Jiao San Xian granule containing prebiotic alleviate functional dyspepsia by regulating intestinal flora and the secretion of inflammatory response

Huimin Pei [a,1], Mengyuan Zhao [a,1], Hongyue Wang [a], Xindi Zhang [b,c], Caihong Shi [a], Xiangrong Zhang [a,*]

[a] Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China
[b] Food Processing Institute, Heilongjiang Academy of Agricultural Sciences, 368 Xuefu Road, Harbin 150086, China
[c] Key Laboratory of Food Processing of Heilongjiang Province, Harbin 150086, China

Abstract

Jiao Sanxian (JSX) consists of three traditional Chinese medicine including charred hawthorn, malt and Liu Shen Qu, they are also from Chinese medicine homologous food. It is commonly used to relieve functional dyspepsia (FD). The present study aims to explore the mechanism of JSX (granule), with the addition of prebiotics for the treatment of FD using mice model. The network pharmacology and molecular docking were used to forecast therapeutic targets for JSX. Based on the protein−protein interaction (PPI) results, thirteen targets exhibited strong interactions with each other. The results of network pharmacology and molecular docking revealed therapeutic activity of JSX in FD regulated by its anti-inflammatory effect, improvement in gastrointestinal function and physiological activity in components-targets-pathways-disease. The pharmacological effects of JSX were further verified by rRNA gene sequencing, enzyme-linked immunosorbent assay (ELISA) and animal study. The *in vivo* results showed JSX, in combination with prebiotics, could mitigate FD symptoms. ELISA study showed that glyceraldehyde-3-phosphate dehydrogenase (GAPDH), AKT1 and tumor necrosis factor (TNF) -α played the roles in reducing the inflammatory response by downregulating inflammation content. The above results were coincided with the network pharmacology prediction. By 16S rRNA, the results showed JSX may alleviate the FD symptom by increasing the diversity of the intestinal flora, adjusting the dominant prebiotic. In conclusion, JSX can alleviate functional dyspepsia in mice. The mechanisms were related to regulating the secretion of inflammatory response, significantly improving the benefits of intestinal flora and ameliorating multi-metabolic pathways.

Keywords: Functional dyspepsia, *In vivo*, Jiao San Xian, Pharmacology network

1. Introduction

Dyspepsia, also known as functional dyspepsia (FD), is a common functional gastrointestinal disorder associated with postprandial fullness, upper abdominal pain, upper abdomen burning, belching and nausea [1]. Generally, the pathogenesis of FD is the dysfunction and disorder caused by digestive tract movement. Reduced gastric motility resulted in slow gastric emptying [2]. At present, the main treatment methods are the use of prokinetic drugs, antacids and antidepressants. Using the pharmacological effects of food for treatment is not only safer in clinical application, but also can reduce many toxic side effects. On the basis of overall regulation, it shows advantages in treating FD [3].

JSX, a traditional Chinese Medicine was composed by charred hawthorn (*Crataegus pinnatifida* Bge. Var. *major* N.E. Br.), charred malt (*Hordeum vulgare* L.) and Liu Shen Qu (*Massa Medicata*

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* Corresponding authors at: Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China.
E-mail address: zhangxr@vip.sina.com (X. Zhang).
[1] Huimin Pei and Mengyuan Zhao contributed equally to this work.

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Fermentata). The combination of the three ingredients can significantly enhance the digestive function. Hawthorn is a key medicine and food to promote digestion, especially stodge. Malt is useful in eliminating starchy food and improving stagnation. Liu Shen Qu is a fermented product of *Artemisia annua L.*, *Polygonum hydropiper* L., *Xanthium sibiricum* L., Semen *Armeniacae Amarum.*, *Vigna umbellate* (Thunb.), mixed at a specific ratio. Prebiotics could maintain the balance of gut microbiota and promote the absorption of herbs in the gastrointestinal tract [4]. The research aimed to prepare JSX granules containing prebiotics and investigate their effect on the treatment of FD.

Granules have many advantages, such as easy to take, carry, store, and suitable for industrial mass production. In order to ensure the safe, effective and stable quality of clinical Chinese medicine granule, it is extremely important to determine the active ingredient. This purpose of this study was to explore the mechanism of JSX (granule) with the addition of prebiotics, for the treatment of FD using mice model.

Network pharmacology is a multidisciplinary research field, which integrates biology, omics, and computational biology. It revealed the mechanism of action from an overall perspective with integrity, synergistic effect and kinetic characteristics [5]. Meanwhile, molecular docking has been widely applied to verify receptor-ligand binding. It can be combined with network pharmacological study to find the targets and active components to explain the underlying mechanisms.

In this study, we aimed to explore the target and mechanism of Jiao San Xian granule in improving FD by computer technology and pharmacodynamic experiment. And the standards were established about the extraction comprehensive scoring and particle production of JSX granule. The mechanisms are related to inflammatory response, gastrointestinal function and disordered intestinal flora.

Abbreviations

JSX	Jiao San Xian
FD	functional dyspepsia
PPI	protein–protein interaction
16S rRNA	16S ribosomal RNA
ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
TNF	tumor necrosis factor
KM mice	Kunming mice
OB	oral bioavailability
DL	drug likeness
GO	gene ontology
KEGG	kyoto encyclopedia of genes and genomes
BPs	biological processes
CCs	cellular components
MFs	molecular functions
SDF	simulation description format
PCA	Principal coordinates analysis
MAPK	mitogen-activated protein kinase
EGFR	epidermal growth factor receptor
IL 6	interleukin 6
VEGFA	vascular endothelial growth factor A

2. Material and methods

2.1. Chemical compounds and reagents

Charred hawthorn, charred malt and charred Liu Shen Qu were purchased from Beijing Tongrentang Co., Ltd (Fuzhou, Fujian, China), and identified by the Lab of Beijing Tongrentang Health Care Productions (Fuzhou) CO., LTD. China. The voucher specimens, TJSY/R-YC-51-2-210501. Chlorogenic acid with 95.0% purity were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Seventy Kunming mice (KM mice, equal gender) weighing 12–15 g were obtained from the Liaoning Changsheng Biotechnology Co., Ltd. 10% fat energy supply control group diet (XTCON50J) and 60% fat provides energy for high-fat diet (XTHF60) were provided from Jiangsu Cooperative Pharmaceutical Bioengineering Co., Ltd. The water was double distilled, and the other chemicals and solvents were analytical grade.

2.2. The preparation of JSX granule containing prebiotic

The active ingredients were extracted from JSX pieces. The solution was obtained by adding 12 times water on base of the JSX, decocting at 70 °C, extracting 2 h and 3 times. The process was evaluated based on the content of chlorogenic acid and the extraction rate (7:3). Then the solution was concentrated and dried at 40 °C. The particles were prepared by mixing dry extract powder, maltodextrin and oligofructose (1.0:0.5:0.25) at spraying 80% alcohol. The process was evaluated based on the particle forming rate. In traditional Chinese medicine preparations, adding prebiotic can improve intestinal flora and the efficiency of metabolism. The oligofructose were taken as the representative to explore the effect of prebiotic in this study. Repeat experiments were verified the stability and feasibility of the formula and preparation method. According to the *Pharmacopoeia of the People's Republic of China 2020*, JSX granule containing prebiotic was up to the standard of the inspection indicators for granule.

