

Original Article

Shengjiang San modification promotes colonic mucosal repair in ulcerative colitis rats by inhibiting lauric acid-Shigella synergistic damage via PKC signaling pathway

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Abstract: Objectives: Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease. Recent research has focused on microbial dysbiosis within the gut environment. Although Shengjiang San modification ameliorates inflammation via activation of the PKC signaling pathway, the specific molecular mechanisms underlying its anti-inflammatory effects remain unclear. This study aimed to systematically investigate the anti-inflammatory mechanisms of Shengjiang San modification. Methods: UC models were established in 50 male SD rats using Dextran Sulfate Sodium Salt (DSS). Rats were divided into model group, mesalazine group, and Shengjiang San modification high/mid/low-dose groups. After 14 days of intervention, samples were collected. General condition, gross colon morphology, and histopathological changes were observed. Untargeted metabolomics was used to detect serum metabolites, 16S rRNA sequencing was performed to analyze gut microbiota, and combined multi-omics analysis was conducted. Results: Shengjiang San modification significantly improved colonic pathological injury and restored body weight. Metabolomics identified 18 aberrant metabolites, with 16 returning to near-normal levels after treatment, primarily involving the protein kinase C (PKC) signaling pathway. Gut microbiota analysis revealed decreased pathogenic bacteria and significantly increased beneficial bacteria. Notably, the lauric acid-Shigella correlation was disrupted. Compared to the control group, the high-dose group showed superior efficacy in restoring the Firmicutes/Bacteroidetes (F/B) ratio. Conclusions: Shengjiang San modification exerts anti-inflammatory effects and promotes colonic mucosal repair in UC rats by blocking lauric acid-Shigella synergistic damage via the PKC signaling pathway. The high-dose formulation demonstrated optimal therapeutic efficacy.

Keywords: Ulcerative colitis, Shengjiang San, intestinal flora, metabolomics, rats

Introduction

UC is a chronic refractory inflammatory bowel disease, with the chronic relapsing type being the most common [1]. The etiology of UC remains unclear, and its pathogenesis involves multiple factors. Long-term chronic inflammation significantly increases the risk of colitis-associated colorectal cancer, which is the most serious complication of UC [2]. Although Western medicine can induce and maintain remission, it has limitations including adverse reactions, unstable efficacy, and high costs. Therefore, many clinicians are urgently seeking new effective therapeutic drugs to reduce the incidence of UC [3].

In recent years, traditional Chinese medicine (TCM) has demonstrated advantages in the treatment of UC through multi-target, multi-pathway, and holistic regulatory effects. Due to its significant efficacy and individualized advantages, TCM has been widely applied, with relevant consensus gradually established and evidence-based medical evidence further supporting its effectiveness [4, 5]. UC can be classified under the categories of “diarrhea”, “chronic dysentery”, “intermittent dysentery”, or “intestinal sepsis” based on its clinical manifestations [6]. Shengjiang San modification consists of 11 herbs: Jiangcan (*Bombyx Batryticatus*), Chantui (*Periostracum Cicadae*), Jianghuang (*Curcuma longa*), Jiudahuang (pro-

cessed *Rheum palmatum*), *Fuling* (*Poria*), *Bai-shao* (*Paeonia lactiflora*), *Huangqi* (*Astragalus membranaceus*), *Hongjingtian* (*Rhodiola rosea*), *Diyu* (*Sanguisorba officinalis*), *Fengweicao* (*Pteris multifida*), and *Muxiang* (*Aucklandia lappa*) [7]. Based on our preliminary network pharmacology analysis, the top three effective components in Shengjiang San modification were apigenin, luteolin, and kaempferol. Although Shengjiang San modification has shown good therapeutic effects in the clinical treatment of UC, its mechanism of action remains unclear. In this study, Shengjiang San modification was used as an intervention in a DSS-induced UC rat model. General condition and pathological indicators were observed. Q-Orbitrap high-resolution liquid chromatography-mass spectrometry was employed for quality control analysis, combined with untargeted metabolomics and 16S rRNA gut microbiota sequencing, to systematically investigate how the active components of Shengjiang San modification influence metabolic regulatory mechanisms and gut microbiota in the treatment of UC. This study aims to provide scientific evidence and theoretical support for the use of TCM in the treatment of UC.

