Y., Liu, X., Deng, S., Feng, X., Lin, H., Yu, H., Chen, S., Zhao, Z., Li, S., Li, K., Wang, L., Wei, Y., He, Z., … Jin, Z. (2018). SMG-1 inhibition by miR-192/-215 causes epithelial-mesenchymal transition in gastric carcinogenesis via activation of Wnt signaling. *Cancer Medicine*, *7*(1), 146–156.

Zhang, Y., Li, D., Dai, Y., Li, R., Gao, Y., & Hu, L. (2019). The role of e-cadherin in helicobacter pylori-related gastric diseases. *Current Drug Metabolism*, *20*(1), 23–28.

How to cite this article: Yuan E, Liu L, Huang M, et al. Effects of complex extracts of traditional Chinese herbs on gastric mucosal injury in rats and potential underlying mechanism. *Food Frontiers* 2021;*2*, 305–315.
https://doi.org/10.1002/fft2.73