2.3. Network pharmacology analysis

2.3.1. Screening active compounds and targets of JSX

The virtual fishing candidate ingredients of the three herbal medicines in JSX were retrieved by TCMSP database (https://old.tcmsp-e.com/tcmsp.php) and Batman-TCM (http://bionet.ncpsb.org/batman-tcm/). The SMILE formats of screened ingredients were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). The active chemical constituents were screened by the conditions of oral bioavailability (OB) value $\geq$ 15%, drug likeness (DL) value $\geq$ 0.18. Then, the standardized gene names of the selected corresponding targets were further screened by Uniprot database (http://www.uniprot.org/) and the Swiss Target Prediction database (http://www.swisstargetprediction.ch) [6].

2.3.2. Screening targets of FD

GeneCards database (https://www.genecards.org/) was used to search genes about "functional dyspepsia" at the screening conditions of "Homeo specifications".

2.3.3. Construct PPI of the core targets

The intersection targets of JSX for FD were obtained by the online toolkit Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). The obtained common targets were put into STRING (https://string-db.org, Version 11. 0) to perform protein–protein interaction (PPI) analysis. The data was stored in a tab-separated value format and uploaded into the Cytoscape 3.8.2 software. We screened core targets according to three main parameters that were the over average betweenness centrality, closeness centrality and a double of average freedom degree. In addition, the edges representing intermolecular interactions was the links between nodes. The wider the edge, the stronger the interaction.

2.3.4. Gene Ontology and pathway analysis

The key targets of JSX for FD were submitted to DAVID database (https://david.ncifcrf.gov/) to analyze Gene Ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment [7]. The database can provide systematic, comprehensive biological functional annotations, including biological processes (BPs), cellular components (CCs), molecular functions (MFs), and pathways. In the GO and KEGG analyses, p is the result of Fisher's exact test. The lower the p value, the stronger the correlation between the pathway and targets, the higher the count and the more targets in the pathway [8]. p value < 0.05 was considered statistically significant. The charts of these analyses were drawn by the bioinformatics platform (http://www.bioinformatics.com.cn/).

2.4. Molecular docking

The compounds obtained from JSX docked with the key targets. The simulation description format (SDF) of active compound was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) and optimized by Chem3D18.0. The core targets were downloaded structures in PDB database (https://www.rcsb.org/). The ligands were put into the PyRx software for energy minimization, and visualizing the docking results. For molecular docking, ligands preparation and receptor grids were performed by Schrodinger Glide (https://www.schrodinger.com/products/glide). The score value represents the binding activity between a compound and a target. The docking score less than −6 kcal/mol indicates good binding activity, and the more negative score, the more stable the ligand-receptor binding.

2.5. Pharmacodynamics and therapeutic mechanism

2.5.1. Animals

All mice were maintained at 22 °C and a 12 h circadian rhythm. After 10 days of acclimatization, mice were randomly separated into seven groups: 1C-blank control group, 2C-JSX containing prebiotic (5 g/kg), 3C-positive control group (5 g/kg), 4C-low dose group of JSX (2.5 g/kg), 5C-medium dose group of JSX (5 g/kg), 6C-high dose group of JSX (10 g/kg), 7C-Model group, 10 mice per group. The blank control group were fed with XTCON50J. The other animals were fed with XTHF60. It was determined that the model was successful if the food intake of 7C group was less than the 1C group 30%. All animals were received humane care according to the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Shenyang Pharmaceutical University under the approval number SYPH-IACUC-S2022-10.06-101.

2.5.2. Administration

From the second day, mice in 2C, 4C, 5C and 6C group were intragastric administrated 20 mL/kg granule once a day. In addition, mice in 3C group received of Dashanzhawan, and the 1C and 7C groups were given distilled water. The granule was administered to mice for 10 days at 20 mL/kg dosing

volume. The mice were allowed to have free access to food and water ad libitum.

2.5.3. General observations

The daily general status, food intake and weight of the mice were recorded. The mice were fasted before the last treatment and then given nutritious semi-solid paste (40 mL/kg) after 1 h. Nutritional semi-solid paste includes sodium carboxymethyl cellulose 10 g, starch 8 g, sucrose 8 g, milk powder 16 g, activated carbon 2 g and distilled water 250 mL, stirring evenly to 40 mL/kg.

2.5.4. 16S rRNA sequencing

The feces of mice were respectively collected for 16S rRNA sequencing by biomarker Biotechnology Co., Ltd (Beijing, China). Bacterial genomic DNA was extracted from the mice feces using the Spin Kit for Soil according to the instruction manuals, specific primers were synthesized according to the full-length primer sequence. PCR amplification was performed, and the purified products were quantified and homogenized to form a sequencing library.

2.5.5. Enzyme-linked immunosorbent assay

The serum was prepared by centrifuging of mice plasma. The serum and gastric supernatant were detected by ELISA. In addition, the gastric residual rate and intestinal propulsion rate were calculated.

3. Results

3.1. Verification of JSX granule containing prebiotic preparation method

The regression equation between the peak area and concentration of chlorogenic acid was: $y = 73875x\text{-}251553$ ($R^2 = 0.9993$), which indicated a good linear relationship. The verification results of extraction process were 96.45%, 100.00%, 96.34%, and the average composite score was 97.60% (Table 1). The verification results of particle preparation were 94.5%, 92.6%, 96.79%, and the average composite score was 97.60% (Table 2). The composite scores of the verification results proved that the formulation has good stability and feasibility.

Table 2. Validation of forming process for JSX granule containing prebiotic.

Number	Paste rate (wt%)	Standard deviation	Average	RSD (%)
1	94.50			
2	92.60	2.10	94.63	2.00
3	96.79			

According to the *Pharmacopoeia of the People's Republic of China 2020*, JSX granule was up to the standard of the inspection indicators for granule (Table 3).

3.2. Network pharmacology

A total of 52 compounds were selected and the basic information of these compounds was showed in Table 4. As showed in Fig. 1A, we can clearly find that there are intersected targets of active compounds. In addition, a compound interacts with multiple targets and different compounds also act on a target at the same time. According to topological analysis, the targets were extracted by String database (Fig. 1B). It revealed the highly tight interaction between targets. The top three targets of degree value will be used for ELISA analysis to check the content changes in serum and gastric homogenate supernatant.

In GO enrichment analysis, BPs were mainly associated with the protein phosphorylation, positive regulation of transferase activity and regulation of mitogen-activated protein kinase (MAPK) cascade, etc. CCs involved in receptor complex, membrane microdomain, focal adhesion and membrane raft, etc. MFs involves phosphotransferase activity, alcohol groups as acceptor, kinase activity and protein tyrosine kinase activity, etc. (Fig. 1C). The KEGG analysis showed pathways of JSX for FD, including the PI3K-Akt signaling pathway, MAPK signaling pathway and pathway in cancer, etc. (Fig. 1D).

