**Salsola collina** ethyl acetate extract alleviates diabetic gastroparesis possibly through oxidative stress inhibition

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**Abstract.** Objective: To investigate the therapeutic significance of ethyl acetate extract of *Salsola collina* (EES) on diabetic gastroparesis (DGP) and its underlying mechanisms. Methods: The composition of EES was analyzed by HPLC and LC/MS. A DGP model was established by streptozotocin injection and irregularly feeding a high-sugar, high-fat (HSHF) diet. Serum nitric oxide (NO), total cholesterol (TC), and triglyceride (TG) and the gastric superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GSH-Px) concentrations were measured by colorimetry and ELISA. The expression of neuronal nitric oxide synthase (nNOS) and protein gene product 9.5 (PGP9.5) in the gastric tissue were examined by Western blot and immunohistochemistry. Results: EES promoted gastric emptying delayed by DGP, which was mainly composed of 10 organic acids. Furthermore, EES increased serum NO, decreased glucose, TC and TG, increased gastric SOD, CAT, and GSH-Px, while decreased MDA, increased nNOS and PGP9.5 expression in the gastric tissue, and showed a concentration dependence. Conclusion: EES promoted gastric emptying in the DGP rats, which might be related to its inhibition of oxidative stress and the associated increase in the gastric neuron population, as well as its hypoglycemic and lipid-lowering activities. These findings suggest that *Salsola* may have potential benefits in the treatment of DGP.

1. Introduction

Oxidative stress refers to the overproduction or reduced elimination of active polymers such as free radicals and an imbalance between oxidation and antioxidation, which results in a variety of chronic diseases. Active polymers mainly include reactive nitrogen species (RNS) and reactive oxygen species (ROS) [1]. RNS are composed of NO and its derivatives (i.e., NO- and NO3-) [2]. ROS, including O2-, H2O2, and •HO [3], are the main substances that induce oxidative stress in the body and are capable of inducing cell trauma, mitosis, apoptosis, and inflamed growth. Under normal conditions, the body can quickly remove excess ROS and RNS to maintain a dynamic equilibrium between oxidation and antioxidation. Once the dynamic equilibrium is destroyed, the overproduction of RNS and ROS results in damage to all components of the cell, including DNA, proteins, and lipids [4], which causes disordered cell function and metabolism. For example, hyperglycemia is a glucose metabolism abnormality that involves oxidative stress. ROS and RNS levels increase during hyperglycemic states through the mitochondrial electron transport chain [5], glucose autooxidation [6], and polyol pathways.
[7], and long-term hyperglycemia aggravates oxidative stress. Numerous studies have shown that natural antioxidants in traditional Chinese medicine can eliminate excess ROS and RNS and block free radical oxidation and damage.

Diabetic gastroparesis (DGP) refers to gastrointestinal neuropathy caused by long-term hyperglycemia in diabetic patients [8, 9]. The main features of DGP are delayed gastric emptying and adverse reactions of the digestive system [10], including bloating, nausea, vomiting, and weight loss [11]. DGP causes unpredictable food intake and malnutrition, which leads to fluctuations in blood glucose and affects quality of life. In addition, delayed gastric emptying affects drug absorption, reduces drug efficacy, and causes water and electrolyte disorders. The only drug approved by the FDA for the treatment of gastroparesis is metoclopramide [12, 13], a D2 receptor antagonist that targets the 5-HT4 receptor. However, this agent is not suitable for long-term use because of its serious adverse effects, especially extrapyramidal symptoms. Further development of more effective and safer drugs for DGP are needed.

*Salsola collina* Pall. is a genus of the subfamily Salsoloideae in the family Amaranthaceae. The genus name *Salsola* was first published in 1753 by Linnaeus in *Species Plantarum*. As an important desert plant, *Salsola* is widely distributed in central and southwestern Asia, the Mediterranean region, and North Africa. Pharmacological research on this species was initially performed in the 1950s and 1960s. Subsequently, increasingly more research has been done on its chemical constituents. This research has revealed that *Salsola* mainly contains alkaloids, flavonoids, sterols, and organic acids [14, 15]. Pharmacological experiments have also identified a clear antihypertensive effect [16] and central inhibition as properties of this plant. Alkaloids contribute to the species’ antihypertensive effects.

