Pingwei capsules improve gastrointestinal motility in rats with functional dyspepsia

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Abstract
OBJECTIVE: To investigate the mechanism of Pingwei capsules (PWC) in improving gastrointestinal motility in rats with functional dyspepsia (FD).

METHODS: We established an FD model by stimulating semi-starvation rats via tail damping, provocation, and forced exercise fatigue. The FD model group was further divided into five groups according to the treatment received: normal saline, domperidone, low-dose PWC, mid-dose PWC, or high-dose PWC. The effect of PWC on FD rat was evaluated by measuring gastrointestinal motility. Changes of leptin and cholecystokinin (CCK) were detected through enzyme-linked immunosorbent assay, reverse transcription-polymerase chain reaction, and immunohistochemistry.

RESULTS: PWC significantly increased gastrointestinal motility in FD rats. Furthermore, PWC significantly increased CCK mRNA and protein concentrations in the duodenum and antrum, decreased leptin protein concentrations in the duodenum, antrum, and hypothalamus, and decreased CCK protein concentration in the hypothalamus.

CONCLUSION: PWC improve gastrointestinal motor function in FD rats by decreasing the leptin concentration in serum and the brain-gut axis, and by increasing the CCK concentration in gastrointestinal tissue. Our findings help to elucidate the mechanism of FD and provide further insight into the pharmacokinetics of PWC.

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Keywords: Pingwei capsules; Functional dyspepsia; Gastrointestinal motility; Brain-gut peptide; Brain-gut axis

INTRODUCTION
Functional dyspepsia (FD) is a common clinical gastrointestinal disorder that is characterized by persistent or recurrent pain and discomfort in the absence of any organic disease. FD has a negative impact on the quality
of life and health of patients, despite causing minimal or no mortality. Although many FD studies have been performed, the mechanism of FD remains unclear. Determining the mechanism of FD is particularly challenging because there are several types of FD, including dysmotility, non-ulcer and reflux dyspepsia, and each FD type has varying clinical symptoms. Previous studies have reported the main clinical symptoms of FD as antral hypomotility and delayed gastric emptying, which is clinically characterized by a feeling of fullness, upper abdominal discomfort, early satiety, or nausea.

Bidirectional brain-gut interactions play an important role in the regulation of physiological activities in gastrointestinal tract disease. For example, dysregulation of the central nervous system (CNS) and enteric nervous system (ENS) in FD leads to alterations in sensation, motility, mood, and affect. Information from the local sensory inputs of the gastrointestinal tract is transmitted to the CNS via various visceral afferent pathways (enteric, spinal, and vagal). These communication pathways between the ENS and CNS may involve brain-gut peptides.

Circulating leptin penetrates the blood-brain barrier via a receptor-mediated transport system and acts on the leptin receptor in the medial hypothalamus to affect the regulation of energy balance and feeding behavior. In contrast, the blood-brain barrier cannot be crossed by cholecystokinin (CCK), which is secreted in the gastrointestinal tract in connection with gastrointestinal motility via the vagal reflex pathway, which is involved in feedback suppression of gastrointestinal motility. In clinical practice, gastrointestinal motility can be increased by prescribing cisapride, itopride, and metoclopramide; however, these drugs are no longer used for this purpose due to associated adverse effects. The gastroprokinetic drug domperidone is used in many countries, but is not available in the USA without a prescription, as it has not been approved by the US Food and Drug Administration. Thus, there is a need for safe and effective prokinetic agents that increase gastrointestinal motility.

Pingwei capsules (PWC) were developed from the Traditional Chinese Medicine Pingwei San (PWS; Pyungwi-San in Korea). PWS is a classic drug used during the Song Dynasty in China; it plays a key role in the treatment of gastritis, esophageal reflux, gastric or duodenal ulcers, and acute or chronic enteritis. The PWS formula was first published in the Prescriptions of Taiping Benezvolent Dispensary and consists of six herbs: Cangzhu (Rhizoma Atractylodis Lanceae), Houpu (Cortex Magnoliae Officinalis), Chenpi (Pericarpium Citri Reticulatae), Gacno (Radix Glycyrrhizae), Shengjiao (Rhizoma Zingiberis Recens), and Dazao (Fructus Jujubae). In the USA, PWS could potentially be used as a substitute for cisapride in the treatment of heartburn and gastritis. The most effective herbal composition and dosage of PWC was developed at the Affiliated Hospital of Gansu University of Chinese Medicine according to modern medicine pathogenesis, pharmacological research, and clinical experience. PWC comprises:

