Jianqu, a traditional Chinese medicine, alleviates functional dyspepsia in high-calorie and high-protein diet mice

Jing-Yan Yang1,2, Xiao-Xing Li3, Juan Chen1, Yan-Jun Liu1, Yue-Hong Wu1, Ning-Yu Luo1, Cai-Xia Yang3, Yang Li3, Si-Jing Liu1,2,∗, Jin-Lin Guo1,3,∗

1College of Medical Technology, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China. 2Chongqing Key Laboratory of Sichuan-Chongqing Co-construction for Diagnosis and Treatment of Infectious Diseases Integrated Traditional Chinese and Western Medicine, Chengdu 611137, China. 3Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China.

∗Correspondence to: Si-Jing Liu, Jin-Lin Guo. Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, No.1166, East Liutai Avenue, Chengdu 611137, China. E-mail: liusijing@cdutcm.edu.cn; guo596@cdutcm.edu.cn.

Author contributions
Guo Ji designed the study. Guo Ji, Liu Si, and Yang JY participated in the study design. Li XX, and Chen J participated in the experiments. Yang JY, and Li XX performed the statistical analysis. Yang JY, Liu Yi, Wu YH, Luo NY, and Yang CX conducts research. Yang JY helped draft the manuscript and helped coordinate funding. Liu Si, and Guo Ji revised the paper.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
FD, functional dyspepsia; HCHP, high-calorie and high-protein diet; HE, hematoxylin-eosin; SCFAs, short chain fatty acids; TCM, traditional Chinese medicine; GAS, gastrin; MTL, motilin; VIP, vasoactive intestinal peptide; OTU, operational taxonomic units.

Citation

Abstract
Background: Jianqu has been used to alleviate symptoms in patients with functional dyspepsia, but its specific anti-functional dyspepsia effect is still unclear. Therefore, our study aimed to investigate the impact of Jianqu on functional dyspepsia in mice.

Methods: The phytochemical profile of Jianqu was analyzed by UPLC-Q-TOF-MS. Subsequently, Kunming mice were fed a high-calorie or high-protein diet (HCHP) for 7 days, and then orally treated with vehicle or Jianqu (1.62 g/kg body weight (b. w.) and 3.25 g/kg b. w.) for 10 days. A carbon powder solution was used to detect the gastric emptying and intestinal transit rate. The pathological changes in stomach and duodenum were evaluated by hematoxylin-eosin staining. Importantly, the serum gastrointestinal hormones were detected by ELISA. In addition, the gut microbiota composition was determined using 16S rRNA gene sequencing. The cecal short chain fatty acids were assessed by gas chromatography.

Results: In general, 17 phytochemical compounds were identified from Jianqu, which significantly improved the gastric emptying rate and intestinal transit rate (p < 0.01), increased the body weight and food intake (p < 0.0001) in HCHP mice as well. Though HCHP did not cause significant pathological lesions in the gastrointestinal tract, increased the expression of CD45 in the duodenum (p < 0.05) was observed. Notably, Jianqu attenuated this abnormal expression of CD45 (p < 0.05). The levels of serum gastrointestinal hormones were significantly normalized by Jianqu intervention (p < 0.05). Moreover, Jianqu increased the relative abundance of Roseburia as well as short chain fatty acids levels in cecum (p < 0.05).

Conclusion: The present results showed that Jianqu alleviated dyspeptic symptoms in HCHP mice possibly through reducing the duodenal leukocyte infiltration, and regulating the expression of gastrointestinal hormones. These effects may be partly related to the increasing cecal short chain fatty acids levels probably via gut microbial modulation.

Keywords: herbal formula; dyspepsia; gastrointestinal hormones; gastric emptying; intestinal transit
Medicine, prokinetic acid, can be obtained from high-calorie varieties. The fruit is rich in enzymes and probiotics, which can help reduce the risk of infection and promote digestion. Moreover, the fruit is rich in digestive enzymes which promote digestive function, and there are several reports on its mechanism of promoting digestion. In this paper, the mechanism of Jianyu invigorating spleen to promote digestive function was confirmed by experiments.

Functional dyspepsia (FD) is a prevalent functional gastrointestinal disease that presents symptoms such as bloating, anorexia, early satiety, and epigastric discomfort. While FD is not fatal, it significantly impacts the quality of life for patients and imposes substantial costs on both individuals and society. The prevalence of FD varies between countries, with reported rates ranging from 7.2% to 40% [2, 3]. In the USA alone, the estimated annual costs of FD are conservatively estimated to be 18.4 billion dollars [4]. The causes of FD can include abnormal gastrointestinal motility, oversecretion of gastric acid, increased immune cell activation, increased duodenal permeability, increased duodenal eosinophilia, gastrointestinal hormones disorder, and infection of Helicobacter pylori [5]. While organic disease is not considered a criterion for FD, recent studies have demonstrated a strong association between low-grade duodenal inflammation, specifically microinflammation characterized by the infiltration of local immune cells (eosinophils), and the development of FD [6–8]. Additionally, the gut microbiota and its metabolites, such as short-chain fatty acids (SCFAs), lipopolysaccharide, peptidoglycan, and sphingolipids, have been implicated in FD [9]. Dysbiosis of gut microbiota may contribute to the development of FD by disrupting the intestinal biological barrier, interfering with the immune function of the intestine, or causing dysregulation of the brain-gut-microbiota axis [9, 10].