Our previous studies have shown that *Salsola* extract significantly promotes gastrointestinal motility [17] and hypoglycemia in vitro [18]. Therefore, it is hypothesized that *Salsola* might be beneficial in the treatment of DGP. In the present study, the alleviation of DGP by *Salsola* and whether its underlying mechanism involves oxidative stress were explored.

## 2. Materials and methods

### 2.1. Preparation and LC/MS analysis of the ethyl acetate extract from *Salsola*

The *Salsola collina* (whose plant name was verified using http://www.theplantlist.org) was provided and certified by Associate Professor Hong Jin (Qingdao University of Science & Technology) in September 2017. A voucher specimen (No. Qust-1612) was deposited at the Herbarium of the Department of Pharmacy, Qingdao University of Science & Technology, China. Ethanol reflux was used to prepare the *Salsola* ethanol extract (at a concentration of 20 mL•g⁻¹ with 70% ethanol and a reflux time of 3.5 h). The extract was dispersed in distilled water and repeatedly extracted with an equal volume of ethyl acetate. The filtrate was collected, concentrated, and dried using a vacuum dryer (OLABODZF-6020, Jinan, China) to obtain the desired ethyl acetate extract of *Salsola collina* (EES).

Chromatographic analysis was performed using an Agilent 1200 series liquid chromatography system (Japan Agilent Technologies), which consists of a quaternary pump, manual sampler, column oven, and degasser. An Agilent C18 column (4.6 mm × 250 mm, 5 μm particle size, Japan) was used for HPLC measurement. The mobile phase was composed of 0.01% (v / v) formic acid solution (A) and methanol (B), and the flow rate was 1 mL • min⁻¹. Gradient elution was performed according to the following elution steps [19, 20]: 0-9 min, 95% A; 10-15 minutes, 90% A; 16-20 minutes, 85% A; 21-30 minutes, 80% A; 31-40 minutes, 70% A; 41-60 minutes, 60% A. The column temperature was 30 °C, the injection volume was 5 μL, and the detection wavelength was 290 nm.

For LC/MS analysis, an Agilent 1260 system (Santa Clara, California, USA) was used. The system consisted of a quaternary pump, an autosampler, and a variable wavelength detector. Separation was performed on a Thermo C18 column (250 mm x 4.6 mm, Massachusetts, USA) at 30 °C. Spectral recording using 6530-TQF ESI–MS system (Santa Clara, California, USA) in negative ion mode.
2.2. Establishment of DGP in rats

Male Sprague-Dawley rats weighing 180-200 g were provided by Qingdao Daren Wealth Animal Technology Co., Ltd., China. All rats were fed adaptively for 7 days and kept in cages at 22±2°C and 55±10% humidity with 12-h light/dark cycles. The experiment was approved by the Animal Protection and Use Committee of Qingdao University of Science and Technology.

One hundred and twenty rats were used in this experiment. Twenty rats were randomized to the normal control group (NC), and the others were used to establish a rat model of DGP. The rats in the DGP group were injected intraperitoneally with 50 mg•kg\(^{-1}\) freshly prepared streptozotocin (Sigma-Aldrich, St. Louis, USA), while those in the NC group were injected with the same volume of saline. After 72 hours, the fasting blood glucose (FBG) values of the rats were measured using an Accu-check glucose meter (Roche Diagnostic, Basel, Switzerland), and the rats with a FBG ≥ 16.7 mmol•L\(^{-1}\) (for 2 weeks) were diagnosed with diabetes [21]. Subsequently, the rats in the DGP group were fed a high-sugar, high-fat diet (HSHF diet; 66.5% base diet, 10% lard, 20% sucrose, 1.5% cholesterol, 1% bile acid, and 1% egg yolk powder) for 10 weeks on an odd-day morning and an even-day afternoon schedule (i.e., an irregular diet) [22], while the rats in the NC group were fed a normal diet. After 10 weeks, 8 rats from the NC group and the DGP group were randomly selected for the gastric emptying test. The difference in gastric emptying, the frequency of defecation, and the characteristics of rat feces were observed to verify the establishment of DGP.