3.3. Molecular docking

It is believed that the lower the binding energy, the greater the protein binding affinity of the ligand. As

Table 1. Validation of extraction process for JSX granule containing prebiotic.

Number	Paste rate (wt%)	Chlorogenic acid (mg/30 g)	Overall score	Average	RSD (%)
1	46.67	10.61	96.45		
2	46.67	11.18	100.00	97.60	1.43
3	45.00	10.77	96.34		

$$\text{Composite Score}=\left(\frac{\text{Extract Rate}}{\text{the max of Extract Rate}}\right) * 0.3 + \left(\frac{\text{the content of chlorogenic acid}}{\text{the max content of chlorogenic acid}}\right) * 0.7.$$

Table 3. Quality standard inspection of JSX granule containing prebiotic.

Items	Standards	Results
Granularity	≤15%	3.20–5.50%
Repose angle	Excellent: 25–30°	26.57° ± 1.38
Moisture content	≤8%	5.17 ± 0.22%
Loss on drying	≤2%	1.59 ± 0.12%
Dissolubility	Completely dissolved or slightly turbid within 5 min	3.00 ± 0.98 min
Alcohol-soluble extract	≥13%	49.33 ± 2.13%
Inventory difference	≤5%	2.00 ± 1.10%

Table 4. Effective ingredients of JSX from the TCMSP database (OB value ≥ 15%, DL value ≥ 0.18).

Name	MOLID	MOLNAME	OB	DL
Charred Hawthorn	Suchilactone	MOL005384	57.52	0.56
	20-Hexadecanoylingenol	MOL002307	28.20	0.68
	12-Oxoarundoin	MOL009360	16.43	0.76
	Vitexin 7-glucoside	MOL008510	16.65	0.78
	Ursolic acid	MOL008498	17.70	0.75
	Ruvoside	MOL004349	18.13	0.63
	5-hydroxymethylfurfural	MOL000748	45.07	0.02
	Isoquercitrin	MOL000437	1.86	0.77
	Citric Acid	MOL001456	56.22	0.05
	Chlorogenic acid	MOL001955	11.93	0.33
	Oleanic acid	MOL000263	29.02	0.76
	5-Methyl furfural	MOL004119	43.92	0.01
Charred Malt	FA	MOL000433	68.96	0.71
	Digallate	MOL000569	61.85	0.26
	Sterigmato-cystin	MOL010857	57.16	0.68
	(+)-catechin	MOL000492	54.83	0.24
	Naphthol aS-bl phosphate	MOL010865	49.88	0.45
	Ent-Epicatechin	MOL000073	48.96	0.24
	Pyrethrin II	MOL002710	48.36	0.35
	5,7-Dihydroxy-3′,4′,5′-trimethoxyflavon	MOL010864	46.01	0.37
	Delta-D	MOL010861	45.66	0.48
	Delphinidin	MOL004798	40.63	0.28
	Sitosterol	MOL000359	36.91	0.75
	Luteolin	MOL000006	36.16	0.25
	α-tocopheryl quinone	MOL010862	35.91	0.5
	Nivalenol	MOL005088	35.68	0.28
	Vitamin-e	MOL007180	32.29	0.7
	Candletoxin A	MOL002671	31.81	0.69
	Deoxynivalenol standard	MOL010846	31.16	0.25
	Isoorientin	MOL000498	23.30	0.76
	Sitogluside	MOL000357	20.63	0.62
	Tryptanthrin	MOL001808	19.28	0.29
	Hovenine A	MOL013375	17.02	0.69
	Violanthin	MOL010860	15.38	0.81
Charred Liu Shen Qu	β-D-Glucopyranose, anhydrous	MOL005441	48.09	0.04
	Lactic acid	MOL002301	44.51	0.01
	Succinic acid	MOL000346	29.62	0.01
	5-hydroxymethylfurfural	MOL000748	45.07	0.02
	Aspterric acid	MOL000065	79.74	0.02
	L-glutamic acid	MOL000052	6.66	0.02
	Sericic acid	MOL000064	83.59	0.01
	Glycine	MOL000050	48.74	0
	Glutamine	MOL009676	87.90	0.02
	L-Histidine	MOL000071	53.18	0.03
	Alanine	MOL000042	87.69	0.01
	Tyrosine	MOL000056	57.55	0.05
	Valine L-Valin	MOL000067	53.33	0.01
	L-isoleucine	MOL000068	59.05	0.02
	Proline	MOL000061	77.57	0.01
	Leucinum	MOL005448	72.92	0.01
	Phenylalanine	MOL000041	41.62	0.04
	L-Lysine	MOL000055	29.33	0.02

