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Proteomic Insights into the Effects of Jianweixiaoshi Tablets on Functional Dyspepsia with Spleen Deficiency in Rats

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Background: Jianweixiaoshi tablets (JWXS) is widely used in traditional Chinese medicine for treating functional dyspepsia with spleen deficiency (SD-FD) in China. However, the molecular mechanisms underlying the therapeutic effects of JWXS remain incompletely understood.

Methods: Functional dyspepsia was induced in rats with spleen deficiency by iodoacetamide in combination with the modified multiple platform method. The SD-FD rats were administered JWXS at both low and high doses, as well as domperidone. We conducted a comprehensive evaluation of the treatment effects of JWXS, including body weight, gastrointestinal motility, immune organ index, biochemical analysis, gastrointestinal hormones, and hematological studies. Quantitative proteomic analysis based on data-independent acquisition (DIA) was used to determine the changes in protein profiles of gastric and duodenal tissues in SD-FD rats and JWXS intervention rats.

Results: The results showed that JWXS effectively alleviated gastrointestinal motility disorders in SD-FD rats, as indicated by accelerated gastric emptying and intestinal propulsion, increased levels of gastrin, motilin, and ghrelin, and reduced levels of cholecystokinin-octapeptide, vasoactive intestinal peptide, and somatostatin. Additionally, JWXS increased the spleen and thymus index, increased %lymphocyte in blood, reduced white blood cell count and %neutrophil, and improved immune function. Through quantitative proteomic analysis of gastric tissues, we identified 333 differentially expressed proteins in the JWXS treatment group and the model group. Notably, the mechanism by which JWXS accelerated gastric emptying may be related to PLC-γ and SERCA2 in the calcium signaling pathway. Furthermore, JWXS treatment altered the expression of 732 proteins in rat duodenal samples. The differentially expressed proteins were enriched in immune-related functions and pathways, including antigen processing and presentation, as well as the intestinal immune network for IgA production.

Conclusion: In conclusion, JWXS exhibits a multi-faceted impact on various pathways, demonstrating its efficacy in treating SD-FD. These findings provide a foundation for the clinical application of JWXS in managing SD-FD.

Keywords: Jianweixiaoshi tablets, functional dyspepsia, spleen deficiency, traditional Chinese medicine, proteomic

Introduction

Functional dyspepsia (FD) is a common disorder of gut-brain interaction that originates in the gastroduodenal region, without a structural abnormality explaining the problems.[1] The prevalence of FD is about 16% worldwide.[2] According to the Rome IV consensus, FD can be categorized into postprandial distress syndrome with postprandial fullness or early satiation, and epigastric pain syndrome with epigastric pain and/or burning.[3] Currently, the pathogenesis of FD is complex and includes factors such as abnormal gastric emptying, increased visceral sensitivity, mild duodenal inflammation, and disturbances in the gut-brain axis.[4] Unfortunately, the symptomatic treatment of FD has limited success in

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fully improving patients' symptoms and quality of life, accompanied by adverse reactions.[5–7] Traditional Chinese medicine (TCM), known for its wide range of components and targets, high safety, and fewer toxic side effects, has shown promising results in treating FD.[8–11]

In TCM, FD is defined by the TCM terms "epigastric pain" and "distension and fullness" based on clinical symptoms and signs.[12] Based on the principle of syndrome differentiation in treatment, the Chinese consensus on the diagnosis and treatment of FD considers spleen deficiency as the primary syndrome type of FD. A clinical survey involving 565 patients with FD revealed that 63.6% of the patients were diagnosed with syndromes related to spleen deficiency.[13] Spleen deficiency is primarily characterized by weakened digestive function, abnormal secretion of gastrointestinal (GI) hormones, and decreased immune function, which overlap with the symptoms of FD.[14] Clarifying the symptoms of FD in Chinese medicine facilitates the selection of appropriate Chinese medicines.

Jianweixiaoshi tablets (JWXS), a traditional Chinese medicine used to treat dyspepsia caused by spleen and stomach weakness, has been used in the clinical treatment of FD in China.[15] It is a TCM modified from the classical prescription "Spleen-invigorating pill", which was recorded in the ancient Chinese medicine book "Zheng Zhi Zhun Sheng" by Wang Kentang of the Ming Dynasty in China.[16] JWXS contains five botanical drugs, including Taizishen (Radix Pseudostellariae), Shanyao (Dioscorea opposita Thunb.), Chenpi (Citri Reticulatae Pericarpium), Shanzha (Crataegus pinnatifida Bunge), and fired Maiya (Hordei Fructus Germinatus). Pharmacological research has demonstrated that polysaccharides extracted from the raw materials for JWXS can promote gastric emptying function in mice.[17] Additionally, previous reports have indicated that Chenpi, Shanzha, and fired Maiya extracts may have beneficial effects on the clinical symptoms of FD.[18–20] Extracts from Taizishen and Shanyao can regulate immunity and alleviate the syndromes of spleen deficiency.[14,21] Although JWXS is extensively utilized for FD treatment in China, there is insufficient information on the key targets and underlying mechanisms through which JWXS relieves FD.

Herbal medicines consist of a variety of components and usually have multiple pharmacological effects, exerting synergistic therapeutic effects. Proteomics plays a key role in investigating disease biomarkers and drug targets and has been extensively utilized in TCM studies to uncover the pharmacological mechanisms of traditional medicines.[22,23] In this study, we established a rat model of functional dyspepsia with spleen deficiency (SD-FD) by using intragastric iodoacetamide, combined with the modified multiple-platform method. To evaluate the pharmacological effects of JWXS in the treatment of SD-FD, this study examined the effects of JWXS on GI motility, hormones, and immune function in rats with SD-FD. Quantitative proteomic analysis was employed to examine alterations in the protein profiles of gastric and duodenal tissues in both SD-FD rats and those treated with JWXS. These findings provide valuable insights for the clinical application of JWXS in managing FD.

Materials and Methods

Drugs and Reagents

JWXS consists of five different herbs (Table 1). These ingredients are prepared by Jiangzhong Pharmaceutical Co., Ltd., China. UPLC-MS/MS was used to identify the major compounds of the JWXS extract (batch number: 22030005).

Domperidone (batch number: KDJ3YSP) and iodoacetamide were obtained from Xi'an Janssen Pharmaceutical Ltd. (Xi'an, China) and Sigma-Aldrich, respectively (St. Louis, Missouri, United States). Concentrations of the gastrin ELISA kit (CSB-E12743r), motilin ELISA kit (CSB-E08208r), vasoactive intestinal polypeptide (VIP) ELISA kit (CSB-

Table 1 Herbal Composition and Dosage of JWXS

Chinese Name	Latin Name	Medicinal Parts and Sources	Mass Percent (%)
Taizishen	Radix Pseudostellariae	Dried radix of *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm.	32.26
Chenpi	Citri Reticulatae Pericarpium	Dried mature pericarpium of *Citrus reticulata Blanco*	3.23
Shanyao	Dioscorea opposita Thunb.	Dried rhizoma of *Dioscorea opposita* Thunb.	24.19
Maiya	Hordei Fructus Germinatus	Germinated and dried processed fruit of *Hordeum vulgare* L.	24.19
Shanzha	Crataegus pinnatifida Bunge	Dried mature fruits of *Crataegus pinnatifida* Bge.	16.13

Note: All plant names were checked using the WFO (https://www.worldfloraonline.org/).

E08355r), ghrelin ELISA kit (CSB-E09816r), and somatostatin (SST) ELISA kit (CSB-E08204r) were purchased from Cusabio Biotech Co. Ltd. (Wuhan, China). The cholecystokinin-octapeptide (CCK-8) ELISA kit (CEB044Ra) was acquired from USCN Life Science Inc (Wuhan, China).

Examination of JWXS Extract by UPLC-MS/MS

About 2.0 g of the JWXS extract was accurately weighed and extracted with 25 mL of 80% v/v methanol for 30 minutes. The 80% methanol extracts were centrifuged at 13000 rpm for 15 minutes at 4°C and the supernatants were filtered through a 0.22 μm filter membrane. The filtrate was collected and stored at 4°C before UPLC-MS/MS analysis.

Sample separation and analysis were performed on a UHPLC-Q Exactive system (Thermo Scientific, Bremen, Germany). The samples were separated at 40°C using a Waters UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The flow rate was set to 0.4 mL/min, and the sample injection volume was set to 5 μL. The gradient program was as follows: 0–11 min, 85–25% A; 11–12 min, 25–2% A; 12–14 min, 2–2% A; 14–14.1 min, 2–8% A; 14.1–16 min, 85–85% A.

Mass spectrometry analysis was conducted using a Q-Exactive mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with Xcalibur software (Thermo Scientific). The samples underwent ionization through electrospray ionization, and the mass spectral signals were collected in positive and negative ion scanning modes, respectively. The data were collected in the m/z range of 70–1050. The mass spectrometric detection mode was Full MS/dd-MS^2 with a resolution of 70,000 for Full MS and 17,500 for dd-MS^2. The optimized source parameters in positive (negative) mode included a capillary temperature of 320°C, auxiliary gas temperature at 400°C, sheath gas flow at 40 arb, auxiliary gas flow at 10 arb, and a spray voltage of 3.5 kV (positive) or −2.8 kV (negative) with collision energies of 20, 40, and 60 eV.

Animals

7-day-old male Sprague-Dawley rats were purchased from SPF (Beijing) Biotechnology Co., Ltd. [SCXK (Jing) 2019–0010]. Rats were housed in a climate-controlled environment with an ambient temperature of 20–25°C and a relative humidity of 40–70%, following a 12-hour light and dark cycle. All rats had ad libitum access to food and water throughout the experiment. All animal care and experimental procedures were conducted in strict accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. This study was approved by the Laboratory Animal Ethics Committee of Jiangzhong Pharmaceutical Co., Ltd. (Nanchang, China).