The DGP model was successfully established in 80 rats, and these rats were randomly divided into 5 groups: the DGP model (DGP) group, the low- (0.016 g•kg\(^{-1}\)), medium- (0.032 g•kg\(^{-1}\)), and high-dose (0.064 g•kg\(^{-1}\)) EES groups (LES, MES, and HES groups, respectively), and the positive treatment (MHC) group, which received metformin hydrochloride (175 mg•kg\(^{-1}\), CTTQ, Jiangsu, China) and cisapride (3.5 mg•kg\(^{-1}\), Jing-xin, Zhejiang, China). The prescribed drugs were administered orally once a day for 4 weeks. Body weight and food intake were recorded weekly.

2.3. Sample preparation

At the end of the experiment, 8 rats in each group were randomly selected, weighed, administered 1.5 mL of a 0.05% phenol red solution after overnight fasting, and then anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg•kg\(^{-1}\)). Half an hour later, the pylori of the rats were laparoscopically ligated. The stomach was removed, cut along the greater curvature, and washed with 0.9% sodium chloride solution, and the contents of the stomach were collected. The volume was adjusted to 20 mL to evaluate gastric emptying. The other rats in each group were fasted overnight, weighed, and anesthetized. Blood samples were obtained by cardiac puncture, and the plasma or serum was prepared by centrifugation at 3500 rpm for 15 min using a centrifuge (Shidaibeili, Beijing, China) and stored at -20°C. Part of the gastric antrum was carefully and immediately separated and stored frozen at -80°C for subsequent measurement of neuronal nitric oxide synthase (nNOS) and protein gene product 9.5 (PGP9.5) expression. The other part of the gastric antrum was embedded in paraffin and subjected to nNOS and PGP9.5 immunohistochemically staining.

2.4. Gastric emptying

The gastric emptying rate was determined by the phenol red label method [23]. A volume of 20 mL of gastric contents was added to 20 mL of 0.5 mol•L\(^{-1}\) NaOH and mixed well. After 1 h, 5 mL of the supernatant was removed, and 0.5 mL trichloroacetic acid (20% w/v) was added to deproteinize. The solution was then centrifuged at 3500 r•min\(^{-1}\) for 10 min. The absorbance value (OD) of the supernatant was measured at 560 nm with a spectrophotometer. A volume of 18 mL of distilled water, 20 mL of 0.5 mol•L\(^{-1}\) NaOH, and 4 mL of trichloroacetic acid (20% w/v) were added to another 2 mL of phenol red solution and mixed to measure the absorbance.

The following equation was used to calculate the gastric emptying rate: gastric emptying rate (%) = (1 - phenol red absorbance in gastric tissue homogenate / blank control phenol red absorbance) × 100.
2.5. Biochemical analysis
The serum nitric oxide (NO), total cholesterol (TC), and triglyceride (TG) levels were determined using biochemical kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to the manufacturer’s instructions.

2.6. Estimation of indicators related to oxidative stress
Indicators related to oxidative stress, including superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GSH-Px), in the gastric tissue homogenate were measured using a commercial kit (Nanjing Institute of Bioengineering, Nanjing, China) according to the manufacturer’s instructions.

2.7. Assessment of nNOS and PGP9.5 expression by Western blot analysis
The rat gastric tissue was removed from the -80°C freezer and added to protein lysate. After cleavage for 30 min, the homogenate was transferred to an EP tube and centrifuged at 12000 rpm for 5 min at 4°C. The protein concentration was determined by the bicinchoninic acid assay (Pierce, Rockford, IL, USA). In total, 50 μg of protein sample was gradient-transferred to a nitrocellulose transfer membrane (NC membrane) via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blocked with 5% skim milk powder in washing buffer for 1 h at room temperature. Membranes were incubated with specific rabbit anti-rat nNOS antibody (Sigma-Aldrich, US, 1:1000) or PGP9.5 antibody (Sigma-Aldrich, 1:800) overnight at 4°C. All transfer membranes were washed 3 times for 10 min each in TBST buffer and incubated with HRP-labeled goat anti-rabbit antibody (Abcam, Cambridge, UK, 1:1000) for 1 h at room temperature. The substrate in the Enhanced Chemiluminescence Reagent kit (ECL Western Blotting Substrate, Abcam) was uniformly applied to the NC membrane and exposed to X-ray film (Kodak X-Omat, Rochester, NY, USA). The film was scanned in a dark room, and the optical density of the tape was measured by autoradiography OptiQuant software (Packard Instruments Company, Meriden, CT, USA) and then normalized by Western blotting parallel to GAPDH.