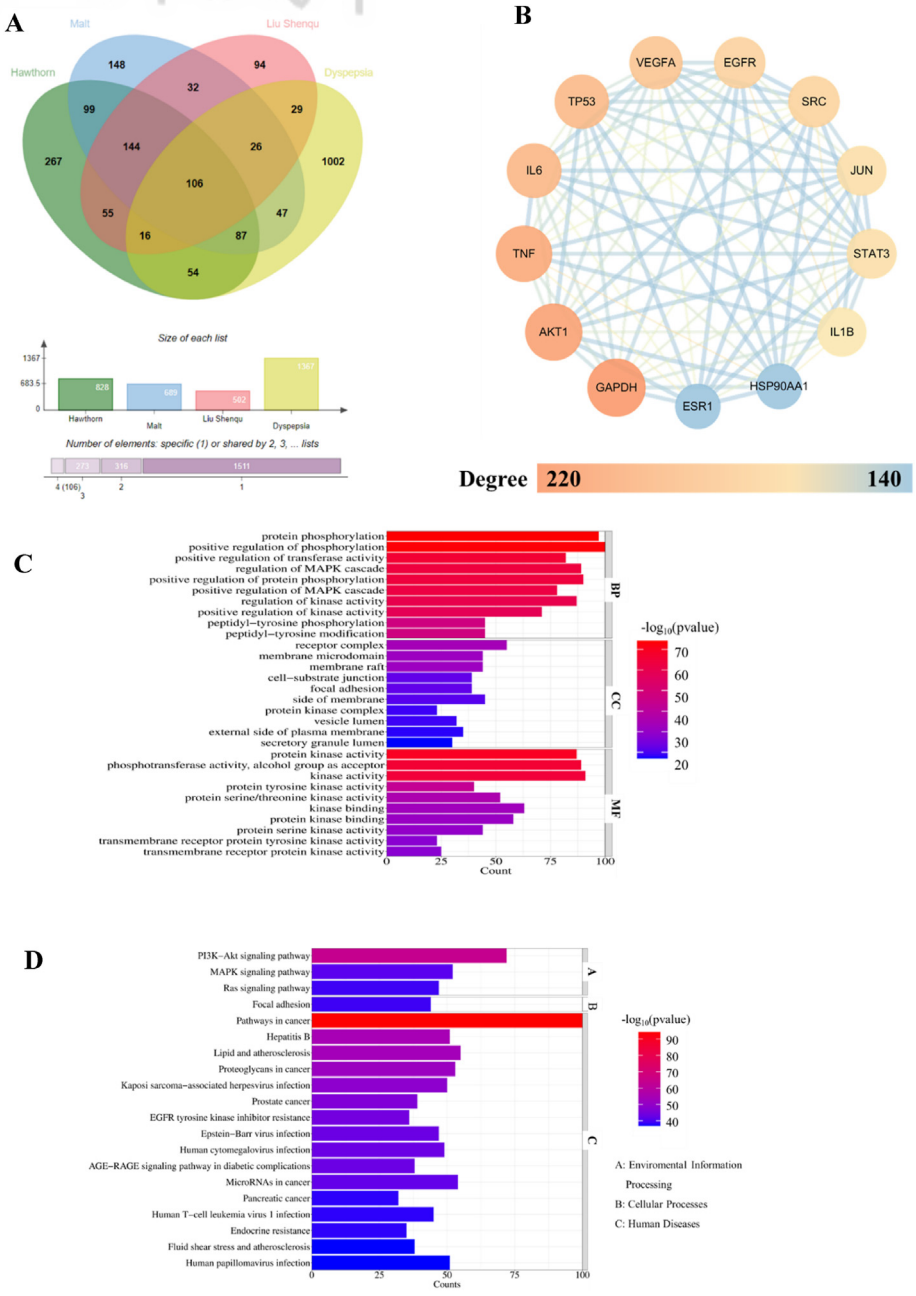

Fig. 1. Network pharmacology analysis revealed the targets and pathways regulated by JSX in FD. (A) Venn diagram of JSX and FD targets. (B) The PPI network diagram from STRING database. (C) GO enrichment analysis based on key targets of JSX for FD. (D) KEGG pathway enrichment analysis based on key targets of JSX for FD.

showed in Table 5, the binding energy of AKT1, epidermal growth factor receptor (EGFR), JUN, ESR1 and MAPK3 with compounds were low, which indicated that the conformation of key targets with compounds are stable. If the docking score were less than −6 kcal/mol, kernel targets and active ingredients would show good binding. Among this, 20-Hexadecanoylingenol had the lowest score with interleukin 6 (IL6) (*5ZO6*), −10.2 kcal/mol. FA combined with GAPDH (*6E08*) formed hydrogen bonds with LYS238, ABG487, GLU504 and π pond with TYR503 (Fig. 2A). Tryptanthrin combined with TNF (*1XU1*) to form hydrogen bonds with AGR186 and THR168 (Fig. 2B). Luteolin combined with AKT1 (*7NH5*) formed three hydrogen bonds (TYR272, ASP292, THR211) and two π ponds (TRP80) (Fig. 2C).

Table 5. The score of active components with target genes by molecule docking.