<table>
<thead>
<tr>
<th>Herb</th>
<th>Therapeutic effects</th>
<th>Proportion (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cangzhu (Rhizoma Atractylodis Lanceae)</td>
<td>Improvement of gastrointestinal motility</td>
<td>6.8</td>
</tr>
<tr>
<td>Houpu (Cortex Magnoliae Officinalis)</td>
<td>Anti-inflammatory and anti-depression</td>
<td>6.8</td>
</tr>
<tr>
<td>Muxiang (Radix Austriacae)</td>
<td>Spasmylytic role in gastrointestinal motility</td>
<td>5.6</td>
</tr>
<tr>
<td>Zhiqiao (Fructus Aurantii Submaturi)</td>
<td>Anti-tumor</td>
<td>6.8</td>
</tr>
<tr>
<td>Chenpi (Pericarpium Citri Reticulatae)</td>
<td>Anti-inflammatory and weight increase</td>
<td>5.6</td>
</tr>
<tr>
<td>Chaihu (Radix Bupleuri Chinessis)</td>
<td>Anti-depression</td>
<td>6.8</td>
</tr>
<tr>
<td>Baishao (Radix Paeoniae Alba)</td>
<td>Anti-inflammatory and anti-depression</td>
<td>5.6</td>
</tr>
<tr>
<td>Baiji (Rhizoma Bletillae Striatae)</td>
<td>Anti-inflammatory and anti-aging</td>
<td>6.8</td>
</tr>
<tr>
<td>Sanleng (Rhizoma Sparganzis)</td>
<td>Anti-tumor and the abdomen pain remission</td>
<td>5.6</td>
</tr>
<tr>
<td>Haipiaoxiao (Endocunoma Sepiellae)</td>
<td>Anti-inflammatory and gastroprotective potential</td>
<td>6.8</td>
</tr>
<tr>
<td>Jinejin (Endothelium Coreneum Gigeriae Galli)</td>
<td>Promotion of digestion and circulation</td>
<td>8.5</td>
</tr>
<tr>
<td>Zhebeimu (Bulbus Frisilliae Thunbergii)</td>
<td>Anti-inflammatory</td>
<td>5.6</td>
</tr>
<tr>
<td>Huangliang (Rhizoma Coptidis)</td>
<td>Anti-inflammatory</td>
<td>3.0</td>
</tr>
<tr>
<td>Pugongying (Herba Taraxaci Mongolici)</td>
<td>Anti-inflammatory and analgesic activity</td>
<td>8.5</td>
</tr>
<tr>
<td>Yanhusuo (Rhizoma Corydalis Yanhusuo)</td>
<td>Anxiolyis</td>
<td>5.6</td>
</tr>
<tr>
<td>Ezh (Rhizoma Curcumae Phaeocaulis)</td>
<td>Anti-inflammatory and anti-tumor</td>
<td>5.6</td>
</tr>
</tbody>
</table>
es 16 herbs that possess clear therapeutic effects (Table 1); clinicians at the Affiliated Hospital of Gansu University of Chinese Medicine have used PWC to treat FD for many years. A paper published by the Affiliated Hospital of Gansu University of Chinese Medicine reported that PWC can significantly improve upper abdominal fullness, early satiety, belching, heartburn, nausea, vomiting, and other symptoms in patients with FD, but the mechanism by which PWC improves FD in rats remains unclear.

The purpose of the present study was to explore the mechanism by which PWC improves gastrointestinal motility in terms of brain-gut peptides and the brain-gut axis. We used a tail damping FD rat model that simulates the development of FD in humans; this rat model displays several key features of human FD, such as reduced food intake, weight reduction, irritability, aggression, and gastrointestinal hypomotility.