2.8. Immunohistochemistry of nNOS and PGP9.5 in gastric antrum
The sections were deparaffinized in xylene, hydrated in a gradient of ethanol solution, and antigen-repaired according to the instructions. The sections were sequentially incubated in 3% hydrogen peroxide to block the activity of endogenous peroxidase. Primary antibodies of rabbit anti-rat PGP9.5 (Sigma-Aldrich, 1:100) or nNOS (Sigma-Aldrich, 1:100) were applied to separate sections. The sections were incubated overnight at 4°C in a humidified, sealed box, rinsed three times in PBS, incubated in the reaction-enhancing solution at 37°C for 20 min, blocked with goat serum albumin (BSA, Abcam, 1:1000) at 37°C for 20 min, and then incubated in freshly prepared 3,3-diaminobenzidine (DAB, Zsbio, Beijing, China) solution. After 5-8 min, the sections were counterstained with hematoxylin, differentiated, dehydrated, and transparently sealed. Positive immunostaining was observed under an Olympus FV500 optical microscope (Olympus, Tokyo, Japan).

2.9. Statistical analysis
The data in our experiment are shown as mean ± standard deviation (X ± SD) and were analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett’s test using SPSS 17.0 software. Values of P < 0.05 were considered statistically significant.

3. Results

3.1. HPLC and LC/MS Characterization of the EES
HPLC and LC/MS analysis was applied to recognize the main components in the EES. 10 peaks were assigned to the EES, as shown in Fig. 1. Table 1 summarizes mass spectrum characteristics of the EES.
It was confirmed by comparing literature data[24, 25] and reference materials[26, 27] that peaks 1-10 were designated as D-galacturonic acid, orsellic acid, protocatechuic acid, caffeic acid, salicylic acid, vanillic acid, syringic acid, 4-hydroxycinnamic acid, ferulic acid, 4-hydroxybenzoic acid.

**Figure 1.** HPLC chromatogram of EES with chemical structures of identified substances

| Peak | RT [min] | m/z [M-H] | Relative molecular mass | Compound | Molecular formula |
|------|----------|-----------|------------------------|----------|------------------|
| 1    | 3.132    | 193.0336  | 194.14                 | D-Galacturonic acid | C₆H₁₀O₇ |
| 2    | 6.027    | 167.0180  | 168.15                 | Orsellic acid    | C₇H₈O₄ |
| 3    | 20.973   | 153.0188  | 154.12                 | Protocatechuic acid | C₇H₆O₄ |
| 4    | 26.910   | 178.0502  | 180.16                 | Caffeic acid     | C₉H₈O₄ |
| 5    | 28.467   | 137.0239  | 138.12                 | Salicylic acid   | C₇H₆O₃ |
| 6    | 38.927   | 167.0344  | 168.15                 | Vanillic acid    | C₇H₆O₃ |
| 7    | 42.653   | 197.0444  | 198.17                 | Syringic acid    | C₉H₁₀O₅ |
| 8    | 49.760   | 163.0392  | 164.16                 | 4-Hydroxycinnamic acid | C₉H₇O₃ |
| 9    | 53.027   | 193.0496  | 194.18                 | Ferulic acid     | C₁₀H₁₀O₅ |
| 10   | 57.160   | 137.0238  | 138.12                 | 4-Hydroxybenzoic acid | C₇H₆O₃ |

Note: Peak numbers according to the chromatogram shown in Fig. 1; RT = retention time, main peak in bold.