Based on these etiological factors, Helicobacter pylori eradication therapy, probiotics, and probiotic drugs, such as acamitamide, itopride, and proton pump inhibitors, are used to treat FD [5, 11]. However, these current treatments have limited efficacy or present safety issues [5, 11]. For example, the eradication therapy of Helicobacter pylori in patients who have FD is modest, dyspepsia symptoms recur about one year after cessation of acamitamide therapy and the recurrence rate was high [5, 12]. In addition, cisapride was the most used prokinetics drug, but it increases the risk of adverse cardiac events [5]. Therefore, there is a need for alternative strategies to alleviate FD with lesser side effects. The promising potential candidate includes traditional Chinese medicine (TCM), which can exert a wider range of pharmacological effects [13]. Many studies have shown the significant therapeutic effects of TCM on FD in clinical trials and animal studies [13–18].

Jianyu is a typical fermented product of Chinese medicine, which was first published in the *A Supplement to Compendium of Materia Medica* [19], According to National Standard for Chinese Patent Drugs, Jianyu is composed of wheat bran, flour and 21 Chinese herbal medicines, including *Polygoni Herba*, *Xanthii Fruits*, *Artemisiae Annuae Herba*, *Armeniacae Semen Amaran*, among others. According to the theory of Chinese medicine, the main etiology of FD is a deficiency of Qi in the spleen and stomach (including decreased function of digestion, absorption, and energy conversion) [20]. In the theory of TCM, the spleen is a comprehensive conception, including not only he spleen in modern anatomy, but also the pancreas and lymphatic systems. The deficiency of Qi in the spleen and stomach is a combination of decreased function of these systems, such as digestion, absorption, and energy conversion [21]. Coincidentally, Jianyu is a traditional remedy utilized to address dyspepsia and enhance gastrointestinal functionality. Moreover, the Jianyu prescription is produced from another famous fermented product from Chinese medicine, Liu Shenqu, with the addition of some herbs that promote digestion, such as hawthorn, wheat bran, and satsuma orange (Citrus Reticulatae Pericarpium) [17, 22, 23]. Notably, many studies have demonstrated that Liu Shenqu is capable of alleviating FD [24–26]. Moreover, Jianyu is the main ingredient of many drugs, such as Jianzhixiangen granules, Guttans capsules, and Xiaoshijiapan tablets, which are widely used in the treatment of FD symptoms, including dyspepsia, abdominal distension, diarrhea, and abdominal accumulation clinically. However, the anti-FD efficacy of Jianyu is still unclear. Therefore, in this study, we set to determine the anti-FD effects of Jianyu and preliminarily explore its potential mechanisms using a high-heat and high-protein diet induced FD mouse model.
number: 2022DL-020). All experiments were conducted in accordance with the guidelines of the Animal Research Committee of Chengdu University of Traditional Chinese Medicine. A total of 40 four-week-old SPF healthy Kunming male mice (18–22 g) (SCXK (Chuan) 2020-030) were purchased from Dashuo Laboratory Animal Co., Ltd. (Sichuan, China). Five animals were housed in each cage and have free access to food and sterile drinking water in a temperature-controlled environment (22 °C ± 2 °C) and humidity-controlled room (50–60%) under 12 h dark-light cycle. After seven days of adaptation, the mice were randomly divided into four groups (n = 10). The normal control mice were fed a standard chow diet (Supplementary Table S1), and other mice were provided with a high-calorie and high-protein diet (HCHP) (Supplementary Table S1), consisting of HCHP pellet and concomitant oral administration of 52% milk solution (0.2 mL/10 g b. w.). The HCHP pellet was made of fish pine, soybean flour, Bour, and milk powder in the ratio of 1:2:1:1, which was mixed with water and dried in a 50 °C oven. The HCHP solution was made from milk powder and HCHP pellet (25:75), and then the mixture was sterilized and stored at −20 °C before administration. The HCHP was used to induce FD in mouse model [27–30]. Seven days later, mice were supplemented daily with 20 µL/g b. w. 0.5% sodium carboxymethylcellulose (vehicle), low dose of Jianqu (1.62 g/kg b. w.) or high dose of Jianqu (3.25 g/kg b. w.) by intragastric gavage. The dose was selected based on “Sichuan Province Standard for Processing Traditional Chinese Medicine Decoction Pieces (2015)” and our previous studies [31]. The dose of 1.62 g/kg b. w. for a mouse would be translated to the dose of 12.5 g Jianqu per day for a 60 kg human adult after adjustment for a body surface area. The interventions were conducted for 10 days. The general state of mice was also observed daily, including fur, fecal state, and activities. At the time indicated, mice were sacrificed. Serum, stomach, small intestine, and cecal contents were collected.