MATERIALS AND METHODS

Animal model

Thirty-six healthy male Wistar rats (clean grade, 7-weeks-old, weighing 180-220 g) were obtained from the Experimental Animal Center of Lanzhou University. PWC were supplied by the Affiliated Hospital of Gansu University of Chinese Medicine (drug approval number: Z1200022224). The rats were housed in a restricted-access laboratory with a controlled temperature of 25 °C and a light: dark cycle of 12 h:12 h. Two pairs of bipolar stainless steel electrodes were implanted 3 mm apart onto the serosal surface of the gastrointestinal tract in each rat. One pair was implanted on the antrum (1 cm distal to the pylorus), and the other was implanted on the small intestine (1-2 cm distal to the pylorus). The free ends of the electrodes were brought subcutaneously to the back of the neck. After 1 week of conventional feeding, the rats were randomized into a control group (n = 8) and an FD model group (n = 28) using a random number table method. The number of laboratory rats necessary was calculated according to previously reported animal experiments. The FD model was established by stimulating semi-starvation rats via tail damping, provocation, and forced exercise fatigue; this stimulation was performed four times a day for 10 d. In detail, we used a long sponge-holding forceps and clamped the distal 1/3 of the tail, without causing damage to the skin. The tail clamping was performed for 30 min each time, after which the rats ran on an experimental treadmill at an appropriate speed for 10 min to induce forced exercise fatigue. These experimental procedures were performed at 9:00, 12:00, 15:00, and 18:00 for 10 consecutive days, with alternate day feeding. Bodyweight and food intake were recorded. If the skin on the tail ruptured, iodophor was applied to the wound. The random number table method was used to randomly divide the 28 model rats into five groups: a model group (n = 8, received 4 mL of normal saline daily), a domperidone-treated group (domp group, n = 5, 10 mg·kg⁻¹·d⁻¹), a low-dose PWC-treated group (PWC I group, n = 5, 1.6 g·kg⁻¹·d⁻¹), a middle dose PWC-treated group (PWC II group, n = 5, 3.2 g·kg⁻¹·d⁻¹), and a high dose PWC-treated group (PWC III group, n = 5, 4.8 g·kg⁻¹·d⁻¹). The control group received 4 mL of normal saline daily. The rats received 4 mL of drug or normal saline daily by oral gavage for 21 d using straight gavage needles (Globalbio, Beijing, China) for 150-300 g bodyweight (16 gauge, 3-inch length, 3-mm ball diameter). Then, the gastrointestinal electrical activity was recorded by a microcomputer with a BL-420S experimental system biological function analyzer (TaiMeng Technology, Chengdu, China). The frequency and amplitude of slow wave and spike activity in the duodenum and antrum were recorded for 1 h.

Ethics approval and consent to participate

All animal procedures carried out in this study were reviewed, approved, and supervised by the Institutional Animal Care and Use Committee of the Ethics Committee of Lanzhou University, China (certificate of quality No. SCXK [gan] 2013-0002).

Measurement of intestinal propulsion and gastric emptying

The rats were fasted for 24 h before the experiment. On the day of the experiment, oral gavage was used to give each rat 5% graphite powder (Tianheta Graphite, Qingdao, China) with milk (Solarbio, Beijing, China) and glucose (Solarbio) in water (weighed and recorded as A₁). After 30 min of absorption time, the rats were euthanized. Blood samples were taken from the femoral arteries, and the serum was separated and frozen at −80 °C for enzyme-linked immunosorbent assay (ELISA). The stomach and attached small intestine were immediately exposed by laparotomy, carefully removed, and placed on a wooden board; the esophagogastric, gastroduodenal, and ileocecal junctions were ligated, and the leading edge of the graphite powder in the intestine was observed. Measurements included the length of the small intestine from the pylorus to the ileocecal junction, and the length of graphite powder movement. The intestinal propulsion was calculated as the length of the graphite powder/the whole length of the small intestine. The stomach was removed, weighed (recorded as A₂), and immersed in 0.9% saline solution (Solarbio) to clean out the remaining graphite powder. The stomach was then blotted dry with absorbent paper to remove any surface moisture before being weighed (recorded as A₃). The gastric emptying rate was calculated as (A₃ - A₄)/A₁.

Tissue preparation

After removing the stomach and small intestine, the duodenum, gastric antrum, and hypothalamus were also removed. Tissue blocks of these samples were rapidly frozen in liquid nitrogen and then stored at −80 °C.
until reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed.

**Hematoxylin-eosin (HE) staining**
The duodenum and stomach were fixed in neutral 4% paraformaldehyde (Solarbio) in 0.1 M sodium phosphate buffer, embedded in paraffin blocks, and then embedded in paraffin blocks. Serial 4-μm-thick sections were prepared and stained with HE by routine methods. All sections were carefully examined to exclude those with substantial microscopic histologic abnormalities.