3.2. Effects of EES on body weight, daily food intake, and blood glucose in DGP rats

During the experiment, the weights of the NC rats steadily increased due to adequate and normal feeding. Their daily food intake was basically unchanged, and their blood glucose values remained within the normal range (Table 2). The indicators in the DGP rats changed significantly compared to those in the NC rats and showed a decrease in weight and an increase in food intake and blood glucose (Table 2, *P* <0.01). After treatment with EES, the rats’ weights increased and the daily food intake and blood glucose decreased in the LES, MES, and HES groups, which differed from the DGP group.
Table 2. Effects of EES on body weight, feeding and blood glucose in diabetic gastroparesis rats. (X ± SD, n=8)

| Group | Weight /g | Ingestion /g | Blood glucose /mmol•L⁻¹ |
|-------|-----------|--------------|-------------------------|
|       | Before    | After        | Before                  | After                  |
| NC    | 350.68 ± 25.90 | 409.45 ± 25.59 | 20.83 ± 4.65           | 23.64 ± 4.61          |
|       | 23.64 ± 20.34 | 23.64 ± 20.34 | 5.21 ± 0.94            | 5.21 ± 0.94           |
| DGP   | 263.28 ± 22.28 | 229.28 ± 15.11 | 38.02 ± 6.21           | 38.02 ± 6.21          |
|       | 229.28 ± 38.02 | 229.28 ± 38.02 | 20.05 ± 4.77           | 20.05 ± 4.77          |
| LES   | 257.14 ± 24.80 | 250.23 ± 30.98 | 37.81 ± 7.79           | 37.81 ± 7.79          |
|       | 250.23 ± 37.81 | 250.23 ± 37.81 | 20.34 ± 3.80           | 20.34 ± 3.80          |
| MES   | 266.36 ± 281.34 | 250.23 ± 30.98 | 37.81 ± 7.79           | 37.81 ± 7.79          |
|       | 250.23 ± 37.81 | 250.23 ± 37.81 | 20.34 ± 3.80           | 20.34 ± 3.80          |
| HES   | 256.71 ± 24.44 | 298.00 ± 38.82 | 35.05 ± 5.51           | 35.05 ± 5.51          |
|       | 298.00 ± 35.05 | 298.00 ± 35.05 | 20.34 ± 3.80           | 20.34 ± 3.80          |
| MHC   | 261.50 ± 29.05 | 309.61 ± 39.11 | 29.06 ± 6.93           | 29.06 ± 6.93          |
|       | 309.61 ± 29.05 | 309.61 ± 29.05 | 20.05 ± 6.28           | 20.05 ± 6.28          |

Note: Before: before drug administration; After: after drug administration; Compared with before, #P < 0.05, ##P < 0.01; Compared with the NC group, *P < 0.05, **P < 0.01; Compared with the DGP group, △P < 0.05, △△P < 0.01; Compared with the LES group, ▲P < 0.05, ▲▲P < 0.01; Compared with the MES group, $P < 0.05, $$P < 0.01

3.3. Effects of EES on gastric emptying in DGP rats

Compared to the NC group, the DGP group experienced a significantly slower gastric emptying rate (Fig. 2, P < 0.01). After treatment with EES, the gastric emptying rate increased significantly in a dose-dependent manner in the LES, MES, and HES groups when compared to that in the DGP group (Fig. 2, P < 0.01). The alleviation of gastroparesis by high-dose EES was as effective as that by the combination of metformin hydrochloride and cisapride (Fig. 2, P > 0.05).

Figure 2. Effect of EES on gastric emptying. Note: Compared to the NC group, *P < 0.05, **P < 0.01; compared to the DGP group, △P < 0.05, △△P < 0.01; compared to the LES group, #P < 0.05, ##P < 0.01; compared to the MES group, $P < 0.05, $$P < 0.01
3.4. Effects of EES on biochemical analysis in DGP rats
Compared to the NC group, the DGP group showed a significant increase in the serum TC and TG levels and a significant decrease in the NO levels (Fig. 3, P < 0.05). During the experiment, EES reduced the serum TC and TG levels and increased the NO levels in a dose-dependent manner (Fig. 3, P < 0.05-0.01) without reaching a significant difference between the levels of the NC group and HES group. Compared to the positive treatment group, the TC level in the high-dose EES group decreased, which shows the significant lipid-lowering effect of EES.