Detection of gastric emptying rate and intestinal transit rate
The detection of gastric emptying rate and intestinal transit rate were performed as previously described with modification [32]. One hour after the last treatment, the mice were fasted for 18 h and given free access to tap water. Then, these fasting mice were gavaged with 0.5 mL carbon powder solution. After 30 min, mice were sacrificed. The stomach samples were immediately removed and weighed. Then the contents in the stomach was removed and the net weight of stomach was weighed. The gastric emptying rate was calculated as follows Equation (1):

\[
\text{The gastric emptying rate} = \frac{\text{The gross weight (mg)} - \text{The net weight (mg) of stomach}}{\text{The weight of carbon powder solution (mg)}} \times 100\% 
\]

After scarification, the intestinal canal samples were gently removed. The total length of small intestinal and the length of powder carbon movement were measured. The intestinal transit rate was calculated as follows Equation (2):

\[
\text{The intestinal transit rate} = \frac{\text{The length of powered carbon movement (cm)}}{\text{Total length of small intestine (cm)}} \times 100\% 
\]

Histology and immunohistochemistry
Tissues of stomach and duodenum were fixed in 4% paraformaldehyde and stained with hematoxylin-eosin (HE). The specimens were evaluated by two pathologists who were blinded to the treatment groups. For immunohistochemical staining, sections of small intestine were incubated with anti-CD45 overnight at 4 °C. And signals were detected with Cy3 conjugated Goat Anti-Rabbit IgG (1:300). Quantification analysis was assessed with Image J software.

Serum biochemical analysis
Serum gastrin (GAS), motilin (MTL), and vasoactive intestinal peptide (VIP) were detected by ELISA (04/2021, Milbio Co., Ltd. Shanghai, China).

Cecal microbiota analysis

### Table 1 Components of Jianqu

<table>
<thead>
<tr>
<th>Common name</th>
<th>Herb name</th>
<th>Family name</th>
<th>Part used</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-pepper</td>
<td>Polygoni Herba</td>
<td>Polygonaceae</td>
<td>Dry stems and leaves</td>
<td>6.6</td>
</tr>
<tr>
<td>Cocklebur</td>
<td>Xanthii Fructus</td>
<td>Asteraeaceae</td>
<td>Dry stems and leaves</td>
<td>6.6</td>
</tr>
<tr>
<td>Celery sagebrush</td>
<td>Artemisiae Annuae Herba</td>
<td>Asteraeaceae</td>
<td>Dry stems and leaves</td>
<td>6.6</td>
</tr>
<tr>
<td>Armenelace semen amaran</td>
<td>Armeniaceae Semen Amaran</td>
<td>Rosaceae</td>
<td>Dry ripe seed</td>
<td>4.0</td>
</tr>
<tr>
<td>Vignae semen</td>
<td>Vignae Senem</td>
<td>Vigna savi</td>
<td>Dry ripe seed</td>
<td>4.0</td>
</tr>
<tr>
<td>Barley</td>
<td>Hordei Fructus Germinatas</td>
<td>Poaceae</td>
<td>Dry sprouting caryopsis</td>
<td>9.0</td>
</tr>
<tr>
<td>Hawthorn</td>
<td>Crataei Fructus</td>
<td>Rosaceae</td>
<td>Dry ripe fruit</td>
<td>9.0</td>
</tr>
<tr>
<td>Satsuma orange</td>
<td>Citri Reticulatae Pericarpium</td>
<td>Rutaceae</td>
<td>Dry pericarp from ripe fruit</td>
<td>6.0</td>
</tr>
<tr>
<td>Wrinkled gianthysop</td>
<td>Pogostemonis Herba</td>
<td>Lamiaceae</td>
<td>Dry stems and leaves</td>
<td>6.0</td>
</tr>
<tr>
<td>Atractylodis rhizoma</td>
<td>Atractylodis Macrocephalae Rhizoma</td>
<td>Asteraceae</td>
<td>Dry rhizomes</td>
<td>6.0</td>
</tr>
<tr>
<td>Officinal magna</td>
<td>Magnoliae Officina Cortex</td>
<td>Magnoliaceae</td>
<td>Dried bark, velamen and branch coat</td>
<td>3.0</td>
</tr>
<tr>
<td>Dolomiae</td>
<td>Vladimiae Radix</td>
<td>Asteraeaceae</td>
<td>Dry root</td>
<td>3.0</td>
</tr>
<tr>
<td>Baizhi angelica</td>
<td>Angelicea Dahuariae Radix</td>
<td>Apiaceae</td>
<td>Dry root</td>
<td>3.0</td>
</tr>
<tr>
<td>Betelnut palm</td>
<td>Arcaeae Semen</td>
<td>Arceraeaceae</td>
<td>Dry ripe seed</td>
<td>3.0</td>
</tr>
<tr>
<td>Seville orange</td>
<td>Aurantii Fructus</td>
<td>Aurantii fructus</td>
<td>Dry immature fruit</td>
<td>3.0</td>
</tr>
<tr>
<td>Common perilla</td>
<td>Perillae Folium/Perillae Caulis</td>
<td>Lamiaceae</td>
<td>Dry stems and leaves</td>
<td>6.0</td>
</tr>
<tr>
<td>Wind mint</td>
<td>Menhiae Haplocalyce Herba</td>
<td>Lamiaceae</td>
<td>Dry stems and leaves</td>
<td>3.0</td>
</tr>
<tr>
<td>Setariae fructus germinatus</td>
<td>Setariae Fructus Germinatas</td>
<td>Poaceae</td>
<td>Dry sprouting caryopsis</td>
<td>9.0</td>
</tr>
<tr>
<td>Cassia bark tree</td>
<td>Cinnamomi Cortex</td>
<td>Lauraceae</td>
<td>Dry bark</td>
<td>1.5</td>
</tr>
<tr>
<td>Nutgrass galingale</td>
<td>Cyperi Rhizoma</td>
<td>Cyperaceae</td>
<td>Dry rhizomes</td>
<td>6.0</td>
</tr>
<tr>
<td>Ural licorice</td>
<td>Glycyrrhae Radix et Rhizoma</td>
<td>Apiaceae</td>
<td>Dry roots and rhizome</td>
<td>1.5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Tritici Aestivi Testa</td>
<td>–</td>
<td>Dry skin</td>
<td>21.2</td>
</tr>
<tr>
<td>Flour</td>
<td>Tritici Aestivi Pulveratum Semen</td>
<td>–</td>
<td>Dry seeds</td>
<td>10.6</td>
</tr>
</tbody>
</table>