**ELISA**
The serum concentrations of leptin protein were quantified by ELISA (R & D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol. The optical densities of the ELISA samples were determined within 30 min on a microplate reader set to 450 nm.

**Immunohistochemistry**
The duodenum, antrum, and hypothalamus were fixed in neutral 4% paraformaldehyde in 0.1 M sodium phosphate buffer, embedded in paraffin blocks, and cut into 4-μm-thick sections on slides. Deparaffinized sections were incubated in 0.01 M citrate buffer (Solarbio) in a microwave oven for heat-induced epitope retrieval. Endogenous peroxidase activity was blocked with 3% H₂O₂ (Zsbio, Beijing, China), or nonspecific binding was blocked by incubation with 5% normal goat serum (Zsbio) in phosphate-buffered saline for 5 min. The samples were then incubated overnight at 4 °C with anti-leptin antibody and anti-CCK antibody (Bioss, Beijing, China). After washing, the slides were incubated for 20 min at room temperature with secondary biotinylated antibody (Zsbio). Visualization was achieved using a DAB detection kit (Solarbio).

**RT-PCR**
Total RNA was isolated from the gastrointestinal tissues and hypothalamus with Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the reverse transcription reaction was performed with a reverse transcription kit (Promega, Madison, WI, USA) according to the manufacturer’s protocol.

The PCR reactions were heated at 95 °C for 2 min, and then immediately cycled 32-36 times through a 30 s denaturing step at 95 °C, a 30 s annealing step at 60 °C, and a 30 s extension step at 72 °C. After the cycling procedure, a final elongation step was performed for 10 min at 72 °C. The reaction products were separated on 2% agarose gel (Invitrogen), and then imaged.

The following primers were used for PCR amplification:
CCK: forward, 5'-CGCAGCTAGCCGATACAGTCA-3'; reverse, 5'-TTTTCATTCCGGCTGCTCTCC-3'.
Actin gene: forward, 5'-TCCTGTGCGCATCCATGAAA-3'; reverse, 5'-GAAGCAGTTCGGTGACGAT-3'.

**Statistical analysis**
The above experiments were repeated for each rat, and the data were pooled. For each test, the experimental unit was the individual animal. The data were presented as the mean ± standard deviation, and were analyzed by one-way analysis of variance or the χ² test using SPSS 21 software (IBM, Chicago, IL, USA). Statistical significance was determined for intragroup differences in comparison with the control group (P < 0.05) and the model group (P < 0.05).

**RESULTS**

**PWC increased bodyweight and food intake in FD rats**
After modeling, bodyweight and food intake were significantly decreased in the model group compared with the control group (Tables 2, 3; P < 0.05). Compared with the model group, the PWC II and PWC III treatments significantly increased both bodyweight and food intake (P < 0.05). PWC I treatment also tended to increase the bodyweight and food intake compared with the control group, but these differences were not significant.

**PWC increased the gastrointestinal electrical activity**

Table 2: Body weight (g) changes in all rats (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before modeling</th>
<th>After modeling</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>232±13</td>
<td>256±8</td>
<td>267±11</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>230±11</td>
<td>214±17</td>
<td>225±8</td>
</tr>
<tr>
<td>Domp</td>
<td>5</td>
<td>232±20</td>
<td>209±18</td>
<td>262±13</td>
</tr>
<tr>
<td>PWC I</td>
<td>5</td>
<td>233±10</td>
<td>211±8</td>
<td>233±4</td>
</tr>
<tr>
<td>PWC II</td>
<td>5</td>
<td>229±13</td>
<td>214±17</td>
<td>241±6</td>
</tr>
<tr>
<td>PWC III</td>
<td>5</td>
<td>231±21</td>
<td>215±7</td>
<td>254±18</td>
</tr>
</tbody>
</table>

Notes: Domp group: received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; PWC I group: received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC II group: received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC III group: received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. Control and model groups: received the same volume of 0.9% sodium daily for 21 d. PWC: Pinglei capsules. All data are expressed as mean ± standard deviation. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).
activity in FD rats

Compared with the control group, gastrointestinal electrical activity was significantly decreased in the model group (Table 4). However, gastrointestinal electrical activity in the antrum was significantly increased in the PWC-treated groups compared with the model group. The migrating motor complex (MMC) consists of four phases, of which phase III is the most prominent and is characterized by a burst of maximal amplitude contractions. Figure 1A and 1B show that the MMC cycle in the model group was disordered compared with the control group, and the phase III activity of the MMC in the model group disappeared in both the duodenum and the antrum. The phase III activity of the MMC was enhanced in the duodenum by the PWC II treatment, and enhanced in the antrum by the PWC I treatment.