SD-FD Model Establishment and Treatment

After acclimatization feeding, the rats were randomly assigned to either the healthy control group (Control) or the model group (Model). SD-FD model rats were established by gastric perfusion with an iodoacetamide solution combined with the modified multiple platform method (MMPM).[24] In brief, 10-day-old model rats were orally administered a mixture of 0.1% iodoacetamide and 2% sucrose at a dosage of 0.2 mL/day for 7 consecutive days. At week 3, the SD-FD rats were randomly divided into the following groups: Model group (distilled water, 10 mL/kg), low-dose JWXS group (JWXS-L, 0.28 g/kg/d, clinical equivalent dose), high-dose JWXS group (JWXS-H, 1.4 g/kg/d) and positive control group (Domperidone, 3 mg/kg/d, clinical equivalent dose). Domperidone, as a dopamine receptor antagonist, is a widely utilized treatment for FD that enhances GI motility. Research has substantiated its effectiveness, and it was utilized as a positive control drug.[25] At week 6, these groups were placed in a water-filled platform box for 14 consecutive days (16 hours per day) to induce spleen deficiency. Concomitantly, the rats received distilled water, low-dose JWXS, high-dose JWXS, or Domperidone by oral gavage for 14 days. The schematic diagram of the experimental design is shown in Figure 1.

At the end of the experiment, the rats were fasted for 18 h with free access to water. The following day, rats were orally administered 2 mL of 10% hydroxymethyl cellulose solution containing 0.04% phenol red, one hour after the last administration. Then the gastric emptying rate and intestinal propulsion rate were determined (refer to the "Gastric emptying and intestine propulsion test"). After 20 minutes, the rats were euthanized, and blood samples were collected. Blood samples were allowed to clot at room temperature for 2 h and then centrifuged at 4000 rpm for 10 minutes at 4°C. The serum was collected and stored at −80°C.

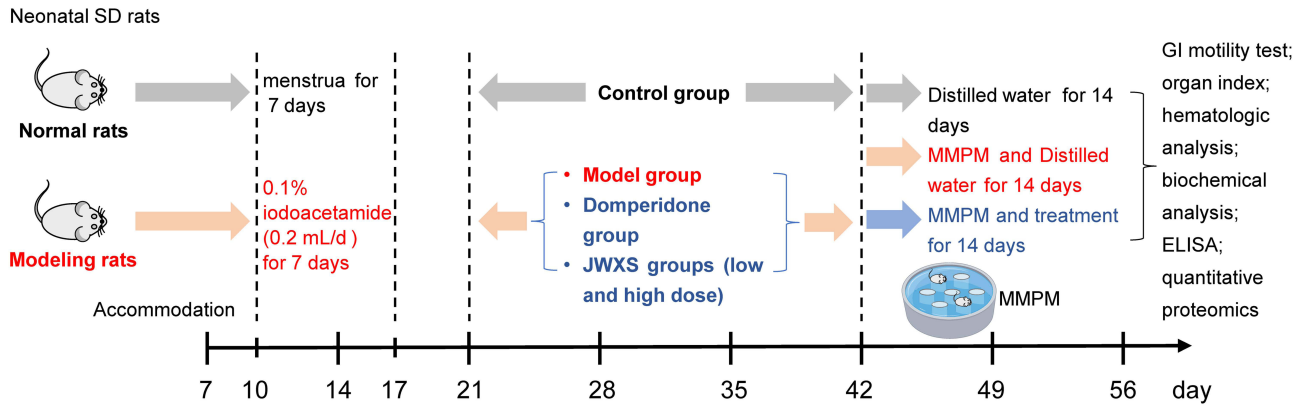

Figure 1 Schematic diagram of the experimental procedure.
Abbreviations: JWXS, Jianweixiaoshi tablets; MMPM, the modified multiple platform method; GI, gastrointestinal; ELISA, enzyme-linked immunosorbent assay.

Gastric Emptying and Intestine Propulsion Test

The rate of gastric emptying and intestinal propulsion was assessed using a phenol red meal, as described in previous studies.[26] After overnight fasting, the rats were given 2 mL of 10% hydroxymethyl cellulose containing 0.04% phenol red by gavage 1 h after the last administration. Twenty minutes later, the rats were euthanized, and their stomachs and small intestines were picked out. After making an incision along the greater curvature of the stomach and thoroughly collecting the gastric contents with 30 mL of 0.5 mol/L NaOH solution, the mixture was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 560 nm using a UV-Vis spectrophotometer. The gastric emptying rate was calculated using the following formula (1).

$$\text{Gastric emptying rate}(\%) = 100\% - \frac{\text{OD560 of test sample solution}}{\text{OD560 of the original solution}} \times 100\% \quad (1)$$

To determine the rate of intestinal propulsion, we measured the distance traveled by phenol red through the small intestine and the total length of the small intestine. The intestinal propulsion rate was calculated using the following formula (2).

$$\text{Intestinal propulsion rate}(\%) = \frac{\text{distance traveled by phenol red}}{\text{length of the small intestine}} \times 100\% \quad (2)$$

Immune Organ Index

Collecting and weighing the immune organs (thymus and spleen) in rats, excess liquid was removed using filter paper to minimize calculation errors. The immune organ index was calculated by the following formula (3).

$$\text{organ index}(\%) = \frac{\text{organ mass}}{\text{body weight}} \times 100\% \quad (3)$$

Hematological Analyses

Whole blood was collected from anesthetized animals and temporarily stored in EDTA-coated tubes. Lymphocytes (Lym), white blood cells (WBC), and neutrophils (Neu) were evaluated using an automatic blood cell analyzer (BC-5000VET, Mindray, Shenzhen, China).

Biochemical Analyses

Serum lactate dehydrogenase (LDH), L-lactic acid (LA), and urea nitrogen (BUN) were measured using the kit from Nanjing Jiancheng Bioengineering Institute (Cat No. A020-2-2, A019-2-1, and C013-2-1, respectively), following the manufacturer's instructions.

Gastrointestinal Hormones Measurement

Serum levels of gastrin, motilin, CCK-8, ghrelin, VIP, and SST were detected using ELISA kits according to the manufacturer's instructions. Briefly, each sample (100 μL) was mixed sequentially with Biotin-antibody, HRP-avidin, TMB Substrate, and stop solution, following the manufacturer's instructions. The mixture was then incubated at the designated temperature for the specified duration. Finally, the absorbance of the sample was measured at 450 nm, and the concentration of the tested GI hormones was determined using the standard curve.

Quantitative Proteomics

Sample Preparation

Data-independent acquisition-based (DIA-based) quantitative proteomics methods were used to analyze the protein changes in the gastric and duodenal tissues of SD-FD rats that were treated with JWXS. Proteomic analysis was conducted at Beijing Qinglian Baio Biotechnology Co. (Beijing, China), following the manufacturer's protocols. Briefly, proteins were extracted from gastric and duodenal tissues, and the concentration of the extracted proteins was determined using the Bradford method. 100 μg of protein was prepared and adjusted to a final concentration of 5 mm DTT (M109-5G, Amresco, USA). The mixture was then incubated at 37°C for 1 h and subsequently returned to room temperature. Next, iodoacetamide IAM (M216-30G, Amresco, USA) was added to achieve a final concentration of 10 mm at room temperature for 45 min. The samples were then diluted 4 times with 25 mm ammonium bicarbonate (A6141-500G, Sigma-Aldrich, USA), and trypsin was added at a ratio of 50:1 of protein to trypsin (V5280/100ug, Promega, USA). The mixture was incubated overnight at 37°C. Formic acid (T79708, Sigma-Aldrich, USA) was added to terminate the reaction and adjusted to pH < 3. Finally, the sample was desalted using a C18 column.

LC-MS/MS Analysis

High-performance liquid chromatography (HPLC) with a RIGOL L-3000 system (Beijing Puyuan Precision Electric Technology, China) was employed for gradient elution. The eluted peptides were subjected to mass spectrometry analysis using a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, USA). Mobile phase A consisted of 100% mass spectrometry water with 0.1% formic acid, while mobile phase B comprised 80% acetonitrile (34851 MSDS, J.T. Baker, USA) with 0.1% formic acid. The separation flow rate was set at 0.7 mL/min, and the elution procedure consisted of the following steps: 0–5 min, 5–8% B; 5–40 min, 8–18% B; 40–62 min, 18–32% B; 62–64 min, 32–95% B; 64–68 min, 95% B. For the mass spectrometry analysis, a Nanospray Flex (NSI) ion source was utilized with an ion spray voltage of 2.0 kV, and the temperature of the ion transfer tube was set to 320 °C. The full scanning range was set as m/z 350–1500 with a primary mass spectrometry resolution of 120,000 (200 m/z). The AGC was set to 300%, and the maximum injection time was set to 50 ms. The secondary mass spectrometry resolution was set at 30,000 (200 m/z), with an AGC of 100%, and the maximum injection time was set to 54 ms. The Spectronaut software was used to search the Rattus norvegicus database.

Proteomics Data Analysis

Difference analysis using the *t*-test method was conducted to analyze proteomic data. Differential proteins were selected based on the criteria of p-value < 0.05 and Fold change > 1.2. Gene ontology (GO) analysis of the non-redundant protein database was conducted using InterProScan-5. We analyzed the protein families and pathways by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Data and Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical significance among multiple groups was analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test, performed with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). An unpaired two-tailed Student's *t*-test was employed to determine statistical significance between the two groups. A significance level of $p < 0.05$ was considered statistically significant for all analyses.

Results

Main Components of JWXS

The total ion chromatogram of JWXS was obtained by UPLC-MS/MS analysis, and a total of 115 main compounds were identified (Figure 2 and Table 2), including 50 flavonoids, 18 terpenoids, and some other compounds.