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Total bacterial genomic DNA of cecal contents was extracted using a QIAamp PowerFecal Pro DNA kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer’s instructions. PCR amplification of the bacteria full-length 16S rRNA gene was performed using the forward primer (5′-AGGTTGGATATGNTGCGTAC-3′) and the reverse primer (5′-TASGGHTACCTGGGACGTG-3′). The cecal microbiota composition was analyzed using PacBio platform with SMRTbell Template Prep kit (Pacific Biosciences, Menlo Park, CA, USA). The raw reads were deposited into the NCBI Sequence Read Archive database. Raw fastq files were demultiplexed, quality-filtered using CULTADAFT (version 1.9.1). OTU (operational taxonomic units)-picking from post-filtering reads was performed using USEARCH, and chimeric sequences were identified and removed using UCHIME (version 4.2).

Cecal SCFAs assessment
A total of 50 mg of cecal contents were added into 50 µL 15% phosphoric acid, 100 µL acetonitrile containing 125 µg/mL internal target (2-ethylbutyric acid). An additional 1.0 mL acetonitrile was added to the mixture, which was then homogenized and centrifuged at 4 °C at 12,000 g for 15 min. The supernatants were collected and then filtered with a 0.22 µm filter membrane and analyzed by GC-FID (Agilent Technologies, Palo Alto, CA, USA). SCFAs were separated using a nitrotetrosphalic acid modified polyethylene glycol column (DB-FFAP, 30 m × 0.53 mm × 1.0 µm; Agilent Technologies, Palo Alto, CA, USA). The GC injector was maintained at 240 °C. The heating procedure was as follow: the initial temperature was maintained at 50 °C for 1 min, the temperature was heated to 180 °C at 20 °C/min and held for 1 min, and then increased to 200 °C at a rate of 20 °C/min and held for 2 min. The carrier gas was nitrogen, and the flow rate of carrier gas was 64.4 mL/min.

Statistical analysis
The statistical analysis was carried out using SPSS 22. The analysis of sequencing data for the 16S rRNA gene sequencing was performed using QIIME and R packages. For parametric analysis, the data was presented as mean ± SD, while for non-parametric analysis, medians ± IQR were utilized. To compare multiple groups, the analysis of variance with Tukey’s multiple-comparison test or Kruskal-Wallis test was employed. The significance level was set at p < 0.05 for all the obtained results.

Results
Identification of compounds in Jianqu
The UPLC-Q-TOF-MS technique was utilized to analyze the primary constituents of Jianqu. This involved a comparison of retention time and fragment ions between the reference compounds and relevant databases and literature. As shown in Figure 1, Table 2, 17 components of Jianqu were identified, including 5 kinds of fatty acid (9-Oct-10(E),12(E)-octadecadienoic acid, (+/-)12(13)-DIHOME, 16-hydroxyhexadecanoic acid, stearic acid, and erucamide), 2 kinds of terpenes (isoalantolactone and dihydroartemisinic acid) and others (octadec-9-ynoic acid, nobiletin, magnolol, phellopterin, tangeritin, acetogenin, α-cyperone, α-eleostearic acid, 2,2′-Methylenebis(4-methyl-6-tert-butylphenol) and Dibutyl phthalate). The chromatograms and MS/MS pictures of these components are presented in Supplementary Figure S1, S2.

Jianqu alleviated dyspeptic symptoms in HCHP mice
To test the effect of Jianqu on FD, we determined the weight, food intake, gastric emptying rate, and intestinal transit rate in the HCHP mice (Figure 2A–2G). HCHP treatment led to the reduction of body weight and food intake in mice (p < 0.0001), while these symptoms were significantly relieved by low and high dose of Jianqu administration (Figure 2B–2E, p < 0.0001). More importantly, compared to the model group, both the Jianqu-L and Jianqu-H intervention significantly increased the gastric emptying rate and intestinal transit rate (Figure 2F, 2G, p < 0.01), suggesting that the gastrointestinal motility of HCHP mice were promoted by Jianqu supplementation. The results of cluster analysis also showed that Jianqu could improve the gastric emptying rate and intestinal transport rate induced by HCHP diet, and could also improve the weight gain and food intake changes induced by HCHP diet to a certain extent, but the changes were not statistically significant (Figure 2H). Taken together, these results demonstrated that Jianqu could alleviate dyspeptic symptoms in HCHP mice. It seemed that the

Figure 1 Chemical components of Jianqu analyzed by UPLC-Q-TOF-MS. (A) Chromatograms and molecular structures of Jianqu in positive ion mode. (B) Chromatograms and molecular structures of Jianqu in negative ion mode.
Figure 2 Jianqu alleviated dyspeptic symptoms in HCHP mice. (A) Schematic for the animal experiment. Kunming mice (n = 10 per group) were fed with chow diet or HCHP diet for 7 days to establish dyspepsia model and then supplemented with vehicle or L/M dose of Jianqu for 10 days by oral gavage. (B) Body weight. (C) Body weight gain. (D) Food intake curves. (E) Food intake during the period of modeling and Jianqu treatment. (F) Gastric emptying rate. (G) Intestinal transit rate. (H) The heatmap of body weight, food intake, gastric emptying rate and intestinal transit rate. Purple indicated high levels and green indicated low levels. All experiments were replicated three times. Data were presented as mean ± SD (n = 10). **p < 0.01, ***p < 0.001 (versus NC group); p < 0.01, ****p < 0.0001 (versus Model group).