PWC increased the rates of intestinal propulsion and gastric emptying in FD rats

The rates of intestinal propulsion and gastric emptying were significantly decreased in the model group compared with the control group (Figure 1C; P < 0.05). Intestinal propulsion tended to be greater in all drug-treated groups compared with the control group, but these differences were not statistically significant. Compared with the model group, the PWC II and PWC III treatments significantly increased gastric emptying (P < 0.05).

HE staining analysis

There was no evidence of ulcer, erosion, metaplasia, infiltration of explicit inflammatory cells, or other organic lesions in the gastric and duodenal mucosa of the rats in each group (Figure 2).

PWC reduced the serum leptin concentration

Leptin concentration was significantly increased in the model group compared with the control group (P < 0.05; Figure 3). Compared with the model group, the serum leptin concentrations were significantly reduced in the Domp and PWC III groups (P < 0.05), but were not changed in the PWC I or PWC II groups (Figure 3).

Effects of PWC on the expression of leptin and CCK

Leptin and CCK were found in the submucosal and muscular layers of the duodenum and antrum (Figure 4). Compared with the control group, the model group had increased leptin expression in the duodenum, antrum, and hypothalamus, while all drug-treat-

Table 3 Changes in food intake (g/d) in all rats (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before modeling</th>
<th>After modeling</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>30.5±1.4</td>
<td>30.9±1.6</td>
<td>33.1±1.8</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>30.4±2.1</td>
<td>23.5±1.0</td>
<td>24.6±1.2</td>
</tr>
<tr>
<td>Domp</td>
<td>5</td>
<td>29.9±3.0</td>
<td>23.4±1.4</td>
<td>29.8±1.1</td>
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<tr>
<td>PWC I</td>
<td>5</td>
<td>30.4±2.0</td>
<td>24.1±1.3</td>
<td>27.0±1.2</td>
</tr>
<tr>
<td>PWC II</td>
<td>5</td>
<td>29.8±1.8</td>
<td>23.6±0.8</td>
<td>28.8±1.2</td>
</tr>
<tr>
<td>PWC III</td>
<td>5</td>
<td>30.9±1.8</td>
<td>24.1±1.4</td>
<td>30.2±1.4</td>
</tr>
</tbody>
</table>

Notes: Domp group: received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; PWC I group: received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC II group: received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC III group: received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. Control and model groups: received the same volume of 0.9% sodium daily for 21 d. PWC: Pingwei capsules; SA: slow wave amplitude; SF: slow wave frequency; SBA: spike bursts amplitude; PSCS: percentage of the slow wave containing spike bursts. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).

Table 4 Effect of PWC treatment on antral and duodenal myoelectric activities in rats with functional dyspepsia (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duodenum</th>
<th>Antrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SA (μV)</td>
<td>SF (num/min)</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>159.5±36.40</td>
<td>37.05±4.03</td>
</tr>
<tr>
<td>Model</td>
<td>8</td>
<td>140.21±58.97</td>
<td>27.93±4.74</td>
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<tr>
<td>Domp</td>
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<td>278.90±180.96</td>
<td>34.8±2.97</td>
</tr>
<tr>
<td>PWC I</td>
<td>5</td>
<td>212.02±93.52</td>
<td>25.76±14.32</td>
</tr>
<tr>
<td>PWC II</td>
<td>5</td>
<td>136.59±31.94</td>
<td>32.34±6.22</td>
</tr>
<tr>
<td>PWC III</td>
<td>5</td>
<td>138.22±26.22</td>
<td>32.17±1.71</td>
</tr>
</tbody>
</table>