Chinese Medicines	Targets PDB / Components	GAPDH *6E08*	AKT1 *7NH5*	TNF *1XU1*	IL6 *5ZO6*	TP53 *6IUA*	VEGFA *1MKK*	EGFR *8A27*	SRC *1U5D*	JUN *6EQ9*	STAT3 *6NJS*	IL1B *6Y8I*	HSP90AA1 *5NJX*	ESR1 *7JKY*	MAPK3 *4QTB*
Charred Hawthorn	Suchilactone	−5.322	−7.382	−3.398	−4.295	−6.049	−5.356	−6.774	−3.849	−8.239	−3.753	−3.487	−4.07	−7.431	−7.377
	20-Hexadecanoylingenol	−5.756	−7.622	—	−10.2	−4.856	−4.235	−8.87	−3.124	−5.703	−4.856	−3.662	−3.478	−5.597	−6.636
	12-Oxoarundoin	−3.071	−4.16	—	—	—	—	−2.121	—	−4.05	−2.501	—	—	−1.686	−6.62
	Vitexin 7-glucoside	−5.888	−7.742	—	—	−4.246	—	−6.042	−4.709	−7.586	−4.246	−3.756	—	−6.954	−5.738
	Ruvoside	−4.511	−7.741	—	—	—	—	−4.531	—	−5.85	−3.546	—	—	−2.759	−3.842
	5-hydroxymethylfurfural	−4.298	−5.522	−4.981	−4.825	−5.021	−4.91	−5.825	−3.857	−4.068	−3.564	−3.536	−4.186	−6.559	−4.343
	Isoquercitrin	−5.007	−8.797	−2.568	−5.244	−5.389	−5.138	−6.561	−4.553	−7.719	−4.397	−4.092	−5.215	−8.267	—
	Citric Acid	−4.775	−4.106	−4.727	−3.761	−4.833	−4.848	−4.865	−7.86	−6.217	−3.412	−4.161	−4.349	−3.928	−4.696
	Chlorogenic acid	−5.673	−7.729	−3.772	−5.139	−4.892	−4.191	−6.362	−6.84	−8.29	−4.853	−4.071	−4.697	−7.196	−6.588
	5-Methyl furfural	−4.828	−5.819	−5.468	−4.778	−4.461	−4.953	−5.862	−4.017	−6.423	−4.134	−4.042	−4.087	−6.288	−4.607
Charred Malt	Ent-Epicatechin	−5.995	−7.583	−4.665	−6.134	−5.294	−5.148	−5.507	−4.758	−7.689	−4.413	−5.559	−6.19	−8.21	−7.001
	FA	−6.344	−7.54	−2.023	−5.61	−6.039	−5.363	−7.413	−6.396	−7.856	−5.741	−6.752	−5.663	−5.74	−6.655
	Digallate	−5.435	−7.706	−3.701	−5.863	−5.635	−5.019	−5.637	−5.868	−7.261	−4.31	−5.657	−5.332	−5.043	−6.73
	Sterigmato-cystin	−4.306	−7.805	−3.338	−4.529	−3.527	−3.456	−5.456	−2.81	−7.633	−3.527	−3.368	−3.059	−7.745	−6.62
	(+)-catechin	−5.697	−8.73	−4.018	−5.65	−5.797	−5.887	−8.73	−4.89	−8.168	−5.797	−5.481	−6.853	−7.811	−6.994
	Naphthol aS-bl phosphate	−4.937	−7.844	−3.424	−4.631	−4.602	−4.038	−5.929	−4.973	−6.443	−5.116	−3.327	−2.706	−5.981	−4.829
	Pyrethrin II	−4.242	−6.891	−4.379	−4.79	−3.371	−4.799	−6.77	−2.298	−5.211	−3.384	−2.49	−3.801	−5.112	−5.68
	5,7-Dihydroxy-3′,4′,5′-trimethoxyflavon	−5.88	−8.472	—	—	−5.791	−5.016	−6.186	−3.228	−7.65	−4.184	−3.826	−5.82	−8.528	−6.778
	Delta-D	−3.597	−6.174	−2.196	−4.546	−2.446	−1.847	−5.162	−2.237	−5.372	−2.873	−2.066	−4.325	−5.102	−5.86
	Delphinidin	−3.696	−8.87	−2.915	−5.459	−4.585	−5.79	−6.042	−4.276	−7.493	—	−5.545	−6.101	−6.361	−8.34
	Sitosterol	−3.304	−5.767	−2.05	−3.872	−1.349	−2.719	−5.878	−0.65	−5.534	−1.9	−2.045	−3.024	−2.464	−4.549
	Luteolin	−6.141	−9.095	−4.128	−5.797	−5.853	−5.705	−6.513	−4.69	−8.199	−5.263	−4.671	−5.677	−8.801	−7.833
	α-tocopheryl quinone	−4.094	−7.843	—	−3.575	—	—	−5.788	−2.09	−5.682	−3.992	−2.128	−4.085	−4.102	−6.37
	Nivalenol	−4.203	−5.179	−3.395	—	−2.73	−3.911	—	−2.615	−5.321	—	−4.264	−4.378	—	−5.131
	Vitamin-e	−4.643	−6.376	−2.243	−6.004	−4.994	−4.626	−7.344	−4.205	−6.454	−4.394	−3.376	−3.891	−7.541	−7.342
	Isoorientin	−5.661	−8.154	—	−4.601	−5.835	−4.292	−6.38	−4.207	−6.892	−4.752	−4.666	−6.446	−6.344	−7.788
	Sitogluside	−2.909	−6.963	—	—	—	—	−6.429	—	−5.904	−4.354	—	—	−3.004	−4.874
	Tryptanthrin	−4.293	−7.658	−5.636	−5.596	−4.777	−5.48	−7.259	−3.554	−7.36	−3.156	−3.829	−4.443	−8.158	−7.053
	Tricin	−5.852	−8.212	—	−4.663	−5.188	−5.713	−6.117	−3.824	−7.733	−4.668	−4.217	−5.815	−8.661	−6.831
	Isovitexin	−4.599	−7.559	—	−5.657	−5.493	−4.209	−6.642	−4.291	−7.212	−4.399	−4.695	−6.213	−8.669	−9.058
	Hovenine A	−2.69	−6.801	−1.201	−4.436	−1.184	−3.614	−4.912	—	−5.127	−1.184	−1.072	−2.981	−4.378	−6.435
Charred Liu Shen Qu	β-D-Glucopyranose, anhydrous	−5.232	−5.556	−4.861	−4.868	−5.118	−5.011	−5.78	−5.475	−5.065	−4.404	−4.086	−6.623	−6.121	−5.203
	Lactic acid	−4.895	−4.61	−4.069	−4.088	−3.87	−4.933	−4.206	−5.744	−4.256	−3.509	−4.314	−4.532	−4.421	−3.931
	Succinic acid	−4.712	−3.454	−3.777	−3.462	−4.462	−4.483	−4.22	−6.377	−4.672	−3.24	−4.42	−3.553	−3.883	−3.152
	Aspterric Acid	−4.234	−6.899	−3.383	−4.901	−4.144	−5.355	−6.25	−3.19	−5.566	−2.783	−3.929	−3.542	−4.532	−3.861
	L-glutamic acid	−3.458	−3.701	−3.702	−3.312	−3.578	−4.238	−3.456	−5.459	−4.293	−2.554	−3.089	−3.646	−4.197	−3.335
	Glycine	−2.92	−2.115	−1.56	−1.84	−1.901	−2.083	−1.87	−2.626	−2.099	−1.666	−1.761	−2.011	−2.141	−2.204
	Glutamine	−4.435	−4.19	−3.461	−3.822	−4.086	−4.38	−4.235	−4.282	−3.617	−3.761	−4.074	−4.114	−4.819	−4.904
	L-Histidine	−4.976	−4.966	−4.458	−4.803	−4.229	−4.839	−5.846	−5.279	−4.485	−4.079	−4.483	−4.376	−6.336	−5.71
	Alanine	−2.742	−2.573	−2.377	−2.316	−2.32	−2.902	−2.301	−3.062	−2.055	−2.115	−2.3	−2.769	−2.756	−2.915
	Tyrosine	−4.329	−6.211	−4.001	−5.229	−4.184	−5.246	−5.757	−3.752	−6.89	−3.625	−4.726	−4.123	−7.41	−5.777
	Valine L-Valin	−4.09	−4.282	−4.341	−3.857	−3.97	−4.426	−4.99	−4.482	−3.706	−3.552	−3.735	−4.394	−3.802	−4.771
	L-isoleucine	−3.611	−3.824	−4.185	−3.51	−3.646	−3.831	−4.569	−3.662	−4.302	−2.81	−2.987	−3.383	−3.335	−4.231
	Proline	−4.793	−4.914	−4.751	−4.409	−4.451	−4.88	−5.096	−4.72	−4.437	−3.964	−3.913	−4.698	−4.604	−4.684
	Leucinum	−3.745	−3.673	−3.865	−3.289	−3.728	−4.194	−4.624	−3.606	−4.081	−2.719	−2.784	−3.859	−3.721	−4.327
	Phenylalanine	−4.618	−5.246	−4.887	−4.971	−3.608	−4.73	−5.525	−3.969	−5.267	−3.739	−3.32	−3.922	−6.386	−5.671
	L-Lysine	−3.009	−4.198	−2.765	−2.909	−3.002	−3.302	−3.598	−3.691	−3.076	−2.222	−3.072	−3.207	−3.73	−4.22
	α-D-Glucoseanhydrous	−5.208	−5.641	−4.085	−4.692	−6.024	−4.796	−5.756	−4.748	−5.003	−3.806	−4.615	−6.441	−6.576	−5.016