![Figure 3](image-url)  
**Figure 3.** Effect of EES on biochemical components in the plasma. a. The concentration of nitric oxide (NO) in each group of rats; b. the total cholesterol (TC) concentration in each group of rats; c. the triglyceride (TG) concentration in each group of rats. Compared to the control group, *P < 0.05, **P < 0.01; compared to the model group, *P < 0.05, △△P < 0.01; compared to the LES group, #P < 0.05, ##P < 0.01; compared to the MES group, $P < 0.05, $$$P < 0.01.

3.5. Effects of EES on oxidative stress
Compared to the NC group, the DGP group demonstrated significantly decreased SOD, CAT, and GSH-Px activity in the gastric tissue (Fig. 4, P < 0.01) but significantly increased MDA levels (Fig. 4, P < 0.01). After treatment with EES, the SOD, CAT and GSH-Px activity increased, while the MDA levels significantly decreased in a dose-dependent manner (Fig. 4, P < 0.05-0.01). Furthermore, these oxidative stress parameters in the gastric tissue in the HES group did not significantly differ from those in the NC group.
Figure 4. Effect of EES on oxidative stress markers in the gastric tissue. a. The concentration of MDA in the stomachs of rats in each group, b. the concentration of SOD in the stomachs of rats in each group, c. the CAT concentration in the stomachs of rats in each group, d. the GSH-Px concentration in the stomachs of rats in each group. Compared to the control group, *P < 0.05, **P < 0.01; compared to the model group, △P < 0.05, △△P < 0.01; compared to the LES group, #P < 0.05, ##P < 0.01; compared to the MES group, $P < 0.05, $$P < 0.01.

3.6. Effects of EES on nNOS and PGP9.5 protein levels in the gastric tissue

The expression levels of nNOS and PGP9.5 in the gastric tissue were determined by Western blot and immunohistochemical analysis. The Western blot showed that the expression of nNOS and PGP9.5 in the gastric tissue significantly decreased in the DGP group (Fig. 5b, 5c) compared to that in the NC group (Fig. 5b, 5c). However, EES significantly increased the expression of nNOS and PGP9.5 in the gastric tissue in a dose-dependent manner (Fig. 5b, 5c) compared to that in the DGP group (Fig. 5b, 5c). The immunohistochemical analysis revealed that there were less nNOS-positive cells in the gastric tissue of rats in the DGP group than in the NC group (Fig. 6). EES significantly increased the expression of nNOS to a higher degree (Fig. 6). PGP9.5 immunoreactivity in the stomach was seen throughout the muscle tissue, especially in the Auerbach plexus (AP) and the circular layer of the external muscular layer (Fig. 7). Compared to the NC rats, the GDP rats had a sparser density of PGP 9.5-positive nerves (Fig. 7a and 7b). In contrast, the density of PGP 9.5-positive nerves in the HES rats resembled that in the NC rats.
Figure 5. Protein expression of nNOS and PGP9.5 in the gastric tissue of rats in each group. a. nNOS, PGP9.5 and GADPH immunoblot bands; b. quantitative analysis of nNOS in the stomach; c. quantitative analysis of PGP9.5 in the stomach. Compared to the control group, *P < 0.05, **P < 0.01; compared to the model group, △P < 0.05, △△P < 0.01; compared to the LES group, #P < 0.05, ##P < 0.01.

Figure 6. The immunoreactivity of nNOS in a group of rat gastric nerves. a. NC group; b. DGP group; c. LES group; d. MES group; e. HES group. The brown particle deposits in the figure demonstrate the positive immunoreactivity of nNOS (represented by the arrow). AP: Auerbach plexus. Scale bar = 100 μm, magnification = x 200.
Figure 7. Immunoreactivity of PGP9.5 in the gastric nerves of each group of rats. a. NC group; b. DGP group; c. LES group; d. MES group; e. HES group. The brown particle deposits demonstrate the positive immunoreactivity of PGP9.5 (represented by arrows: PGP 9.5 positive nerve fibers (thin arrows) and positive nerves (thick arrows)). Scale bar = 100 μm, magnification = x 200.