JWXS Alleviates the Symptoms of the Rats with SD-FD

The SD-FD model was established by gastric infusion of iodoacetamide combined with the modified multi-platform method (MMPM) to evaluate the intervention effect of JWXS in the SD-FD model. At the end of the experiment, the model rats showed significant weight loss and decreased food consumption ($p < 0.05$ or $p < 0.001$, Figure 3A and B), as well as symptoms such as lackluster hair and fatigue, which were consistent with the spleen deficiency syndrome.[14] Furthermore, the body weight of rats in the JWXS treatment group increased compared to the model group; however, no statistically significant

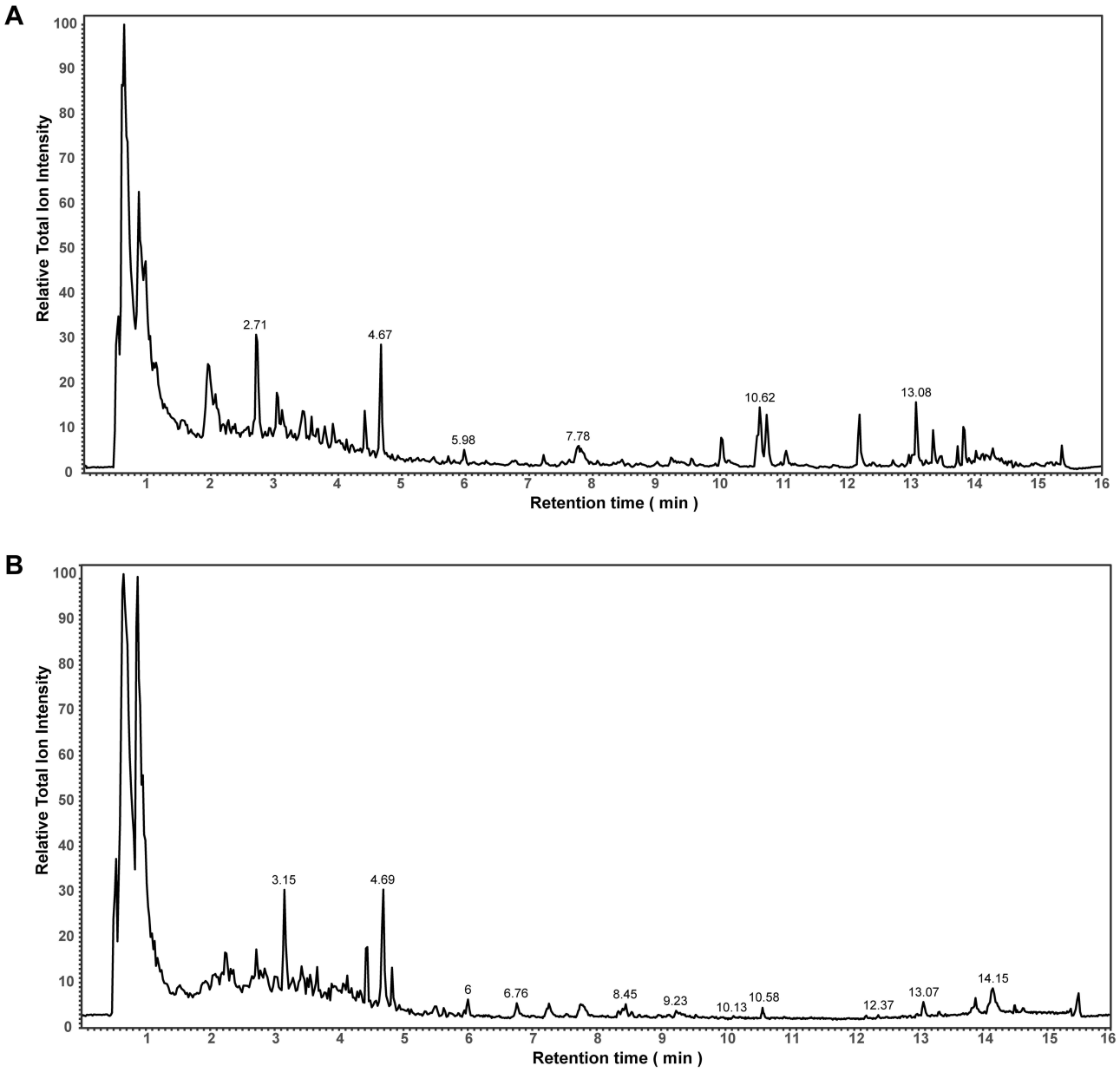

Figure 2 Total ion chromatogram (TIC) of JWXS (**A**) +ESI; (**B**) -ESI.

Table 2 Identification of the Main Chemical Constituents of JWXS Extract by UPLC-MS/MS

No.	Rt/ min	Compound	Adducts	Formula	Molecular Weight
1	0.60	Inositol	$[M-H]^-$	$C_6H_{12}O_6$	179.05
2	0.61	Betaine	$[M+H]^+$	$C_5H_{11}NO_2$	118.09
3	0.63	2-Pyrrolidinecarboxylic acid	$[M+H]^+$	$C_5H_9NO_2$	116.07
4	0.67	Cytosine	$[M+H]^+$	$C_4H_5N_3O$	111.04
5	0.70	L-Pyroglutamic acid	$[M+H]^+$	$C_5H_7NO_3$	130.05
6	0.85	Citric acid	$[M-H]^-$	$C_6H_8O_7$	191.02
7	0.88	L-Tyrosine	$[M+H]^+$	$C_9H_{11}NO_3$	182.08
8	0.92	Uracil	$[M+H]^+$	$C_4H_4N_2O_2$	113.03
9	0.94	Uridine	$[M-H]^-$	$C_9H_{12}N_2O_6$	243.06
10	0.97	Adenosine	$[M+H]^+$	$C_{10}H_{13}N_5O_4$	268.10
11	0.99	Isoguanosine	$[M+H]^+$	$C_{10}H_{13}N_5O_5$	284.10
12	1.00	Guanosine	$[M-H]^-$	$C_{10}H_{13}N_5O_5$	282.08
13	1.08	L-Leucine	$[M+H]^+$	$C_6H_{13}NO_2$	132.10
14	1.76	Lamiide	$[M-H]^-$	$C_{17}H_{26}O_{12}$	421.13
15	1.93	Cinnamic acid	$[M+H]^+$	$C_9H_8O_2$	166.09
16	2.07	6-Methylcoumarin	$[M+H]^+$	$C_{10}H_8O_2$	178.09
17	2.54	1-O-Caffeoylquinic acid	$[M-H]^-$	$C_{16}H_{18}O_9$	353.09
18	2.56	Daphnetin	$[M-H]^-$	$C_9H_6O_4$	177.02
19	2.65	Asperulosidic acid	$[M-H]^-$	$C_{18}H_{24}O_{12}$	431.12
20	2.85	4-Hydroxycinnamamide	$[M+H]^+$	$C_9H_9NO_2$	164.07
21	2.87	Loganin	$[M-H]^-$	$C_{17}H_{26}O_{10}$	389.15
22	3.00	Chlorogenic Acid	$[M+H]^+$	$C_{16}H_{18}O_9$	355.10
23	3.00	Caffeic acid	$[M+H]^+$	$C_9H_8O_4$	163.04
24	3.00	Umbelliferone	$[M-H]^-$	$C_9H_6O_3$	161.02
25	3.22	Aesculetin	$[M-H]^-$	$C_9H_6O_4$	177.02
26	3.28	Isoferulic acid	$[M+H]^+$	$C_{10}H_{10}O_4$	177.05
27	3.39	Vicenin-2	$[M+H]^+$	$C_{27}H_{30}O_{15}$	595.16
28	3.42	(-)-Epicatechin	$[M-H]^-$	$C_{15}H_{14}O_6$	289.07
29	3.50	Shikimic acid	$[M-H]^-$	$C_7H_{10}O_5$	173.04
30	3.52	Eugenol	$[M+H]^+$	$C_{10}H_{12}O_2$	165.09
31	3.52	Isocorydine	$[M+H]^+$	$C_{20}H_{23}NO_4$	342.17
32	3.57	Troxerutin	$[M-H]^-$	$C_{33}H_{42}O_{19}$	741.22
33	3.61	Quercetin 3-O-sophoroside	$[M-H]^-$	$C_{27}H_{30}O_{17}$	625.14
34	3.66	Vicenin-1	$[M+H]^+$	$C_{26}H_{28}O_{14}$	565.15
35	3.68	Schaftoside	$[M-H]^-$	$C_{26}H_{28}O_{14}$	563.14
36	3.75	6-Hydroxyindole	$[M+H]^+$	C_8H_7NO	134.06
37	3.77	Orientin	$[M-H]^-$	$C_{21}H_{20}O_{11}$	447.09
38	3.81	Methyl cinnamate	$[M+H]^+$	$C_{10}H_{10}O_2$	163.07
39	3.90	Vanillin	$[M-H]^-$	$C_8H_8O_3$	151.04
40	3.90	Harmine	$[M-H]^-$	$C_{13}H_{12}N_2O$	211.09
41	3.95	2”-O-Rhamnosylvitexin	$[M-H]^-$	$C_{27}H_{30}O_{14}$	577.16
42	3.97	Rutin	$[M-H]^-$	$C_{27}H_{30}O_{16}$	609.15
43	3.97	Apigenin 6-C-alpha-L-arabinopyranosyl-8-C-beta-D-xylopyranoside	$[M-H]^-$	$C_{25}H_{26}O_{13}$	533.13
44	4.05	Kaempferol-3-O-rutinoside	$[M+H]^+$	$C_{27}H_{30}O_{15}$	595.16
45	4.05	Engeletin	$[M+H]^+$	$C_{21}H_{22}O_{10}$	435.13
46	4.07	Morin	$[M+H]^+$	$C_{15}H_{10}O_7$	303.05
47	4.09	Kaempferol	$[M+H]^+$	$C_{15}H_{10}O_6$	287.05
48	4.15	Scopoletin	$[M+H]^+$	$C_{10}H_8O_4$	193.05
49	4.17	Lariciresinol-4-O-beta-D-glucopyranoside	$[M-H]^-$	$C_{26}H_{34}O_{11}$	521.20

(*Continued*)

Table 2 (Continued).