Table 2 Compounds of Jianqu identified by UPLC-Q-TOF-MS

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>RT (min)</th>
<th>Area (max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Cyperone</td>
<td>C_{15}H_{22}O</td>
<td>218.17</td>
<td>27.49</td>
<td>4.76 × 10^{10}</td>
</tr>
<tr>
<td>2</td>
<td>Isolantolactone</td>
<td>C_{15}H_{20}O_2</td>
<td>232.15</td>
<td>26.04</td>
<td>4.08 × 10^{10}</td>
</tr>
<tr>
<td>3</td>
<td>Magnolol</td>
<td>C_{16}H_{30}O</td>
<td>266.13</td>
<td>27.85</td>
<td>2.28 × 10^{10}</td>
</tr>
<tr>
<td>4</td>
<td>9-Oxo-10(E),12(E)-octadecadienoic acid</td>
<td>C_{17}H_{30}O_2</td>
<td>294.22</td>
<td>30.76</td>
<td>1.34 × 10^{10}</td>
</tr>
<tr>
<td>5</td>
<td>Tangeretin</td>
<td>C_{18}H_{32}O</td>
<td>372.12</td>
<td>22.15</td>
<td>7.96 × 10^{10}</td>
</tr>
<tr>
<td>6</td>
<td>Nobiletin</td>
<td>C_{19}H_{30}O</td>
<td>402.13</td>
<td>20.08</td>
<td>7.85 × 10^{10}</td>
</tr>
<tr>
<td>7</td>
<td>(+/−)12(13)-DIHOME</td>
<td>C_{19}H_{30}O</td>
<td>296.24</td>
<td>29.07</td>
<td>6.96 × 10^{9}</td>
</tr>
<tr>
<td>8</td>
<td>2′,2′-Methylethyl(bis-4-methyl-6-tert-butylphenol)</td>
<td>C_{20}H_{34}O</td>
<td>340.24</td>
<td>38.98</td>
<td>6.24 × 10^{9}</td>
</tr>
<tr>
<td>9</td>
<td>Phellopterin</td>
<td>C_{20}H_{32}O</td>
<td>300.10</td>
<td>25.36</td>
<td>6.15 × 10^{9}</td>
</tr>
<tr>
<td>10</td>
<td>Erucomide</td>
<td>C_{20}H_{36}O</td>
<td>337.33</td>
<td>48.64</td>
<td>5.71 × 10^{9}</td>
</tr>
<tr>
<td>11</td>
<td>Dibutyl phthalate</td>
<td>C_{20}H_{30}O</td>
<td>278.15</td>
<td>32.11</td>
<td>3.19 × 10^{9}</td>
</tr>
<tr>
<td>12</td>
<td>Acetophenone</td>
<td>C_{12}H_{10}O</td>
<td>120.06</td>
<td>28.02</td>
<td>3.95 × 10^{9}</td>
</tr>
<tr>
<td>13</td>
<td>α-Eleostearic acid</td>
<td>C_{18}H_{32}O</td>
<td>278.22</td>
<td>50.06</td>
<td>3.89 × 10^{9}</td>
</tr>
<tr>
<td>14</td>
<td>octadec-9-ynoic acid</td>
<td>C_{19}H_{34}O</td>
<td>262.23</td>
<td>43.27</td>
<td>2.65 × 10^{9}</td>
</tr>
<tr>
<td>15</td>
<td>16-Hydroxyhexadecanoic acid</td>
<td>C_{18}H_{32}O</td>
<td>272.23</td>
<td>39.89</td>
<td>5.32 × 10^{9}</td>
</tr>
<tr>
<td>16</td>
<td>Dihydroartemisinic acid</td>
<td>C_{18}H_{32}O</td>
<td>236.18</td>
<td>30.19</td>
<td>5.31 × 10^{9}</td>
</tr>
<tr>
<td>17</td>
<td>Stearic acid</td>
<td>C_{18}H_{36}O</td>
<td>284.27</td>
<td>47.46</td>
<td>4.40 × 10^{7}</td>
</tr>
</tbody>
</table>
high dose had a better effect on gastric emptying rate and intestinal transit rate than the low dose group, but there was no significant difference between the two groups. So, we chose the low dose group (equal to the recommended human dose) for use in the subsequent experiments.