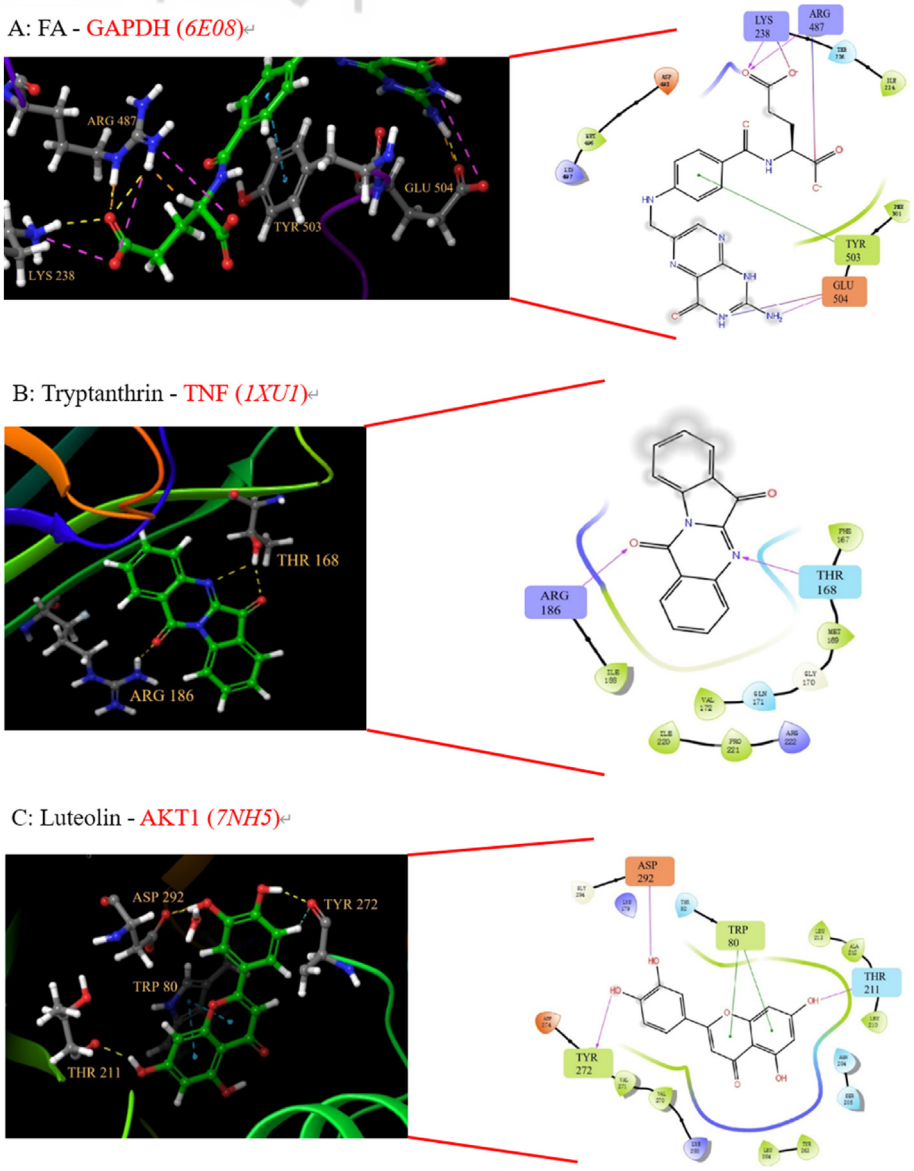

Fig. 2. The molecular docking modes of active compounds with target genes. (A) Action mode of FA with GAPDH (6E08); (B) Action mode of tryptanthrin with TNF (1XU1); (C) Action mode of luteolin with AKT1 (7NH5).

3.4. Pharmacodynamics experiment results

3.4.1. General observations

The food intake and weight recovery in the 2C to 6C groups were exceed than model group from 2nd day to 11th day. Compared with the blank control group, the food intake of the model group was significantly reduced from the 2nd day (Fig. 3A and B). The fecal number and weight of 2C and 3C groups were the closest to 1C (Fig. 3C and D). In addition, the mice in 7C were depressed, reduced in activity and curled up together. They had yellow and dull fur, swollen and full abdomen, the yellow urine. Their feces were sticky soft, yellow and brown. The anatomy of the model group showed that most of the stomach and intestinal cavity were enlarged and bloated, and the stomach contained a large amount of residue. The above symptoms gradually recovered after medication treatment. We found the residual rate of gastric contents and small intestine propulsion rate in mice recovered after treatment in 2C, 3C, 5C groups (Table 6). The above indicated that the effect of adding prebiotic was better.

3.4.2. ELISA

To verify the network pharmacology and molecular docking prediction results, the targets of

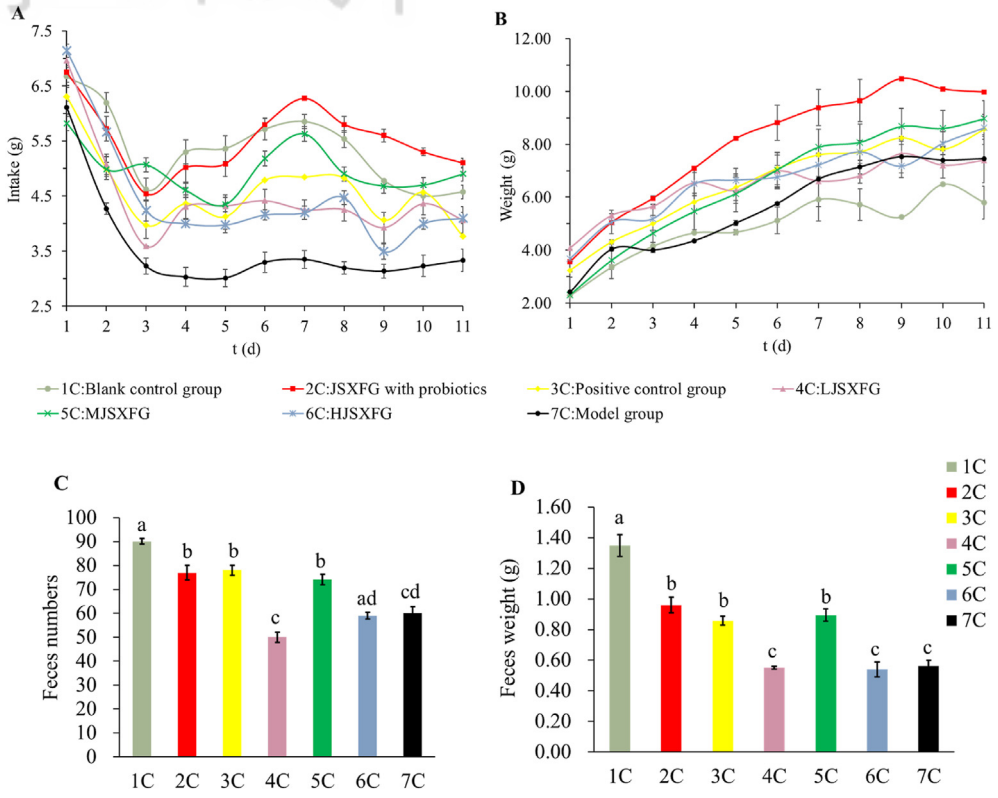

Fig. 3. Daily observations in mice. (A) Changes of food intake in rats at different periods. (B) Changes of body weight in rats at different periods. The values represent the means ± SD (n = 10/group). (C) Changes of feces number in rats at different groups. (D) Changes of feces weight in rats at different groups. The values represent the means ± SD (n = 10/group). a~g Different superscripts letters within a column indicate significant differences among formulations (p < 0.05).

GAPDH, AKT1, and TNF-α play the roles in JSX for FD by ELISA (Fig. 4). Compared with control group, the level of GAPDH, AKT1 and TNF-α were significantly increased in model group, while the content in 2C, 3C, and 5C group significantly decreased after treatment. Among them, the JSX granule containing prebiotic had the best effect. GAPDH is a key enzyme involved in glycolysis and a standardized internal reference for experimental procedures in Western blot. TNF-α is a multipotent cellular molecule that plays a central role in inflammation, apoptosis, and immune system development. Anti TNF-α analysis has been used successfully in the treatment of inflammatory bowel disease. AKT1 plays a key role in cell growth and apoptosis.

Table 6. Residual rate of gastric contents and small intestine propulsion rate in mice.

Groups	Intragastric residual rate (%)	Small intestine propulsion rate (%)
1C	58.29^{c}	65.77^{a}
2C	78.82^{b}	64.13^{a}
3C	70.82^{b}	65.00^{a}
4C	94.66^{a}	47.13^{b}
5C	80.07^{b}	67.35^{a}
6C	92.50^{a}	47.84^{b}
7C	92.23^{a}	45.75^{b}

Note:n = 10, a ~ g Different superscripts letters within a column indicate significant differences among formulations (p < 0.05).