No.	Rt/ min	Compound	Adducts	Formula	Molecular Weight
50	4.19	Indole-3-acetic acid	$[M-H]^-$	$C_{10}H_9NO_2$	174.05
51	4.22	L-Phenylalanine	$[M+H]^+$	$C_9H_{11}NO_2$	166.09
52	4.22	Costunolide	$[M+H]^+$	$C_{15}H_{20}O_2$	233.15
53	4.26	Isoscoparin	$[M-H]^-$	$C_{22}H_{22}O_{11}$	461.11
54	4.31	Fragransin A2	$[M+H]^+$	$C_{20}H_{24}O_5$	327.16
55	4.31	Isofraxidin	$[M+H]^+$	$C_{11}H_{10}O_5$	223.06
56	4.31	Secoisolariciresinol beta-D-glucoside	$[M-H]^-$	$C_{26}H_{36}O_{11}$	523.22
57	4.41	Narirutin	$[M+H]^+$	$C_{27}H_{32}O_{14}$	581.18
58	4.42	Naringin	$[M-H]^-$	$C_{27}H_{32}O_{14}$	579.17
59	4.44	Massoniresinol	$[M-H]^-$	$C_{20}H_{24}O_8$	391.14
60	4.66	Hesperidin	$[M-H]^-$	$C_{28}H_{34}O_{15}$	609.18
61	4.67	Neohesperidin	$[M+H]^+$	$C_{28}H_{34}O_{15}$	611.20
62	4.69	Naringenin-7-O-beta-D-glucoside	$[M-H]^-$	$C_{21}H_{22}O_{10}$	433.11
63	4.81	Nardosinone	$[M+H]^+$	$C_{15}H_{22}O_3$	251.16
64	5.20	Paprazine	$[M-H]^-$	$C_{17}H_{17}NO_3$	282.11
65	5.37	Pinobanksin	$[M-H]^-$	$C_{15}H_{12}O_5$	271.06
66	5.42	N-trans-Feruloyltyramine	$[M+H]^+$	$C_{18}H_{19}NO_4$	314.14
67	5.51	Obacunone	$[M+H]^+$	$C_{26}H_{30}O_7$	455.20
68	5.62	Methylnissolin	$[M-H]^-$	$C_{17}H_{16}O_5$	299.09
69	5.64	Eriodictyol	$[M-H]^-$	$C_{15}H_{12}O_6$	287.06
70	5.72	Luteolin	$[M-H]^-$	$C_{15}H_{10}O_6$	285.04
71	5.73	2-Methoxycinnamaldehyde	$[M+H]^+$	$C_{10}H_{10}O_2$	163.07
72	5.77	Quercetin	$[M-H]^-$	$C_{15}H_{10}O_7$	301.03
73	5.86	Prunetin	$[M-H]^-$	$C_{16}H_{12}O_5$	283.06
74	5.87	Pinocembrin	$[M+H]^+$	$C_{15}H_{12}O_4$	257.08
75	5.98	Poncirin	$[M-H]^-$	$C_{28}H_{34}O_{14}$	593.19
76	6.00	Isosakuranetin	$[M+H]^+$	$C_{16}H_{14}O_5$	287.09
77	6.00	Licoagroside D	$[M+H]^+$	$C_{22}H_{24}O_{10}$	449.14
78	6.09	Acacetin	$[M-H]^-$	$C_{16}H_{12}O_5$	283.06
79	6.09	Genkwanin	$[M+H]^+$	$C_{16}H_{12}O_5$	285.07
80	6.22	Dihydroresveratrol	$[M-H]^-$	$C_{14}H_{14}O_3$	229.09
81	6.36	Isoalantolactone	$[M+H]^+$	$C_{15}H_{20}O_2$	233.15
82	6.67	Hexahydrocurcumin	$[M-H]^-$	$C_{21}H_{26}O_6$	373.17
83	6.75	Naringenin chalcone	$[M-H]^-$	$C_{15}H_{12}O_5$	271.06
84	6.75	Apigenin	$[M-H]^-$	$C_{15}H_{10}O_5$	269.05
85	6.89	Rebaudioside A	$[M-H]^-$	$C_{44}H_{70}O_{23}$	965.42
86	6.95	Stevioside	$[M-H]^-$	$C_{38}H_{60}O_{18}$	803.37
87	6.95	Rubusoside	$[M-H]^-$	$C_{32}H_{50}O_{13}$	641.32
88	7.00	Tricin	$[M-H]^-$	$C_{17}H_{14}O_7$	329.07
89	7.07	3'-Methoxyapigenin	$[M-H]^-$	$C_{16}H_{12}O_6$	299.06
90	7.27	Hesperetin	$[M-H]^-$	$C_{16}H_{14}O_6$	301.07
91	7.28	Ganhuangenin	$[M-H]^-$	$C_{17}H_{14}O_8$	345.06
92	7.38	Medicarpin	$[M-H]^-$	$C_{16}H_{14}O_4$	269.08
93	7.51	Rebaudioside C	$[M-H]^-$	$C_{44}H_{70}O_{22}$	949.43
94	8.08	Isosinensetin	$[M+H]^+$	$C_{20}H_{20}O_7$	373.13
95	8.09	Neoandrographolide	$[M-H]^-$	$C_{26}H_{40}O_8$	479.26
96	8.17	Lunularic acid	$[M-H]^-$	$C_{15}H_{14}O_4$	257.08
97	8.46	L(-)-Carvone	$[M+H]^+$	$C_{10}H_{14}O$	151.11
98	8.69	Phenyl benzoate	$[M-H]^-$	$C_{13}H_{10}O_2$	197.06

(*Continued*)

Table 2 (Continued).

No.	Rt/ min	Compound	Adducts	Formula	Molecular Weight
99	9.02	Sinensetin	$[M+H]^+$	$C_{20}H_{20}O_7$	373.13
100	9.11	1-(3-Hydroxy-5-methoxyphenyl)-2-(3-hydroxyphenyl)ethane	$[M+H]^+$	$C_{15}H_{16}O_3$	245.12
101	9.22	5,7,8,4'-Tetramethoxyflavone	$[M+H]^+$	$C_{19}H_{18}O_6$	343.12
102	9.29	Limonin	$[M-H]^-$	$C_{26}H_{30}O_8$	515.19
103	9.38	Formononetin	$[M+H]^+$	$C_{16}H_{12}O_4$	269.08
104	9.58	Ganoderic acid B	$[M-H]^-$	$C_{30}H_{44}O_7$	515.30
105	9.60	Glycyrrhizic acid	$[M-H]^-$	$C_{42}H_{62}O_{16}$	821.40
106	10.13	Licochalcone B	$[M-H]^-$	$C_{16}H_{14}O_5$	285.08
107	10.13	Chrysosplenetin B	$[M+H]^+$	$C_{19}H_{18}O_8$	375.11
108	10.25	Galangin 3-methyl ether	$[M+H]^+$	$C_{16}H_{12}O_5$	285.07
109	10.27	Wogonin	$[M-H]^-$	$C_{16}H_{12}O_5$	283.06
110	10.59	Cholic acid	$[M-H]^-$	$C_{24}H_{40}O_5$	407.28
111	10.62	3',4',3,5,6,7,8-Heptamethoxyflavone	$[M+H]^+$	$C_{22}H_{24}O_9$	433.15
112	11.02	Tangeretin	$[M+H]^+$	$C_{20}H_{20}O_7$	373.13
113	11.46	Medicagenic acid	$[M-H]^-$	$C_{30}H_{46}O_6$	501.32
114	11.67	5-O-Demethylnobiletin	$[M+H]^+$	$C_{20}H_{20}O_8$	389.12
115	13.80	Eucommiol	$[M+H]^+$	$C_9H_{16}O_4$	189.11

difference was observed ($p > 0.05$). Food consumption significantly increased in all treatment groups compared to the model group ($p < 0.05$). The gastric emptying rate and intestinal propulsive rate of the model group were significantly decreased compared to the control group ($p < 0.05$, or $p < 0.001$, Figure 3C and D). After JWXS and Domperidone treatment, the gastric emptying rate and intestinal propulsion rate of the treatment group significantly increased ($p < 0.05$, or $p < 0.01$). Concomitantly, the spleen and thymus indices in the model group of rats decreased, suggesting a potential impairment in immune function for the SD-FD rats (Figure 3E and F). In contrast, the spleen and thymus indices of rats in the high and low-dose groups showed significant improvement compared to the model group ($p < 0.05$, or $p < 0.01$).

Effects of JWXS on Fatigue-Related Biochemical Indexes and Inflammatory Cell Numbers in SD-FD Rats

The characteristics of the spleen deficiency model include low appetite, fatigue, and reduced immune function. Fatigue in modern medicine encompasses damage to the central nervous system and decreased function of the motor system.[27] To assess the effects of JWXS on fatigue and immune function in FD-SD rats, we measured serum levels of LDH, LA, and BUN, along with blood inflammatory cell count. As shown in Figure 4, serum levels of LDH, LA, and BUN were significantly increased ($p < 0.05$, $p < 0.01$, or $p < 0.001$) in the model group compared to the normal group. Additionally, blood WBC count and %Lym were significantly decreased ($p < 0.05$ or $p < 0.001$), while %Neu was significantly increased ($p < 0.001$). In comparison to the model group, LDH, BUN, and %Neu were significantly decreased in the JWXS-L and JWXS-H groups ($p < 0.05$, $p < 0.01$, or $p < 0.001$), whereas %Lym was extremely significantly increased ($p < 0.05$). Furthermore, LA was significantly decreased in the JWXS-H treatment group ($p < 0.05$), while WBC count was significantly increased ($p < 0.01$).

JWXS Improves SD-FD via the Regulation of Gastrointestinal Hormones

The balance of hormones related to GI regulation plays an important role in regulating GI motility.[28] Our study quantified the levels of six hormones associated with GI regulation: gastrin, motilin, cholecystokinin-octapeptide (CCK8), vasoactive intestinal peptide (VIP), somatostatin (SST), and ghrelin. As shown in Figure 5, serum levels of gastrin, motilin, and ghrelin were significantly increased in all treatment groups ($p < 0.05$, $p < 0.01$, or $p < 0.001$), while CCK8 and SST levels were significantly decreased ($p < 0.05$ or $p < 0.01$). JWXS-H and Domperidone significantly decreased VIP levels ($p < 0.05$).