**Jianqu decreased the levels of CD45 in duodenum**

FD is closely related to the increased duodenal permeability and mucosa inflammation [6–8]. To investigate whether the protective effect of Jianqu was associated with the duodenal permeability or the anti-inflammatory effect in HCHP mice, we analyzed the alterations of several histological parameters of stomach and duodenum in the Jianqu-L group by HE staining and measured the expression of duodenal permeability markers, occludin, claudin-1 and ZO-1 in the duodenum by immunofluorescence. We also detected the levels of CD45, which is a surface marker of leukocytes, in the duodenum by immunofluorescence. As shown in Figure 3, the mucosal layer, submucosal layer, muscle layer, and the intestinal villi of gastric and intestinal tissues in mice of different groups were intact. No significant pathological changes in the stomach and duodenum of HCHP mice were observed. Similar results were also observed in the expression of occludin, claudin-1 and ZO-1 in the duodenum (Figure 4). These results indicated that the HCHP dietary intervention did not alter the structure and permeability of the duodenum. However, the increased expression of CD45 in the duodenum of HCHP mice suggested that a HCHP diet might lead to mild small intestinal inflammation (Figure 5A, 5B, p < 0.05). Importantly, treatment of Jianqu attenuated the abnormal productions of intestinal CD45 (Figure 5A, 5B, p < 0.01). This implied that the function of Jianqu to improve FD may be partly related to the inhibition of the inflammatory response in the small intestine.

**Jianqu regulated the expression of gastrointestinal hormones**

Gastrointestinal hormones play an important role in the regulating gastric motility. To decipher the effects of Jianqu on the gastrointestinal hormones, we utilized ELISA assays to evaluate the serum GAS, MTL, and VIP concentrations. It was indicated that HCHP treatment decreased the serum GAS and MTL concentrations (Figure 6A, 6B, p < 0.05). However, these upregulations were markedly suppressed by Jianqu (p < 0.01). On the contrary, an increment in the level of serum VIP was induced by HCHP but suppressed with Jianqu intervention (Figure 6C, p < 0.0001). Thus, these results suggested that Jianqu could regulate the expression of serum gastrointestinal hormones, which may be the main mechanism for its treatment of FD.

**Jianqu increased the relative abundance of Roseburia**

To determine if the effectiveness of Jianqu in relieving FD is linked to alterations in gut microbiota, we conducted a 16S rRNA sequencing analysis. As shown in Figure 7A, the number of OTUs in the gut microbiota of the Jianqu group was higher than that of the Model group (p < 0.01). Compared to the model group, the Chao1 and Shannon indexes were increased in Jianqu group (Figure 7B, 7C, p < 0.05), indicating that the Jianqu treatment improved the diversity and abundance of microbial community. In addition, principal coordinates analysis of microbial taxa revealed distinct clusters, suggesting that the Jianqu treatment induced rearrangements in microbial composition (Figure 7D). Moreover, analysis of the cecal microbiota at the phylum, family, and genus levels demonstrated that Jianqu slightly altered the bacterial composition in HCHP mice (Figure 7E–7G). As shown in Figure 7H–7J, Jianqu administration did not change relative abundance of the two main phylum, Firmicutes and Bacteroidetes, as well as the ratio of them. But we found that Jianqu treatment was able to increase the abundance of *Roseburia* significantly (Figure 7K, p < 0.05).

![Figure 3](https://www.tmrjournals.com/tnr)

**Figure 3** The histological morphology of the stomach and duodenum in mice. (A) Representative photographs of the stomach and duodenum tissue sections with HE staining using a 200× magnification objective lens. Scale bar, 100 μm. (B–D) The muscle thickness, submucosa thickness, and the mucosa thickness of the stomach sections. (E) The length of intestinal villi. Data were presented as mean ± SD (n = 5).
Figure 4 Jianqu did not affect the levels of occludin, claudin-1 and ZO-1 in duodenum. (A) Representative immunofluorescence staining of occludin, claudin-1 and ZO-1 of mouse duodenum. (B–D) The occludin (B), claudin-1(C) and ZO-1(D) positive area in the duodenum sections. Data were presented as mean ± SD (n = 5).

Figure 5 Jianqu suppressed the levels of CD45 in duodenum. (A) Representative immunofluorescence staining and the CD45 positive area of CD45 of mouse duodenum. (B) The CD45 positive area of CD45 of mouse duodenum. Data were presented as mean ± SD (n = 5). *p < 0.05 (versus NC group); **p < 0.01 (versus Model group).
Jianqu increased the relative abundance of *Roseburia* in HCHP mice. DNA from the genome was extracted and microbial profiling based on the 16S rRNA was subsequently conducted. (A) OTUs numbers. (B, C) α-Diversity represented by the Chao1 index (B) and Shannon index (C). (D) Principal component analysis score plots based on the relative abundance of OTUs. (E–G) Relative abundance of taxa at the phylum (E) and at the family (F) and at the genus (G). (H, I) Relative abundance of Firmicutes (H) and Bacteroidetes (I). (J) The ratio of Firmicutes and Bacteroidetes. (K) Relative abundance of *Roseburia.* Data were presented as mean ± SD (n = 5). *p* < 0.05 (versus Model group).

Jianqu increased the cecal SCFAs levels

Since *Roseburia* is a typical SCFA-producing bacterium and SCFAs can modulate intestinal neurons and affect gastrointestinal motility, the cecal SCFAs levels were determined. As shown in Figure 8, HCHP treatment exerted a decline trend in the fecal acetic acid, propanoic acid, butyric acid, and isobutyric acid, especially valeric acid and isovaleric acid levels (*p* < 0.05). Compared with the model group, the concentrations of utric acid, isobutyric acid, alicer acid, and isovaleric acid were significantly increased in the mice with the Jianqu intervention (*p* < 0.05). Furthermore, we found that some genera were significantly correlated with the SCFAs levels as well as the expression of gastrointestinal hormones and dyspeptic indicators by Spearman correlation analysis (Figure 9). These results indicated...
that Jianqu altered the abundance of specific microbial taxa and restored the level of SCFAs in the cecum contents of HCHP mice to that of normal mice, which might be another mechanism for Jianqu to relieve FD.