Materials and methods

Test drugs

Dextran sulfate sodium (500 g, Yeasen Biotechnology (Shanghai) Co., Ltd., batch number: 60316ES76), Modified Shengjiang San: *Bombyx Batryticatus* 12 g (batch number: 1050053), *Cicadae Periostracum* 6 g (batch number: 1041813), *Curcuma Longae Rhizoma* 12 g (batch number: 1040873), *Rhei Radix et Rhizoma* (wine-processed) 3 g (batch number: 1090813), *Poria* 20 g (batch number: 1080313), *Paeoniae Radix Alba* 20 g (batch number: 1094573), *Astragali Radix* 12 g (batch number: 1094023), *Rhodiola Crenulatae Radix et Rhizoma* 12 g (batch number: 1097283), *Sanguisorbae Radix* 15 g (batch number: 1037333), *Pteridis Multifidae Herba* 15 g (batch number: 0116503), *Aucklandiae Radix* 6 g (batch number: 1043103). The herbal formula granules were provided by Guangdong Yifang Pharmaceutical Co., Ltd. Mesalazine enteric-coated tablets (specification: 0.5 g/tablet), approved by National Medical Products Administration with standard number H2016-

3124, were manufactured by Shanghai Ethypharm Pharmaceutical Co., Ltd.

Animals

Sixty 8-week-old male SPF-grade Sprague-Dawley (SD) rats were purchased from SPF (Beijing) Biotechnology Co., Ltd. (animal production certificate number: SCXK (Jing) 2019-0010). The rats were housed in the Animal Experiment Center of Hebei University of Chinese Medicine, with room temperature maintained at 20-25°C and relative humidity of 40-70%. This animal experiment was approved by the Ethics Committee of Hebei University of Chinese Medicine (animal experiment ethics approval number: DWLL202208001), and all procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China.

Animal model establishment and related procedures

After one week of acclimatization, 60 rats were randomly divided into a blank group (10 rats) and a modeling group (50 rats) using a random number table method. Rats in the blank group were fed a normal diet. Rats in the modeling group were allowed free access to 2.5% DSS solution for 10 days, after which two rats were randomly selected for pathological examination of colon tissue. Both rats showed pathological changes including congestion, edema, erosion, and ulceration, indicating successful model establishment. The remaining rats were then randomly divided into five groups: Shengjiang San modification high-dose group (SJSJJH, 9 rats), Shengjiang San modification mid-dose group (SJSJJM, 9 rats), Shengjiang San modification low-dose group (SJSJJL, 9 rats), mesalazine group (5-ASA, 9 rats), and model group (DSS, 9 rats). The blank group (Ctrl) consisted of 10 rats.

The DSS group received intragastric administration of normal saline at a volume of 1 mL/100 g. The SJSJJH, SJSJJM, and SJSJJL groups were administered at doses of 55 g/kg/d, 27.5 g/kg/d, and 13.75 g/kg/d (crude drug), respectively. The 5-ASA group was given mesalazine suspension at a dose of 0.5 g/kg/d by gavage. All treatments were administered

Table 1. Gradient elution program parameters for binary solvent system

Time (min)	Acetonitrile (0.1% formic acid) (%)	5 mmol/L Ammonium Acetate in Water (0.1% formic acid) (%)
0-1	85	15
1-9	85	15
9-12	15	85
12-13.5	15	85
13.5-14	85	15
14-20	85	15

once daily for 14 consecutive days, and DAI scores were recorded according to standard criteria.

After two weeks of treatment, the rats were fasted with free access to water for 24 hours. The animals were placed into the induction chamber, and isoflurane gas was delivered at a concentration of 3-3.5%. Then, 1% sodium pentobarbital (50 mg/kg) was intraperitoneally injected, and 3-5 mL of blood was collected from the heart. The blood samples were centrifuged at 3000 r/min for 15 minutes at 4°C, and the separated supernatants were stored in a -80°C refrigerator (Thermo Fisher Scientific). The rats were laparotomized, and colon tissues from 8 cm proximal to the anus to the ileocecal junction were collected, rinsed, and fixed in 4% paraformaldehyde. After 24 hours, the tissues were subjected to routine paraffin embedding and staining. All experimental rats were subjected to harmless disposal.

The colon length, mucosal folds, color, and the presence of congestion, erosion, or masses were observed. Paraffin-embedded sections of rat colon mucosal tissue were placed on glass slides and incubated in a 60°C oven for 3 hours. After drying, the sections were sequentially placed in xylene I and II for dewaxing, 10 min each, and then rehydrated through graded ethanol (95%, 85%, and 75%) for 2 min each, followed by immersion in distilled water for 2 min. After dewaxing, the sections were stained with hematoxylin and eosin staining solution. Following dehydration, clearing, and mounting, the staining results were observed under a microscope (OLYMPUS), and images were captured.

Statistical analyses were performed using IBM SPSS 20.0 software. Data are expressed as mean $\pm$ standard deviation ($\bar{x} \pm sd$). For normal-

ly distributed data, one-way analysis of variance (ANOVA) was used for inter-group comparisons, with Least Significant Difference (LSD) test for pairwise comparisons. For non-normally distributed data, rank sum tests were used for inter-group comparisons, with Kruskal-Wallis test for pairwise comparisons. $P < 0.05$ was considered statistically significant.