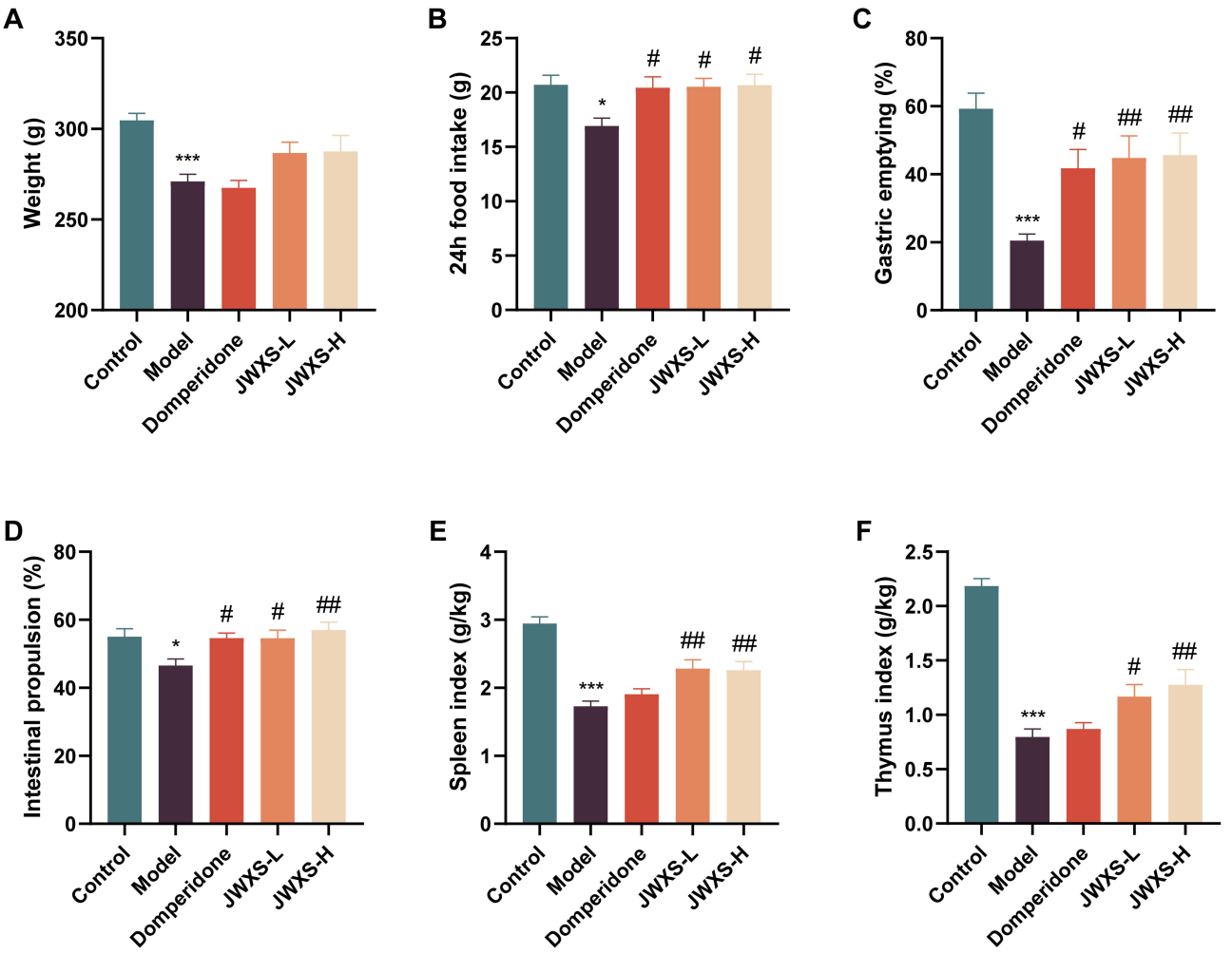

Figure 3 JWXS alleviates the symptoms of the rats with SD-FD. (**A**) Weight, (**B**) 24-hour food intake,(**C**) gastric emptying, (**D**) intestinal propulsion, (**E**) spleen index, (**F**) thymus index (n=8 rats per group). $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the model group.
Abbreviations: JWXS-L, low dose of Jianweixiaoshi tablets; JWXS-H, high dose of Jianweixiaoshi tablets.

Proteomics Analysis of Differentially Expressed Proteins in Gastric Tissues

The studies mentioned above demonstrated that JWXS-L significantly enhanced gastric motility, and the effect was similar to positive drug. Consequently, in the subsequent experiments, JWXS was orally administered at a dose of 0.45 g/kg (JWXS-L). To further elucidate the mechanisms behind the therapeutic effect of JWXS on SD-FD, we conducted DIA-based quantitative proteomic analysis on stomach tissues from three groups of rats: Control, Model, and JWXS (JWXS-L). A total of 28,647 peptides and 5400 proteins were detected using DIA-based quantitative proteomics. Volcano plots were used to visualize the distribution of differentially expressed proteins (DEPs) by applying filtering criteria of fold change (>1.2 for up-regulated or <0.83 for down-regulated) and p-value < 0.05. Between the Model and Control groups, 728 DEPs were identified, comprising 516 upregulated proteins and 212 downregulated proteins (Figure 6A and B). Furthermore, a total of 333 DEPs (120 upregulated and 213 downregulated) were identified in the JWXS group compared to the Model group. The Venn plot displayed an overlap of 126 DEPs between the Model vs Control and JWXS vs Model groups (Figure 6C). Cluster analysis showed significant differences between the model and control groups (Figure 6D). Additionally, the protein expression was significantly changed after JWXS intervention in SD-FD rats.

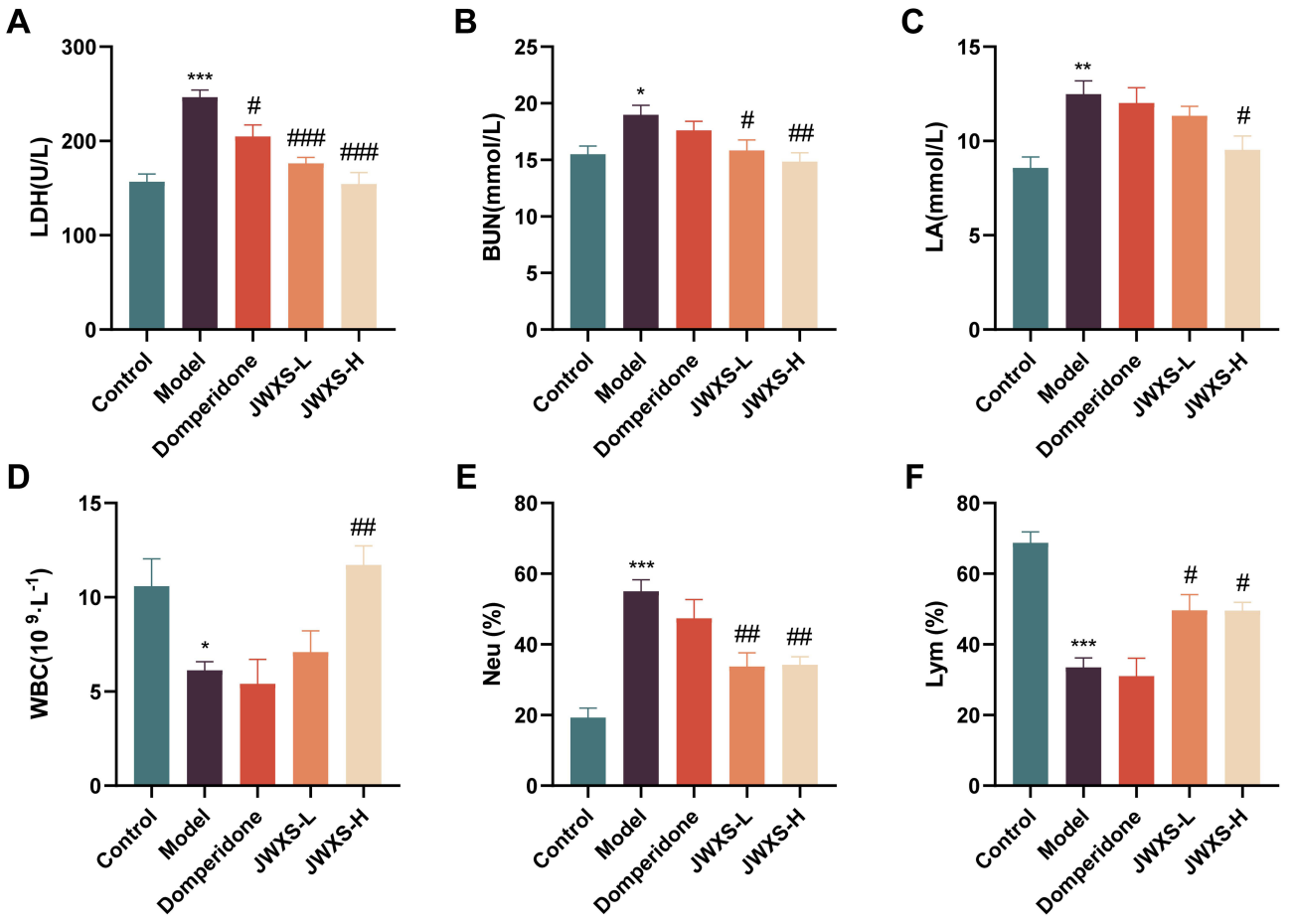

Figure 4 Effects of JWXS on fatigue related biochemical indexes and inflammatory cell numbers in SD-FD rats. (**A**) LDH, (**B**) BUN, (**C**) LA, (**D**) WBC, (**E**) % Neu, (**F**) %Lym (n=6 rats per group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the model group.

Bioinformatics Analysis of DEPs in Gastric Tissues

To enhance comprehension of the biological properties of the differentially expressed proteins, we conducted a bioinformatics analysis (Figure 7A). The results of the GO enrichment analysis of differential proteins are presented in the form of a bar chart, which displays the top 10 terms for cellular component (CC), biological process (BP), and molecular function (MF). In biological processes, JWXS could improve energy metabolism by regulating the tricarboxylic acid cycle, succinyl-CoA metabolic process, respiratory electron transport chain, and the regulation of cytochrome-c oxidase activity. The results of the KEGG enrichment analysis of differential proteins are also displayed as a bar chart, which shows the top 20 pathways (Figure 7B). The JWXS-regulated differential proteins are primarily associated with the Citrate cycle (TCA cycle), Glutathione metabolism, the calcium signaling pathway, and others. In the calcium signaling pathway, the sequencing results revealed that the main differential proteins were SERCA2, PLC-γ2, Vdac1, Vdac2, and Vdac3. Among these proteins, the abundance of PLC-γ2 significantly increased after JWXS treatment (Figure 7C, $p < 0.05$), while the abundance of SERCA2, Vdac1, Vdac2, and Vdac3 markedly decreased ($p < 0.05$).