4. Discussion

In this experiment, a rat model of diabetic gastroparesis was established via streptozotocin injection and irregular HSHF feeding for 10 weeks. After the rats received EES by intragastric administration, gastric emptying, serum biochemical parameters, tissue oxidative stress indicators, and the expression of gastric nervous system neuron markers were detected. These measurements revealed that EES promoted gastric emptying in the DGP rats, and this promotion might involve the inhibition of oxidative stress, the protection of tissue nerves, and a reduction in blood glucose and lipids.

Streptozotocin was used in this experiment to model DGP and is an antibiotic derived from Streptomyces achromogenes [28] and a nitrosourea analog. Streptozotocin, which is toxic to pancreatic insulin-induced β-cells [29], can independently enter cells through glucose transporter type 2 (GLUT2) and damage tissues, such as gastrointestinal and renal tissue [30]. As a DNA alkylating agent, streptozotocin breaks DNA strands and increases poly-ADP ribose synthase activity [31], thereby destroying cells. A small amount of streptozotocin induces insulin insensitivity in peripheral tissues, and irregular feeding with a HSHF diet destroys islet β-cell function and results in gastric motility disorders. The above pathophysiological changes are consistent with diabetic gastroparesis in humans with stable and sustained hyperglycemia [32]. Diabetic gastroparesis rats showed symptoms such as nausea, weight loss, and constipation, which were accompanied by fur disorder and apathy. Currently, DGP is recognized as a significant health problem and affects many diabetic patients worldwide. However, the treatment options for DGP are extremely limited, and the currently available treatments are frequently ineffective and cause intolerable adverse effects. Treatment for diabetic gastroparesis has remained largely unchanged over a decade and relies primarily on the use of prokinetic agents, such as erythromycin, metoclopramide, and domperidone, which are the cornerstones of DGP treatment. In long-term DGP patients, prokinetic and antiemetics are often used in combination to alleviate symptoms. However, these medications are often interrupted because of their short-term efficacy and poor patient response. The discovery of more effective and safer drugs to treat DGP is critical. Fortunately, traditional Chinese medicines show great potential because of their safety and multi-targeted effects. In the present study, we observed the effects of EES, which was identified as a prokinetic drug in our previous study, on DGP rats. The results revealed that EES
promoted gastric emptying, inhibited food intake, and induced weight gain in the DGP rats. Moreover, the hypoglycemic and lipid-lowering effects of EES were also seen in the DGP rats.

The pathogenesis of DGP is mainly related to hyperglycemia, neuropathy, serum gastrointestinal hormonal abnormalities, microvascular disease, and metabolic disorders [33, 34]. Studies have shown that gastric motility and emptying are regulated by blood glucose concentrations [35, 36]; an increase in the blood glucose concentration in patients with type I and type II diabetes has been shown to delay gastric emptying (i.e., an increase in blood glucose can decrease the rate of gastric emptying, and delayed gastric emptying makes blood glucose concentrations difficult to control, thus forming a vicious cycle). Accumulating studies reveal that hyperglycemia not only directly affects autonomic function and gastrointestinal hormone secretion but also inhibits the interdigestive migrating motor complex and antral motility in healthy people and diabetic patients, which thus negatively affects gastric motility. Therefore, gastric motility drugs are used clinically in combination with hypoglycemic drugs to treat DGP. In the present study, EES exhibited gastric motility promotion as well as blood glucose concentration reduction, which alone was effective in DGP.

In addition to hyperglycemia, the other main feature of diabetes is hyperlipidemia, which has been reported in the streptozotocin-induced diabetes model [37]. The TC and TG levels in the DGP rats increased significantly, which was confirmed in our present study. Surprisingly, EES decreased the TC and TG levels in the DGP rats, which might demonstrate its benefit in relieving gastroparesis.