Metabolomics used to detect serum metabolic markers in rats

Serum sample preparation: An appropriate amount of serum was placed in a 2 mL centrifuge tube (Servicebio, Wuhan), and 400 μ L of methanol solution (stored at -20°C) was added, followed by vortexing for 1 min. The sample was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was transferred to a new 2 mL centrifuge tube and concentrated to dryness. Then, 150 μ L of 2-chloro-L-phenylalanine (4 ppm) solution prepared with 80% methanol in water (stored at 4°C) was added. After filtration, the supernatant was transferred to a sample vial for LC-MS analysis.

Chromatographic conditions: A Thermo Vanquish ultra-high performance liquid chromatography system (Thermo Fisher Scientific, USA) was used, equipped with an ACQUITY UPLC HSS T3 column (2.1 $\times$ 150 mm, 1.8 μ m) (Waters, Milford, MA, USA). The flow rate was 0.25 mL/min, the column temperature was 40°C, and the injection volume was 2 μ L. The gradient elution conditions are shown in **Table 1**.

Mass spectrometry conditions: A Thermo Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific, USA) equipped with an electrospray ionization (ESI) source was used. The spray voltages were set at 3.50 kV for positive ion mode and -2.50 kV for negative ion mode. The capillary temperature was 325°C, the sheath gas pressure was 30 arb, and the auxiliary gas pressure was 10 arb.

Data processing and analysis: The raw mass spectrometry data files were converted to mzXML format using the MSConvert tool in the Proteowizard software package (v3.0.8789). Data processing was performed using the XCMS software package to obtain a quantita-

tive feature list, with parameters set as follows: bw = 2, ppm = 15, peakwidth = c(5,30), mzwid = 0.015, mzdiff = 0.01, method = "centWave". Metabolite identification was carried out using the HMDB, MassBank, LipidMaps, mzCloud, KEGG, and an in-house database, with the parameter set to ppm < 30 ppm. Data correction was performed using the LOESS signal correction method to eliminate systematic errors, and features with relative standard deviation (RSD) > 30% in quality control (QC) samples were filtered out. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed on the sample data using the R package Ropls. Permutation tests were applied. Statistical analysis was conducted to calculate *P*-values, variable importance in projection (VIP) values derived from OPLS-DA, and fold changes, which assisted in the screening of potential metabolite markers. Metabolites were considered statistically significant when *P* value < 0.05 and VIP value > 1. All analyses were performed using GraphPad Prism 9 software. Continuous variables following normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s.d.$). Inter-group comparisons were conducted using one-way analysis of variance (ANOVA), with *P* < 0.05 considered statistically significant.

16S rRNA analysis of the gut microbiome

Sample collection and processing: At the end of the second week of treatment, fecal samples were collected from the rats. Each rat was gently lifted by the tail to stimulate defecation. After defecation, the feces were promptly placed into sterile centrifuge tubes, with at least three fecal pellets collected from each rat. The tubes were then frozen in liquid nitrogen and subsequently transferred to a -80°C refrigerator for storage until further use.

Total DNA extraction: DNA was extracted from the fecal samples of UC model rats using a stool DNA kit. DNA concentration was determined using a Nanodrop spectrophotometer, and DNA quality was assessed by 1.2% agarose gel electrophoresis.

PCR amplification of the target fragment: Expansion parameters: Initial denaturation 98°C 2 min, denaturation 98°C 15 s, annealing

55°C 30 s, extension 72°C 30 s, final extension 72°C 5 min, 10°C hold. 25-30 Cycles.

Quantification of PCR products: A microplate reader and the Quant-iT PicoGreen dsDNA Assay Kit were used for fluorescence quantification of the PCR amplification products. Based on the results, the samples were mixed in proportion.

Mixing and purification of PCR products: The PCR products mixed in equal density ratios were purified using an AxyPrep DNA Gel Extraction Kit.

Library preparation and sequencing: Library preparation was performed using the TruSeq Nano DNA LT Library Prep Kit (Illumina). Sequencing was conducted on the Illumina MiSeq/NovaSeq platform using paired-end sequencing of community DNA fragments.

Information analysis process: The raw sequencing data were preliminarily screened, and the libraries and samples were demultiplexed with barcode sequences removed. Sequence denoising and OTU clustering were then performed to display the taxonomic composition at different levels. Alpha diversity was evaluated, and rarefaction curves were used to assess sequencing depth. Further analyses were conducted to evaluate beta diversity, statistical significance, and differences in species abundance among groups to identify signature species. Finally, an association network was constructed to identify key species.