Proteomics Analysis of Differentially Expressed Proteins in Duodenal Tissues

The duodenum is a key region in the pathophysiology of FD. Disruption of the duodenal barrier has been demonstrated in FD patients, and the underlying damage-associated molecular pattern can enter the compromised intestinal barrier and trigger host innate immunity.[29] To elucidate the therapeutic mechanism of JWXS on the duodenum of SD-FD rats, we conducted a DIA-based quantitative proteomic analysis on the duodenal tissues of three groups of rats. A total of 29682 peptides and 4421 proteins were detected using DIA-based quantitative proteomics. Volcano plots were used to visualize

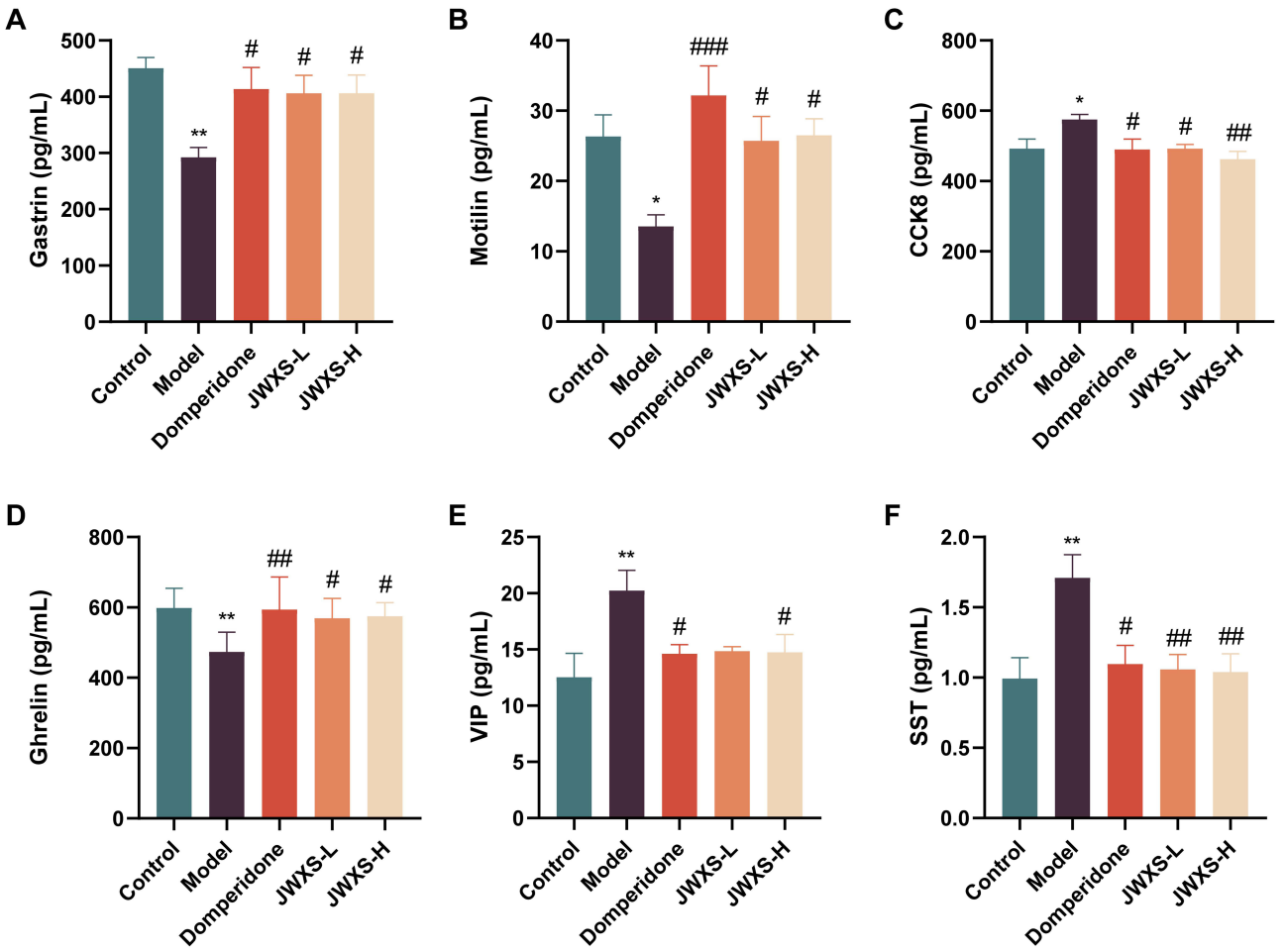

Figure 5 JWXS modulates the secretion of gastrointestinal hormones in SD-FD rats. (**A**) Gastrin, (**B**) Motilin, (**C**) Cholecystokinin-octapeptide (CCK8), (**D**) Ghrelin, (**E**) Vasoactive intestinal peptide (VIP), and (**F**) Somatostatin (SST) in serum are shown (n=6 rats per group). $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.01$ compared to the model group.

the distribution of differentially expressed proteins (DEPs) by applying filtering criteria of fold change (>1.5 for up-regulated or <0.67 for down-regulated) and *p*-value < 0.05 (Figure 8A). There was a total of 276 differentially expressed proteins (DEPs) identified between the Model and Control groups, of which 108 proteins were upregulated and 51 proteins were downregulated (Figure 8B). Meanwhile, a total of 732 DEPs (376 up-regulated and 277 down-regulated) were identified in the JWXS group compared to the Model group. The Venn plot showed that there were 64 overlapping DEPs found between the Model vs Control and the JWXS vs Model groups (Figure 8C). Cluster analysis showed significant differences between the model and control groups (Figure 8D). Additionally, the protein expression was significantly changed after JWXS intervention in SD-FD rats.

Bioinformatics Analysis of DEPs in Duodenal Tissues

To better classify the functional properties of differential proteins, the DEPs were subjected to GO analysis (Figure 9A). In biological processes, JWXS could respond to immune activation via antigen processing and presentation of exogenous peptide antigen via MHC class II, negative regulation of hydrogen peroxide-induced cell death, positive regulation of T cell-mediated cytotoxicity, protection from natural killer cell-mediated cytotoxicity. Furthermore, we performed the KEGG pathway analysis of differentially expressed proteins (Figure 9B). The results indicated that the KEGG pathways enriched in the JWXS group mainly included antigen processing and presentation, staphylococcus aureus infection, oxidative phosphorylation, intestinal immune network for IgA production, among others. In the antigen processing and presentation and intestinal immune network for IgA production, the sequencing results revealed that the main differential

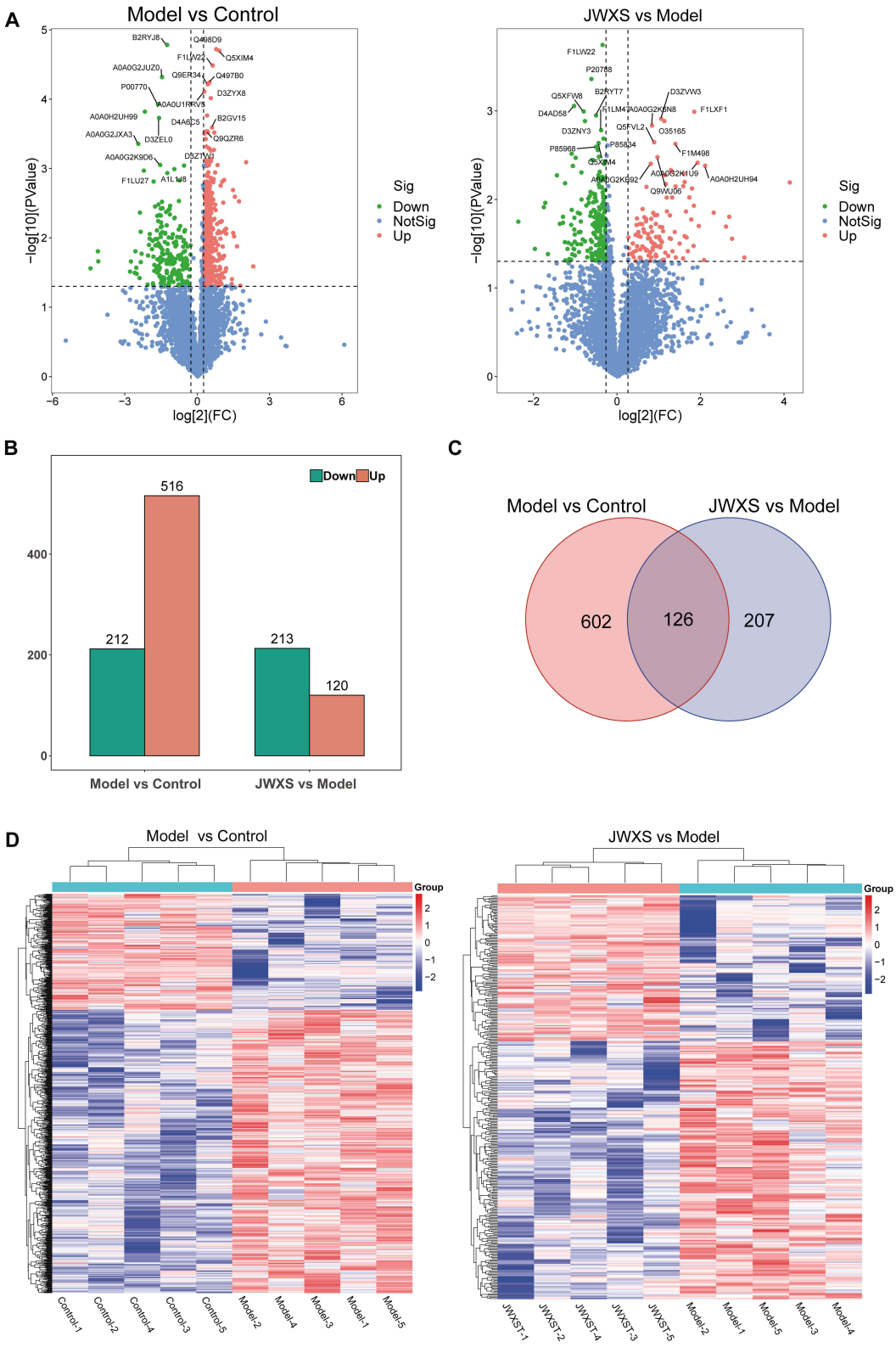

Figure 6 Quantitative proteomics was employed to assess differentially expressed proteins in gastric tissues among Control, Model, and JWXS rat groups. (**A**) Volcano plots of differentially expressed proteins in the Model vs Control and JWXS vs Model groups. Red represents up-regulated expression, green represents down-regulated expression and Blue represents no significant difference. (**B**) Histogram of the distribution of differentially expressed proteins in the Model vs Control and JWXS vs Model groups. (**C**) Venn diagram of DEPs between the Control and Model groups. (**D**) Heatmap illustrating the expression levels of DEPs in the Model vs Control and JWXS vs Model groups. Red represents up-regulated expression and blue represents down-regulated expression.