Neuropathy has been recognized to have an essential role in the pathogenesis of DGP. This role is manifested by most diabetic patients having neuropathy accompanied by varying degrees of gastrointestinal dysfunction. nNOS is a neuronal enzyme that produces nitric oxide [38], and a decrease in nNOS expression is associated with changes in gastrointestinal motility. PGP9.5, a cytoplasmic ubiquitin carboxy terminal hydroxylase present in all neurons, can be used as a highly specific pan-neuronal marker [39, 40]. Therefore, nNOS and PGP9.5 immunohistochemical staining were used to estimate nerve fiber density. NO has vasodilating properties and is synthesized from L-arginine NO synthase (NOS) [41]. As a highly diffusible small molecule, NO is released from endothelial cells mainly to cope with shear stress and other receptors, which leads to vascular smooth muscle cell relaxation. NOS reduces the production of NO or the decomposition of ROS [42], which impairs the bioavailability of NO and causes endothelial dysfunction. *Salsola* may act by enhancing NOS activity, maintaining NO production, and restoring gastric smooth muscle function. Our experiments found that EES increased the gastric neuron population in DGP rats by upregulating the expression of nNOS and PGP9.5. Hosseini [42] reported that diabetic neuropathy develops during hyperglycemia and related metabolic imbalances (especially oxidative stress). Hyperglycemia-induced free radical overproduction has been recognized to further aggravate neuropathy [34]. Mitochondrial damage occurs due to the excessive formation of ROS and RNS, which are toxic to cells and damage peripheral nerves. Oxidative stress and ROS link metabolic initiators to physiological mediators, thereby leading to progressive neurofibrillary dysfunction, damage and loss in diabetic neuropathy. Meanwhile, there is a direct correlation between oxidative stress and blood glucose variability [43]. When excess glucose causes excessive production, cellular oxidative stress is further enhanced, which results in more cell damage. Some evidence suggests that oxidative stress, accumulating free radicals, and changes in antioxidant defense enzymes are key factors in the pathogenesis of diabetes. Furthermore, lipid peroxidation can damage the tissue nerves and aggravate the symptoms of gastroparesis. As the final product of lipid peroxidation, MDA is also considered to be one of the most sensitive markers of lipid peroxidation. The inhibition of SOD, CAT and GSH-Px plays a crucial role in protecting cells from oxidative damage. As a protective enzyme, SOD can reduce the scavenging of free radicals [7]; CAT and GSH-Px hydrolyze SOD to form disproportionate superoxide anions (H₂O₂) [44]. The above oxidative stress mechanisms were seen in the present experiment. In addition, EES increased gastric SOD, CAT, GSH-Px activity and decreased the concentration of MDA in the DGP rats.

*Salsola collina* is rich in organic acids, polysaccharides and similar molecules. Studies have shown that natural organic acids have antibacterial, anti-inflammatory, hypoglycemic, anti-oxidant, and
immunity regulating activities. Furthermore, they can increase coronary blood flow, inhibit the generation of lipid peroxides in the brain, soften blood vessels [45], promote calcium and iron absorption, and promote metabolism [46]. Organic acids can reduce the formation of free radicals by inhibiting the generation of free radicals. In addition, by deactivating free radicals, organic acids can protect antioxidant enzymes and increase the activity of antioxidant enzymes in the body, thereby enhancing antioxidant activity [47, 48]. Analysis of LC-MS results showed that EES is mainly composed of 10 organic acids with a high content. They not only reduce the blood glucose and lipids of animals, but also increase the expression of antioxidant enzymes (i.e., SOD and CAT) and nNOS and PGP9 in gastric tissues. In summary, our results suggest that EES promotes gastric emptying in DGP rats, which may be due to its ability to inhibit oxidative stress and increase the number of gastric neurons, combined with its hypoglycemic and lipid-lowering effects. It is hypothesized that the presence of organic acid in the EES may help alleviating DGP. Simultaneously, the synergistic effects of other compounds in the extract cannot be ignored.

5. Conclusion
In summary, our results suggest that EES promotes gastric emptying in DGP rats, which may be due to its ability to inhibit oxidative stress and increase the number of gastric neurons, combined with its hypoglycemic and lipid-lowering effects. It is hypothesized that the presence of organic acid in the EES may help alleviating DGP. Simultaneously, the synergistic effects of other compounds in the extract cannot be ignored.

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