Notes: Domp group: received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; PWC I group: received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC II group: received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC III group: received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. Control and model groups: received the same volume of 0.9% sodium daily for 21 d. PWC: Pingwei capsules; SA: slow wave amplitude; SF: slow wave frequency; SBA: spike bursts amplitude; PSCS: percentage of the slow wave containing spike bursts. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).
Figure 1 Effects of PWC treatment on gastrointestinal motility in FD rats
A: effects of PWC on duodenal MMC. B: effects of PWC on antral MMC. Ⅲ denotes phase III of the MMC. C: effects of PWC treatment on the rates of intestinal propulsion and gastric emptying. Domp group (n = 5): received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; PWC Ⅰ group (n = 5): received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC Ⅱ group (n = 5): received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC Ⅲ group (n = 5): received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. Control and model groups (n = 8, respectively): received the same volume of 0.9% sodium daily for 21 d. PWC: Pingwei capsules; FD: functional dyspepsia; MMC: migrating motor complex. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).

Figure 2 Hematoxylin and eosin staining of the duodenum and stomach (× 40)
A1-A6: duodenum; B1-B6: stomach; A1 and B1: control group (n = 8); received the same volume of 0.9% sodium daily for 21 d; A2 and B2: model group (n = 8); received the same volume of 0.9% sodium daily for 21 d; A3 and B3: domperidone group (n = 5): received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; A4 and B4: PWC Ⅰ group (n = 5): received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; A5 and B5: PWC Ⅱ group (n = 5): received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; A6 and B6: PWC Ⅲ group (n = 5): received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. PWC: Pingwei capsules.

Figure 3 Effects of PWC on leptin in serum
Serum leptin protein concentrations detected by ELISA. Domp group (n = 5): received 10 mg·kg⁻¹·d⁻¹ domperidone for 21 d; PWC Ⅰ group (n = 5): received 1.6 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC Ⅱ group (n = 5): received 3.2 g·kg⁻¹·d⁻¹ PWC for 21 d; PWC Ⅲ group (n = 5): received 4.8 g·kg⁻¹·d⁻¹ PWC for 21 d. Control and model groups (n = 8, respectively): received the same volume of 0.9% sodium daily for 21 d. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05). PWC: Pingwei capsules; ELISA: enzyme-linked immunosorbent assay.

Effect of PWC on CCK mRNA concentration in FD rats
Compared with the control group, the model group had a significantly lower CCK mRNA concentration in the duodenum and antrum, and a significantly higher CCK mRNA concentration in the hypothalamus (P < 0.05; Figure 5). Compared with the model group, the PWC Ⅱ group had a significantly increased concentration of CCK in the duodenum (P < 0.05), while
In longitudinal column, A1, A7, A13, and C1, C7, C13: control group (n = 8); received the same volume of 0.9% sodium daily for 21 d; A2, A8, A14, and C2, C8, C14: model group (n = 8); received the same volume of 0.9% sodium daily for 21 d; A3, A9, A15 and C3, C9, C15: domperidone group (n = 5); received 1.6 g · kg⁻¹ · d⁻¹ PWC for 21 d; A4, A10, A16 and C4, C10, C16: PWC group (n = 5); received 3.2 g · kg⁻¹ · d⁻¹ PWC for 21 d; A6, A12, A18 and C6, C12, C18: PWC group (n = 5); received 4.8 g · kg⁻¹ · d⁻¹ PWC for 21 d. b: proportion of leptin positive cells; D: proportion of CCK positive cells. PWC: Pingwei capsules; FD: functional dyspepsia; CCK: cholecystokinin. The differences in the proportion of positive cells at each area of interest were tested by the χ² test. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).
the PWC I and PWC II groups had a significantly increased concentration of CCK in the antrum (P < 0.05). However, CCK concentration was not changed in the hypothalamic group of the Domp-treated and PWC-treated groups compared with the control group (Figure 5).