3.4.3. Gut microbiota analysis

It necessary to reveal the effects of JSX on the richness, evenness, uniformity and diversity about the microbial community in the gut of FD mice. The samples were performed the analysis of dilution curve, rank-abundance curve, venn diagram, beta diversity and alpha diversity index. Venn diagram (Fig. 5A) illustrated the number of common and unique features between different groups. Compared with model group, control group and 2C group had larger horizontal axis curve range and smoother curve, indicating higher species richness and uniformity in control group and 2C (Fig. 5B). The results unmasked that the intestinal flora in 2C group was closer to control than other groups, which implied it would revitalize better (Fig. 5C). Compared with the control group, the intestinal flora relative abundance of the model group decreased such as *Coprococcus*, *Alistipes*,

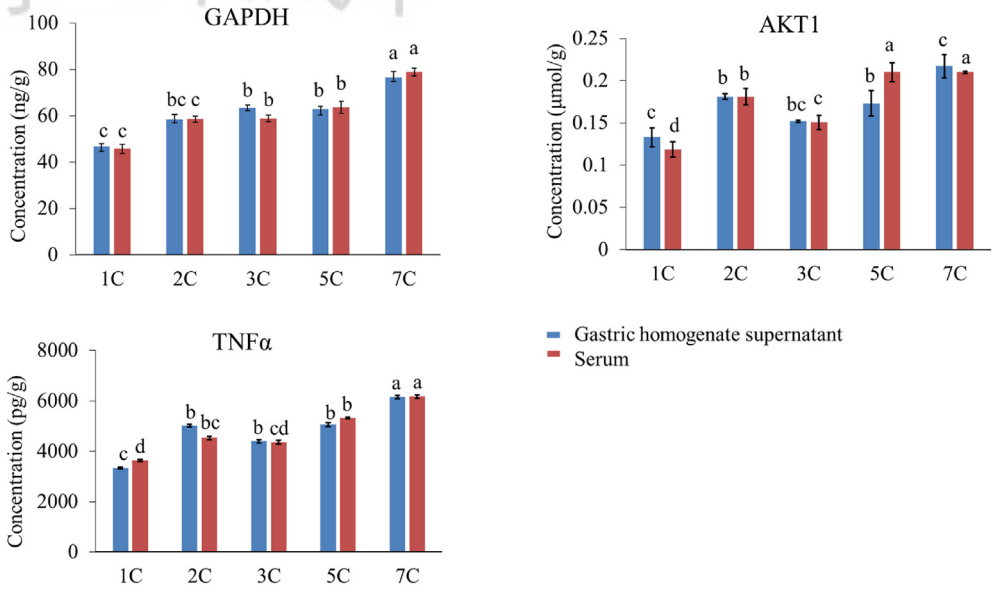

Fig. 4. The results of ELISA. The values represent the means ± SD (n = 10/group). a~g Different superscripts letters within a column indicate significant differences among formulations (p < 0.05).

Pseudomonas or increased such as *Allobaculum*, *Helicobacter*, *Sodalis*. After treatment, the number of beneficial bacteria increased in 2C group, including *Dorea*, *Prevotella*, *Odoribacter* and so on (Fig. 5D). The indexes of colony in model group were significantly lower than control group, such as *Chao1*, *Simpson*, *Shannon*, *Pielou_e*, *Observed_species*. And 2C group were closer to the control group (Fig. 5E).

4. Discussion

The study is aimed to identify the preparation method of JSX granule containing prebiotic, moreover, explore the targets and mechanisms of JSX for FD by computer technology and pharmacodynamic experiment. The conditions of the extraction and preparation were determined. Among this, we have confirmed a comprehensive scoring system for the JSX extraction process, which contains the paste rate and the content of chlorogenic acid. We have added prebiotic into Chinese medicine granule.

The computer technology was used to predict the targets and pathways of JSX for FD. The core targets were obtained by PPI, which provided the information for later pharmacodynamic experiments. The mechanisms of JSX for FD were related to three modules, including inflammatory response, gastrointestinal function and cell physiological activity, which reacted in the cycle of components-targets-pathways-disease.

This result demonstrates that suchilactone could tightly bind with the protein to relieve constipation [9,10], such as JUN. The 20-hexadecanoylingenol is positively associated with diarrhea controlling, especially IL-6. The flavonoid compound vitexin 7-glucoside interacts with part of receptors, and it could decrease inflammation in mice, may be related to the inactivation of p38, ERK1/2 and JNK signalling pathways [8,11]. Citric acid and chlorogenic acid are antibacterial agents and show anti-inflammatory function. They can maintain intestinal homeostasis by affecting the intestinal epithelial barrier, intestinal mucin expression, flora structure and immune cells [12]. In addition, the triterpenoid 12-oxoarundoin is an analog of arundoin, which could inhibit the proliferation of prostate cancer cells and induce apoptosis. In the current study, inhibition of MAPK pathway could increase intestinal moisture and promote intestinal peristalsis [9]. TNF and TNF signaling pathway are associated with inflammation [13]. HSP90AA1 is a protein-coding gene and overexpressed in colon cancers [14–16]. The research suggested that SRC may promote the occurrence of intestinal inflammation by mediating the release of inflammatory factors, inducing the homing, activation of inflammatory cells and angiogenesis [17]. Meanwhile, vascular endothelial growth factor A (VEGFA) contributes significantly in the pathogenesis of FD, and its overexpression can mediate inflammation and promote angiogenesis [18]. Moreover, EGFR, participate in receptor tyrosine-protein kinase, has the activity of tyrosine-protein kinase. A further investigation showed that SRC can increase VEGFA expression in a mechanism that implicates the EGFR signal pathway [19]. JUN participates in inflammation reaction through EGFR