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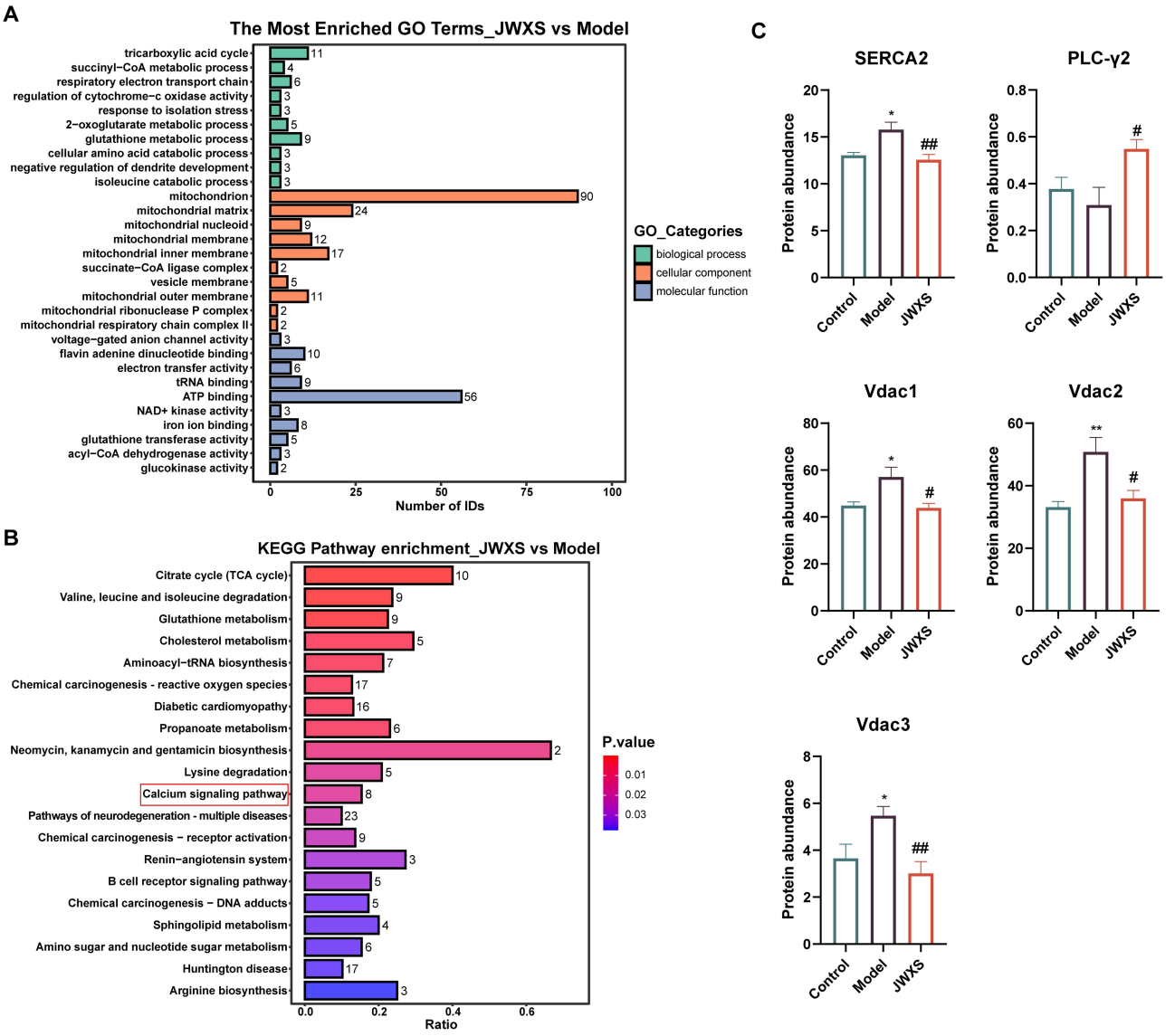

Figure 7 GO enrichment and KEGG pathway analyses of the differentially expressed proteins in gastric tissues among the model and JWXS rat groups. (**A**) Enriched GO terms were identified for biological process (BP), cellular component (CC), and molecular function (MF), respectively. (**B**) Significantly enriched KEGG pathways based on the differentially expressed proteins. (**C**) The protein abundance of SERCA2, PLC-γ2, Vdac1, Vdac2, and Vdac3 (n=5 rats per group). $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the model group.

proteins were PIgR, RT1-A1, RT1-CE1, MHC II, Tap1, and Tap2. Among these proteins, the abundance of PIgR significantly increased after JWXS treatment (Figure 9C, $p < 0.05$), while the abundance of RT1-A1, RT1-CE1, MHC II, Tap1, and Tap2 markedly decreased ($p < 0.05$).

Discussion

Gastric motor dysfunction is considered the primary mechanism that causes symptoms of FD. Previous studies have shown that administering iodoacetamide to neonatal rats for gastric stimulation may result in gastric hypersensitivity and gastric motor dysfunction in adult rats.[30] In TCM, the term "spleen" encompasses not only the anatomical spleen but also functions related to digestion and absorption, hematopoiesis, muscle growth, energy metabolism, and the regulation of brain-gut peptides and neurotransmitters.[31] Spleen deficiency is a common clinical syndrome that involves the deterioration of digestion, absorption, lassitude, energy conversion, and immune system function. Under the guidance of the "fatigue hurts the spleen" theory in TCM, MMPM may induce spleen deficiency by causing fatigue. In our study, we

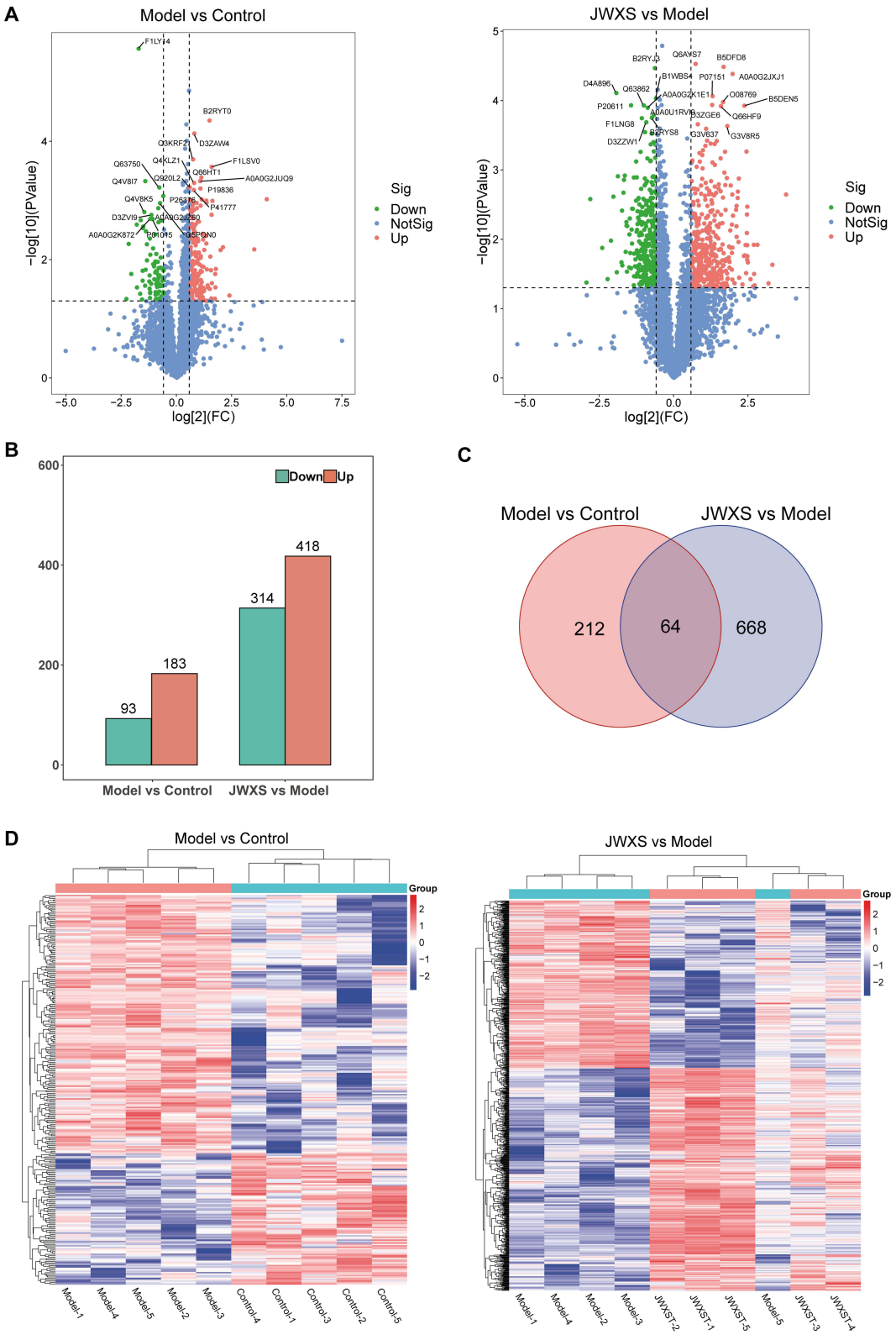

Figure 8 Quantitative proteomics was employed to assess differentially expressed proteins in duodenal tissue among Control, Model, and JWXS rat groups. (**A**) Volcano plots of differentially expressed proteins in the Model vs Control and JWXS vs Model groups. Red represents up-regulated expression, green represents down-regulated expression and Blue represents no significant difference. (**B**) Histogram of the distribution of differentially expressed proteins in the Model vs Control and JWXS vs Model groups. (**C**) Venn diagram of DEPs between the Control and Model groups. (**D**) Heatmap illustrating the expression levels of DEPs in the Model vs Control and JWXS vs Model groups. Red represents up-regulated expression and blue represents down-regulated expression.