DISCUSSION

The dysmotility-like FD rat model established in the present study by stimulating semi-starvation rats via tail damping was more suitable for our purpose than a 0.1% iodoacetamide-induced model. As iodoacetamide-treated rats have edema and erythema in the mucosa and submucosal neutrophilic infiltrates. After tail damping for 10 d, the model group appeared irritable and aggressive, had lost weight, and their food intake had decreased. Furthermore, measurements of the gastrointestinal electrical activity, intestinal propulsion, and gastric emptying revealed hypomotility in the model group. However, HE staining revealed no organic lesions in the duodenum or stomach of any of the rats, which indicates that the experimental model was successfully established. In our study, PWC increased bodyweight, food intake, gastrointestinal electrical activity, phase III of the MMC, intestinal propulsion, and gastric emptying, which may be the mechanisms underlying the effect of PWC in the treatment of FD. Interactions between the brain and the gut in the brain-gut axis occur via four major pathways. These pathways involve immune, neural, and endocrine messages released by hormones and the nervous system. Brain-gut peptides and related signalling molecules may be involved in interactions between the ENS and CNS. Previous studies have shown that bidirectional regulation of the brain-gut axis plays an important role in patients with FD, and brain-gut peptides such as leptin and CCK are produced in the gastrointestinal tract and then released into the circulation. The hypothalamus receives hormonal signals from the gastrointestinal tract and peripheral neural messages from the ENS, and then relays this information to regulate physical functions.

Leptin in serum may play a crucial role in the pathogenesis of FD; the serum concentration of leptin is increased in dysmotility-like dyspepsia, which is characterized by symptoms such as early satiety, nausea, and a feeling of fullness. CCK may contribute to the relaxation of the fundus, and the inhibition of antral motility. In addition, intravenous infusions of CCK octapeptide and CCK-33 prevent gastric emptying. A positive feedback loop between leptin and CCK promotes their effects in the rat model. In this positive feedback loop, leptin in the gastrointestinal tract stimulates the production of CCK, and the increased concentration of CCK further stimulates leptin concentrations in the gastrointestinal tract and in the circulation.

In the present study, the leptin concentrations in the serum, duodenum, antrum, and hypothalamus in the model group were higher than those in the control group, which is consistent with previous reports. Furthermore, the leptin concentration in the PWC-treated group was lower than that in the model group. This indicates that the improvements in early satiety, fullness, and gastrointestinal motility seen after PWC administration may be because of the decrease in serum leptin.

![Figure 5 Effect of PWC on CCK mRNA in FD rats](image)

Expression of CCK mRNA in homogenates of the duodenum (A and B), antrum (C and D), and hypothalamus (E and F). 1: control group (n = 8): received the same volume of 0.9% sodium daily for 21 d; 2: model group (n = 8): received the same volume of 0.9% sodium daily for 21 d; 3: Domp group (n = 5): received 10 mg · kg⁻¹ · d⁻¹ domperidone for 21 d; 4: PWC I group (n = 5): received 1.6 g · kg⁻¹ · d⁻¹ PWC for 21 d; 5: PWC II group (n = 5): received 3.2 g · kg⁻¹ · d⁻¹ PWC for 21 d; 6: PWC III group (n = 5): received 4.8 g · kg⁻¹ · d⁻¹ PWC for 21 d; 7: PWC IV group: Pingwei capsules; FD: functional dyspepsia; CCK: cholecystokinin. Statistical significance was determined in comparison with the control group (P < 0.05) and the model group (P < 0.05).
concentration caused by PWC. Compared with the control group, the CCK concentrations in the model group were lower in the duodenum and antrum, and higher in the hypothalamus. Although this observation conflicts with studies that have shown that CCK inhibits gastric motility, it is similar to a study that showed that FD patients exhibited a lower CCK concentration in response to duodenal lipids. The lower gastrointestinal CCK concentration in the model group may be due to a higher release of CCK or a lower basal CCK content compared with the control group. Additionally, the satiety action of CCK appears to be dependent on leptin signaling. In addition, two CCK receptor subtypes (CCKAR and CCKBR) have been identified within the CNS and ENS. CCKAR is widely distributed in the gastrointestinal tract and regulates gastrointestinal motility, while CCKBR is more widely distributed in the CNS and regulates stress and emotion. Our detection of a higher CCK concentration in the hypothalamus of the model group indicates that the FD rats were anxious and nervous, and that regulation of CCK in the hypothalamus may involve multiple pathways in the CNS and ENS. Furthermore, PWC increased the CCK mRNA and protein concentrations in gastrointestinal tissues, and reduced the CCK protein concentration in the hypothalamus. This indicates that PWC can increase CCK concentrations in the ENS, and can improve the symptoms of anxiety and stress by decreasing the CCK protein concentration in the CNS. In conclusion, our results demonstrate that PWC improve gastrointestinal motility in FD rats. This effect is probably due to changes in leptin and CCK concentrations, which may be used as effective criteria and indicators of FD diagnosis and treatment.

REFERENCES

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