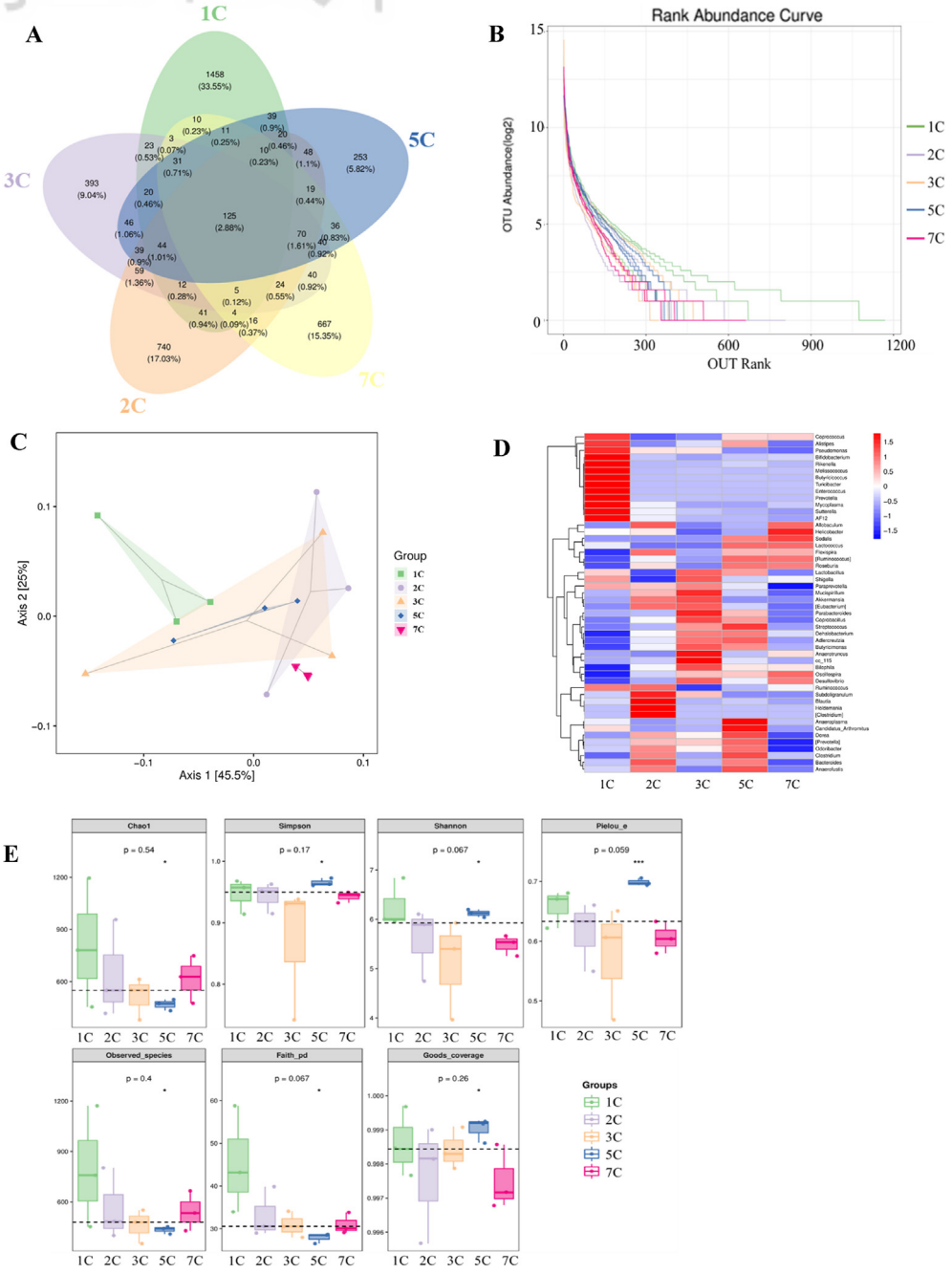

Fig. 5. The diversity analysis of gut microbiota analysis (n = 10/group). (A) Venn diagram. (B) Rank-abundance curve. (C). PCoA of beta diversity analysis. (D) Heatmap of dominant species. (E) Microbial diversity index.

signal pathway by regulating angiogenesis [20]. It is suggested that stimulation of JUN gene may consequently activate the expression of VEGFA [21]. Furthermore, AKT1 plays an important role in cell survival, growth and proliferation. It is controlled by PI3K directly through PI3K-Akt signaling pathway that participates in the release of inflammatory mediators and the proliferation of inflammatory cells. These results suggest that targets could produce a combination effect on inflammation reaction. The formula of JSX can exert the effect on inflammatory of FD by inhibiting the expression level of SRC and JUN and then regulating the negative activation of VEGFA. Protein phosphorylation modification is one of the classical ways to activate protein to participate in cell activities [22]. In the pathogenesis of FD, inflammatory cells will proliferate and differentiate on account of the damage of gut barrier function.

The animal model of FD was established with high-fat diet, and animal signs were observed during the modeling process. The experimental results displayed that the weight and food intake of mice recovered after administration. Among them, the effect of 2C group (containing prebiotic) was more prominent. ELISA test showed that GAPDH, AKT1 and TNF-α played the roles to reduce inflammatory response by downregulating its content. It validated the prediction of the network pharmacological. In recent years, more and more studies show the balance of intestinal flora is closely related to gastrointestinal function [23]. And regulating the intestinal flora and reconstructing the intestinal microecological balance play an important role in the treatment of FD. *Bacteroides* are the dominant bacteria in the human intestine, and the ratio of *Bacteroides* is related to body weight and inflammation [24]. The experiment results showed that the ratio of *Bacteroides* in the model group were remarkable reduced, while the phenomena were reversed after treatment. In the present study, compared with control mice, the beneficial bacteria in the intestinal flora were decrease but recovery after treatment, such as *Lactobacillus*, *Eubacterium*, *Bifidobacterium*, *Anaerofustis*, *Parabacteroides* and *Akkermansia*. *Allobaculum* and *Eubacterium* can produce short chain fatty acids, which can prevent inflammation, protect the intestinal barrier function, and regulate human metabolism and immunity and so on [25]. *Bifidobacterium* and *Lactobacillus*, meanwhile, can inhibit the growth of harmful bacteria in the human body [26,27], resist the infection of pathogenic bacteria, synthesize vitamins, and produce organic acids to stimulate intestinal peristalsis. *Parabacteroides* has the function of bile acid conversion, thereby improving lipid metabolism disorders [28]. *Christensenellaceae* is negatively related to metabolic diseases such as inflammation and fat deposition. Studies have revealed that the level of *Akkermansia* is inversely correlated with body weight [29], and *Gastranaerophilales* can produce various toxins. This explains that the mice lost weight after treatment compared to the model group. Compared with the control group, the ratio of *Ruminococcus* increase, while that of *Bifidobacterium* and *Turicibacter* reduced in FD induced obese mice. Compared with the model group, treatment groups increased the ratio of *Prevotella*, but reduced that of *Helicobacter* and *Ruminococcus*. It could be speculated that JSX meliorate the FD state and regulate the normal operation of physiological functions by increasing the species diversity of the intestinal flora, adjusting the dominant prebiotic, and changing the composition of the intestinal flora.

5. Conclusion

In conclusion, JSX extraction and granules were prepared. The effects of JSX on the components-targets-pathways-disease cycle of FD were studied through network pharmacology and molecular docking techniques. The target and mechanism were investigated by pharmacological experiments. The results showed that JSX granule containing prebiotic could regulate inflammatory response, gastrointestinal function, and improve the disordered intestinal flora. The research could provide theoretical basis for the development and application of JSX functional products.

Availability of data and materials

The data and materials generated or analyzed during this study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The experimental protocol was established according to the ethical guidelines and was approved by the Experimental Animal Ethical Committee of Shenyang Pharmaceutical University. Approval number is SYPH-IACUC-S2022-10.06-101.

Contributions of authors

Huimin Pei: Data analysis, Writing - original draft. Mengyuan Zhao: Data analysis, Writing - Review & Editing. Hongyue Wang: Investigation, Visualization. Xindi Zhang: Investigation, Visualization. Caihong Shi: Conceptualization, Project administration, Supervision. Xiangrong Zhang: Conceptualization, Project administration, Supervision.

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Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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