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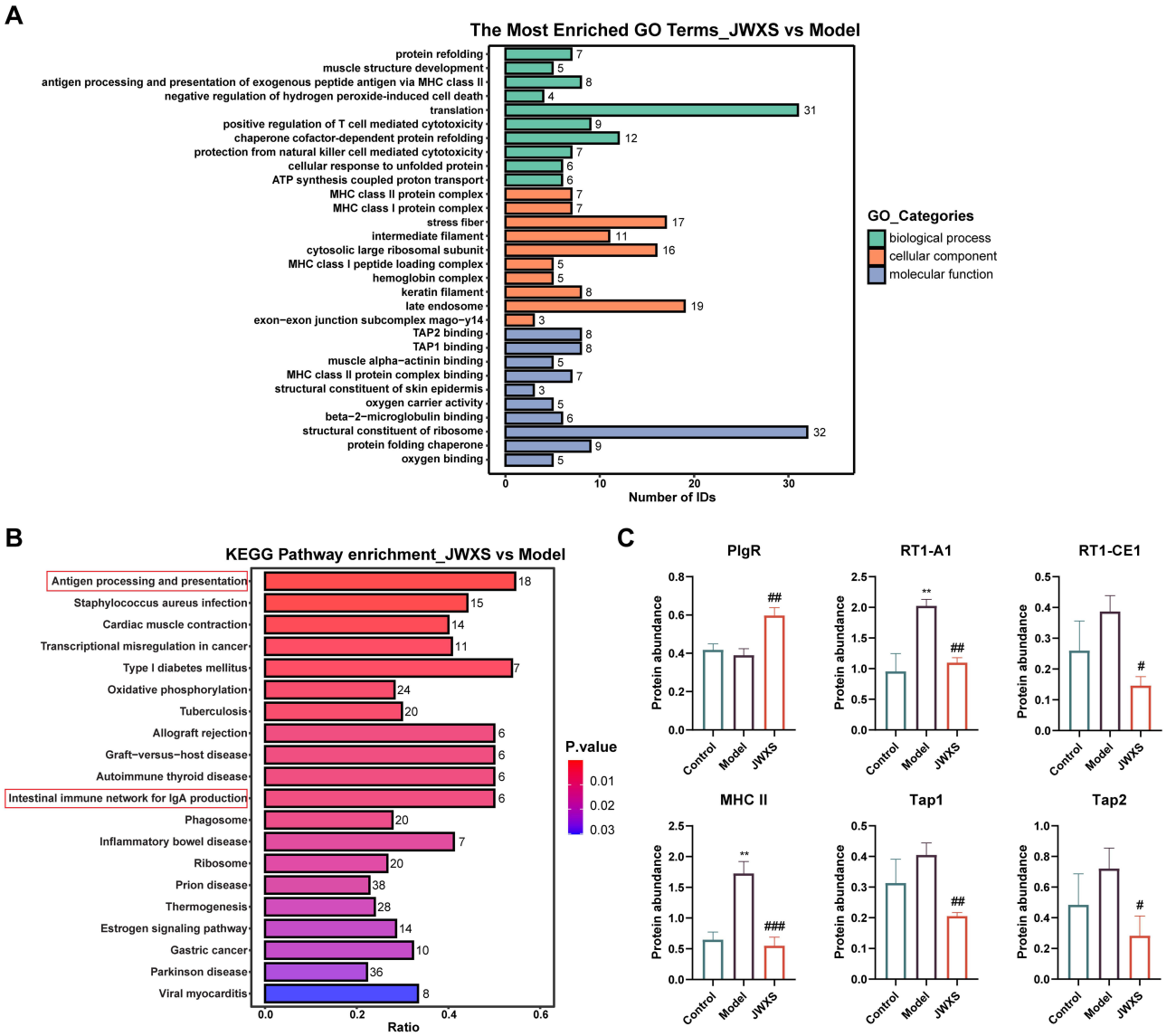

Figure 9 GO enrichment and KEGG pathway analyses of the differentially expressed proteins in duodenal tissue among the model and JWXS rat groups. (**A**) Enriched GO terms in terms of biological process (BP), cellular component (CC), and molecular function (MF), respectively. (**B**) Significantly enriched KEGG pathways based on the differentially expressed proteins. (**C**) The protein abundance of PIgA, RT1-A1, RT1-CE1, MHC II, Tap1, and Tap2 (n=5 rats per group). $^{**}p < 0.01$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the model group.

observed weight loss, fatigue, inactivity, and dry hair in model rats, which are similar to the symptoms of spleen deficiency in TCM.[32] The lower gastric emptying rate and intestinal propulsive rate of model rats, compared to healthy rats, suggest the successful construction of the FD model. In addition, SD-FD rats also had lower spleen and thymus weights than healthy rats, decreased WBC count and %Lym in blood, and increased %Neu, suggesting immune dysfunction. The administration of JWXS enhanced the aforementioned symptoms in SD-FD rats, implying an improvement in both GI motility as well as immune function.

Gastrointestinal (GI) absorption and movement are regulated by GI hormones secreted by endocrine cells located within the intestines of the GI tract.[33] Numerous studies have examined the impact of FD on GI hormones, highlighting their significance in this context. Motilin and gastrin promote smooth muscle contraction, increase GI motility, stimulate gastric acid secretion and pepsin secretion, and regulate food intake.[34,35] Meanwhile, ghrelin increases appetite, aids in gastric emptying, and protects GI mucosa.[36] VIP, SST, and CCK serve as inhibitory neurotransmitters in both the central and enteric nervous systems, effectively reducing GI motility, gastric

emptying, and gastric acid secretion.[37–39] In comparison to healthy rats, SD-FD rats exhibited an increase in serum levels of SST, VIP, and CCK, and a decrease in the expression of motilin, gastrin, and ghrelin. After the administration of JWXS treatment, all of the aforementioned phenomena were almost completely restored to their normal levels. We hypothesize that JWXS enhances digestion by regulating the homeostasis of GI hormones.

Proteomics offers a comprehensive understanding of protein composition, which facilitates the identification of signaling pathways for environmental stimuli and molecular mechanisms of drug action.[40] The potential mechanism of JWXS in the treatment of SD-FD was further investigated using DIA-based proteomic analysis of gastric tissues. Interestingly, our proteomic analysis identified significant alterations in proteins related to the calcium signaling pathway in gastric tissues. These findings align with previous animal studies that have demonstrated the crucial role of intracellular Ca^{2+} concentration ($[Ca2^{+}]_i$) in regulating GI smooth muscle contraction.[41–43] The process of elevated intracellular Ca^{2+} concentration involves Ca^{2+} entering from the extracellular into the intracellular and the release of Ca^{2+} from intracellular stores.[44] An increase in $[Ca^{2+}]_i$ initiates smooth muscle contraction, while a decrease in $[Ca^{2+}]_i$ leads to smooth muscle relaxation and delayed gastric emptying. When comparing the JWXS group with the Model group, we observed a decrease in the abundance of sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2) and an increase in the abundance of phospholipase C-γ (PLC-γ) in the JWXS group. SERCA2 is primarily located in the endoplasmic reticulum (ER) and is responsible for transporting Ca^{2+} from the cytoplasm into the ER against a concentration gradient, requiring significant ATP consumption.[45] On the other hand, the activation of PLC-γ leads to the hydrolysis of phosphatidylinositol 4.5-bisphosphate in the cell membrane, generating inositol trisphosphate (IP3) and diacylglycerol as secondary messengers.[46] The released IP3 then binds to receptors on the ER membrane, triggering the release of intracellular stored Ca^{2+}.[47] These findings suggest that JWXS intervention in SD-FD rats may improve gastric emptying disorders by reducing SERCA2 expression, inhibiting Ca^{2+} storage, and increasing PLC-γ expression thereby enhancing IP3-mediated Ca^{2+} release and contributing further to the regulation of intracellular $[Ca^{2+}]_i$. However, the mechanism by which JWXS improves gastric emptying delay by regulating calcium signaling pathways needs to be further verified.

An increasing number of studies indicate that duodenal immune activation is crucially involved in the pathophysiology of FD.[48] To examine the regulatory impact of JWXS on intestinal immunity, the current study utilized a proteomic approach to investigate the underlying molecular mechanism of JWXS in SD-FD rats. After analyzing the proteomic profile of the duodenum, we observed that the administration of JWXS has an impact on proteins involved in the antigen processing and presentation pathway, as well as the Intestinal immune network for IgA production pathway. Duodenal mucosal integrity impairment and low-grade inflammation are associated with systemic immune activation, which can ultimately lead to dyspeptic symptoms. Previous clinical studies have also indicated that inflammation of the duodenal mucosa and the presence of increased T-cells in the small intestine indicates intestinal inflammation, which is associated with delayed gastric emptying and the severity of dyspeptic symptoms.[49,50] In the present study, JWXS may play a therapeutic role by modulating the expression of two proteins, polymeric immunoglobulin receptor (pIgR) and major histocompatibility complex (MHC) class II antigen. PIgR is an immune factor produced on the surface of mucous membranes (such as intestinal tissues), which can combine with immunoglobulin IgA and transport it to the mucosal surface, leading to the formation of secretory IgA (SIgA).[51] SIgA plays a crucial role in maintaining the immune function of the intestinal mucosa. One of its main functions is to prevent the adhesion of pathogens, thereby inhibiting their colonization and spread on the surface of the intestinal mucosa, and limiting infection.[52] In the present study, the expression of pIgR was reduced in SD-FD rats, while treatment with JWXS restored pIgR expression to normal levels. MHC II is a significant group of immune system molecules primarily found on the surface of antigen-presenting cells. The elevated expression of MHC II in the intestine has been firmly linked to intestinal inflammation.[53] The study results indicate that the expression of MHC II in the model rats was increased, and the administration of JWXS was able to reduce MHC II expression. However, there are some limitations to this study. For example, whether impaired intestinal immunity affects intestinal barrier function requires further study.

Conclusion

Our study suggests that JWXS may be a promising drug for the treatment of FD-SD, as it appears capable of improving gastric motility disorders and regulating immune function. We hypothesize that its mechanism of action involves the regulation of gastric smooth muscle contraction in FD-SD through calcium signaling pathways and the modulation of duodenal immune function. However, the precise mechanism by which JWXS exerts its effects remains unclear, necessitating further validation studies to explore specific targets and pathways.

Abbreviations

JWXS, Jianweixiaoshi tablets; SD-FD, functional dyspepsia with spleen deficiency; FD, functional dyspepsia; CCK8, cholecystokinin-octapeptide; VIP, vasoactive intestinal peptide; SST, somatostatin; Lym, lymphocyte; WBC, white blood cell; Neu, neutrophil; LDH, lactate dehydrogenase; LA, L-lactic acid; BUN, urea nitrogen; TCM, Traditional Chinese medicine; MMPM, modified multiple platform method; GI, gastrointestinal; DEPs, differentially expressed proteins; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; PLC-γ, phospholipase C-γ; ER, endoplasmic reticulum; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; IP3, inositol trisphosphate; PIgR, polymeric immunoglobulin receptor; MHC II, major histocompatibility complex class II; SIgA, secretory IgA.

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Disclosure

The authors Xiaoying Cheng, Jianhua Wan, Denglong Sun, Yang Zhan, Jingtong Yu, Yingmeng Li, Yanxia Xiong, and Wenjun Liu are employed by Jiangzhong Pharmaceutical Co., Ltd. This employment has not influenced the design, data analysis, results, or conclusions of this study.

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