**Discussion**

Qu, such as Liu Shenqu, Hongqu, and Jianqu, is a kind of fermented traditional Chinese herbal formula, which has been widely used to treat indigestion, weakness of spleen and stomach in China for a long time [17, 25, 33]. While there have been many studies confirming the efficacy and mechanism of Liu Shenqu, research on Jianqu is still lacking [17, 25, 33]. As shown in Table 1, Jianqu is made from 23 kinds of substances fermented. Most of them were widely used to treat gastrointestinal diseases, such as indigestion, spleen-deficiency (nutrient absorption disorder), diarrhea, vomiting, abdominal pain, diarrhea, spleen and stomach qi stagnation (including abdominal bloating, belching and acid regurgitation, loss of appetite, etc.) for hundreds of years [23, 34–42]. These may be the material basis for FD treatment with Jianqu. However, TCM theory is concerned with multi-component and multi-target. Whether the effect of Jianqu for FD is better than that of a single herb or Liushen qu deserves further investigation.

To explore the components of Jianqu, we analyzed the chemical compounds of Jianqu using UPLC-Q-TOF-MS and 17 components were identified (Table 2, Figure 1), such as α-cyperone, magnolol, tangeretin, among others. α-cyperone and isoalantolactone are the main components of dolomiaea, which was reported to alleviate ethanol-induced acute gastric mucosa injury through anti-oxidation and anti-inflammation regulation [38]. Magnolol, the main component of Magnoliae Officinalis Cortex, is a commonly used TCM for treating dyspepsia [43]. Previous studies have shown that magnolol was able to improve the gastric emptying rate and intestinal propulsive activity in mice [43, 44]. Additionally, tangeretin and nobiletin are derived from Satsuma orange or Seville orange. A recent study has also shown that tangeretin was able to inhibit inflammatory response and restore intestinal barrier function [45]. However, we didn’t find out which herbs 9-Oxo-10(E), 12(E)-octadecadienoic acid, (+/−)12(13)-DiHOME, 2,2'-Methylenebis(4-methyl-6-tert-butylphenol), erucamide, α-oleostearic acid, octadec-9-ynoic acid, 16-hydroxyhexadecanoic acid and dihydroartemisinic acid came from. Considering that Jianqu is a fermentation product, it is speculated that these substances may be produced by the fermentation of herbs and microorganisms. Overall, these components supported the conjecture that Jianqu could alleviate FD.

![Figure 8](https://www.tmrjournals.com/tmr)  
**Figure 8** Jianqu increased fecal short chain fatty acid levels. (A–F) Fecal acetic acid (A), propanoic acid (B), butyric acid (C), isobutyric acid (D), valeric acid (E) and isovaleric acid (F) concentrations. Data were presented as mean ± SD (n = 10). *p < 0.05 (versus NC group); p < 0.05, ′p < 0.01 (versus Model group).

![Figure 9](https://www.tmrjournals.com/tmr)  
**Figure 9** The relationship between microbiota and gastrointestinal hormones, SCFAs and dyspeptic indicators. (A) The heatmap relationship between microbiota and other indicators. The top 20 genus were analyzed. Red indicated positive correlation and blue indicated negative correlation. (B) The key network of relationship between microbiota and other indicators. Green circles represented bacteria at the genus level, orange circles represented other indicator, green lines indicated negative correlation and orange lines indicated positive correlation. ′FDR-p < 0.05, ″FDR-p < 0.01. GAS, gastrin; MTL, motilin; VIP, vasoactive intestinal peptide.
To investigate the role and potential mechanisms of FD alleviation by Jianqu, we used a HCHP-induced FD mouse model. As expected, supplementation with Jianqu resulted in increased body weight and food intake in mice (Figure 28–2E), providing preliminary evidence of its ability to relieve FD symptoms. Importantly, Jianqu attenuated the delayed gastric emptying and intestinal transit (Figure 2F, 2G), which are important indicators to evaluate the digestive function. Furthermore, gastrointestinal inflammatory response is closely related to FD [46, 47]. Although the HCHP intervention did not cause pathological damage or intestinal barrier damage, we observed increased CD45 expression in the duodenum, suggesting mild mucosal inflammation characterized by leukocyte aggregation [46]. Encouragingly, our study revealed that the administration of Jianqu significantly attenuated this inflammatory response (Figure 5). Previous studies have shown that *Polygonal Herba, Artemisiae Annuae Herba, Glycyrrhizae Radix et Rhizoma*, and α-cyperone, exhibited anti-inflammatory activities through multiple pathways, like PI3K/Akt, NF-κB, and NLRP3 [42, 48–52]. These results indicated that Jianqu may also exert its anti-inflammatory effects through multiple pathways, but its specific mechanism needs to be further investigated.

Additionally, gastrointestinal hormones, such as GAS, MTL, and VIP, play a crucial role in regulating gastric motility [53, 54]. GAS, secreted by G cells in the gastric antrum and duodenum, is regulated by both central and peripheral nerves [53, 54]. It stimulates gastric acid secretion and may accelerate gastrointestinal motility [54]. Similarly, MTL is now indisputably implicated in the gastrointestinal motility by contracting the smooth muscle of the gastrointestinal tract [55]. On the contrary, VIP has the capability to impede the movement of the gastrointestinal tract. This effect is primarily attributed to its various gastrointestinal roles, which encompass the regulation of gastric acid secretion, secretion of anions in the intestines, release of pancreatic enzymes, cellular motility, vasodilatation, and augmentation of the force involved in intestinal contractions [56]. In this study, Jianqu treatment resulted in an increase in GAS and MTL, while induced a reduction in serum VIP levels (Figure 6). Therefore, the relief of FD symptoms after Jianqu supplementation may be explained by promotion of gastrointestinal motility and reduced intestinal inflammation likely via regulating gastrointestinal hormones.

On the other hand, a growing number of studies have demonstrated the significant role of gut microbiota in gastrointestinal function [9, 16, 47]. In this study, we observed that Jianqu interacted with gut microbiota, leading to improvements in α-diversity, β-diversity, and significant changes in bacteria at the phylum and genus levels (Figure 7A–7F). To the best of our knowledge, this is the first study on the alteration of gut microbiota by Jianqu. This effect may be attributed to its chemical components, such as magnolol, *Atractylidis Macrocephalae* *Rhizoma*, fermented barley, and *Glycyrrhizae Radix et Rhizoma*. have all been reported to regulate intestinal flora [36, 57–61]. Notably, we found that Jianqu increased the relative abundance of *Roseburia* at the genus levels (Figure 7J). It is a well-known SCFAs-producing strain which has been implicated in immune modulation and inflammatory regulation [62]. SCFAs are known to participate in mucus, water, and duodenal bicarbonate secretion, regulate intestinal pH value, and provide energy for intestinal epithelial cells [9]. Additionally, SCFAs have been reported to inhibit the growth of pathogenic bacteria and improve intestinal barrier function [9]. Our findings revealed upregulated levels of cecal SCFAs, specifically butyric acid, isobutyric acid, aleric acid, and isovaleric acid (Figure 8). These results suggest that the increased gastrointestinal motility observed in Jianqu-treated mice may be attributed to the regulation of gut microbiota and SCFAs production. Consistent with this hypothesis, we observed a positive correlation between SCFAs-producing bacteria (*Allobaculum*) or a certain probiotic (*Lactobacillus*) and SCFAs, gastrointestinal hormones, or gastrointestinal motility indices following Jianqu intervention (Figure 9). However, contrary to our expectations, we found a negative correlation between *Akkermansia* and these indicators in our study (Figure 9). Previous studies have shown that *Akkermansia* has potential as a probiotic for alleviating metabolic diseases [63, 64]. However, recent research has also indicated that an over-enrichment of *Akkermansia* in the specific intestinal environment of mice can exacerbate intestinal inflammation caused by epithelial barrier damage. For instance, both Song CH et al. and Lang M et al. observed a higher abundance of *Akkermansia* in the intestines of colorectal cancer patients or mice compared to healthy controls [65]. Furthermore, pathogenic infections have been found to increase the abundance of *Akkermansia* [66]. These findings suggest that the gut microbiota likely plays a significant role in the effect of Jianqu on FD. Nevertheless, it is important to note that whether the changes in the gut microbiota are a cause or a consequence requires further confirmation.

**Conclusions**

In conclusion, our results of the present study demonstrated that Jianqu could effectively improve the gastrointestinal motility of FD mice. The main mechanisms corresponding to these effects may involve the regulation of gastrointestinal hormones, modulation of gut microbiota, and increase of SCFAs (Figure 10). In addition, we preliminarily analyzed the chemical composition of Jianqu and its anti-FD effect may attribute to these components. We can, therefore, speculate that Jianqu might be a promising herbal medicine for the treatment of FD.

![Figure 10 A comprehensive strategy diagram for Jianqu in FD treatment.](https://www.tmrjournals.com/tnr)
References


29. Li ZN, Xu GS, Sun ZP. Experimental study of Jianpheiweiyin on animal model of dyspepsia. Shuaxi Tradit Chin Med. 1996;7(3):330–331. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=ZxhFRRmSliUWopKk1J2zHRfBfmZm2dRihgeoeejiH1Jy1gfY3PBza04ZGcnElWj8j9xNyxKpFlmpqld0pR0w5ksy0zEJvJVB1yb0hCk3Qbrq0OeavKAsAT4F7HeM8loACFviWi0q=&uniplatform=NZKPT&language=CHS

30. Peng SZ, Luo YJ, Huang XD, et al. Study on the regulation of immune function and its mechanism of Xiaoxia Oixing Tea on dyspepsia model mice. Chin Med Res. 2018;16(32):179–181. (Chinese) Available at: http://kns.cnki.net/kcms2/article/abstract?v=ZxhFRRmSliUWopKk1J2zHRfBfmZm2dRihgeoeejiH1Jy1gfY3PBza04ZGcnElWj8j9xNyxKpFlmpqld0pR0w5ksy0zEJvJVB1yb0hCk3Qbrq0OeavKAsAT4F7HeM8loACFviWi0q=&uniplatform=NZKPT&language=CHS


54. Camilleri M. Gastrointestinal hormones and regulation of...


56. Iwasaki M, Akiha Y, Kaunitz JD. Recent advances in vasoactive intestinal peptide physiology and pathophysiology: focus on the gastrointestinal system. *FI000Res.* 2019;8:1629. Available at: http://doi.org/10.12688/fi000research.18039.1