Statistical methods: Statistical analysis was performed using GraphPad Prism 9 software. One-way ANOVA was used for normally distributed continuous data, which were expressed as mean ± standard deviation ($\bar{x} \pm s.d.$), to determine changes in markers and gut microbiota abundance among groups. A significance level of *P* < 0.05 was considered statistically significant.

Results

Shengjiang San modification prevents mortality and improves survival rate in UC rats

During the DSS modeling period, two rats from the model group died, likely due to gavage administration errors or DSS intolerance. On day 14, pathological examination of colon tis-

Table 2. Effect of Shengjiang San modification on DAI scores in UC rats ($\bar{x} \pm \text{sd}$)

Group	N	DAI
Ctrl	10	$0.10 \pm 0.16^*$
DSS	9	3.43 ± 0.39
5-ASA	9	$0.97 \pm 0.64^*$
SJSJJH	9	$1.30 \pm 0.66^*$
SJSJJM	9	$1.50 \pm 0.76^*$
SJSJL	9	$1.50 \pm 0.76^{*,\Delta}$

Note: Compared with the DSS group, $^*P < 0.05$; compared with the 5-ASA group, $^{\Delta}P < 0.05$.

sues from two model group rats confirmed successful model establishment with congestion, edema, erosion, and ulceration. Post-modeling, rats were randomized into five groups ($n = 9$ per group): DSS, 5-ASA, SJSJJH, SJSJJM, and SJSJL. No deaths occurred during the 14-day treatment period.

Shengjiang San modification improves general clinical condition and restores colon length

Rats in the Ctrl group displayed normal food intake, responsive behavior, good mental status, smooth fur, normal defecation, and gradually increased body weight with normal colon morphology. In contrast, DSS group rats exhibited poor mental status, dull fur, reduced activity and food intake, weight loss, increased defecation frequency, loose stools around the anus, and mucoid bloody stools in some cases. Colonic tissue in the DSS group was tortuous with markedly dilated intestinal lumen, rough mucosa, congestion, edema, and significant shortening of colon length. Treatment groups showed varying degrees of recovery in all parameters. The 5-ASA and SJSJJH groups demonstrated the most significant improvements in body weight and colon length restoration (Table 2; Figure 1A, 1E).

Shengjiang San modification restores colonic mucosal integrity and reduces inflammatory infiltration

In the Ctrl group, the colonic mucosa epithelium was intact with normal morphology and distribution of goblet cells and no inflammatory cell infiltration. The DSS group exhibited colonic mucosal epithelial defects (red arrows), reduced goblet cells, and abundant lymphocyte infiltration (black arrows). The 5-ASA and

SJSJJH groups showed almost complete mucosal epithelial restoration, relatively regular glandular arrangement, increased goblet cell numbers, and only mild inflammatory cell infiltration. The SJSJJM group displayed mild crypt structure destruction, restored goblet cell numbers, and scattered inflammatory cells. The SJSJL group still exhibited multiple mucosal interruptions, severe crypt destruction, marked decreases in goblet cells, and extensive lymphocyte infiltration (Figure 1B-D).

Shengjiang San modulation reverses 18 differential metabolites in UC rats

PCA of overall samples: Principal component analysis generated new characteristic variables by linearly combining metabolite variables to classify data and remove samples with poor repeatability. The R^2X value (> 0.5) was acceptable for model interpretability. The PCA score plots demonstrated distinct clustering between Ctrl and DSS groups under both positive and negative ion modes, indicating significant differences in serum metabolites (Figure 2A).

PLS-DA and OPLS-DA analysis: PLS-DA analysis revealed clear separation between the DSS group and other groups under both ion modes, while traditional Chinese medicine groups clustered relatively closely, suggesting similar metabolic changes among SJSJJH, SJSJJM, and SJSJL groups (Figure 2B). OPLS-DA further reduced model complexity while maintaining predictive ability. Permutation tests confirmed that the Q^2 values were below the original values, indicating no overfitting and good model stability (Figure 2C).

Differential metabolite screening: Differential metabolites were identified with $\text{VIP} > 1$ (OPLS-DA) and $P < 0.05$ (Student's t-test). Eighteen differential metabolites were reversed after Shengjiang San modification treatment: 1-methylindole-3-carboxaldehyde, HC toxin, 2,4-dinitrophenol, 6-methylmercaptopyrine, 8-hydroxyquinoline, aspartame, β -asarone, buprenorphine, lauric acid, glycerophosphocholine, L-erythrose, L-gulose, N-acetylleucine, O-phosphoethanolamine, pseudouridine, pyridaben, nitrazepam, and tryptamine. Lauric acid and tryptamine exhibited anti-inflammatory effects and functions in colonic mucosal barrier repair (Figure 2D-G).

SJS modification blocks LA-Shigella via PKC in UC

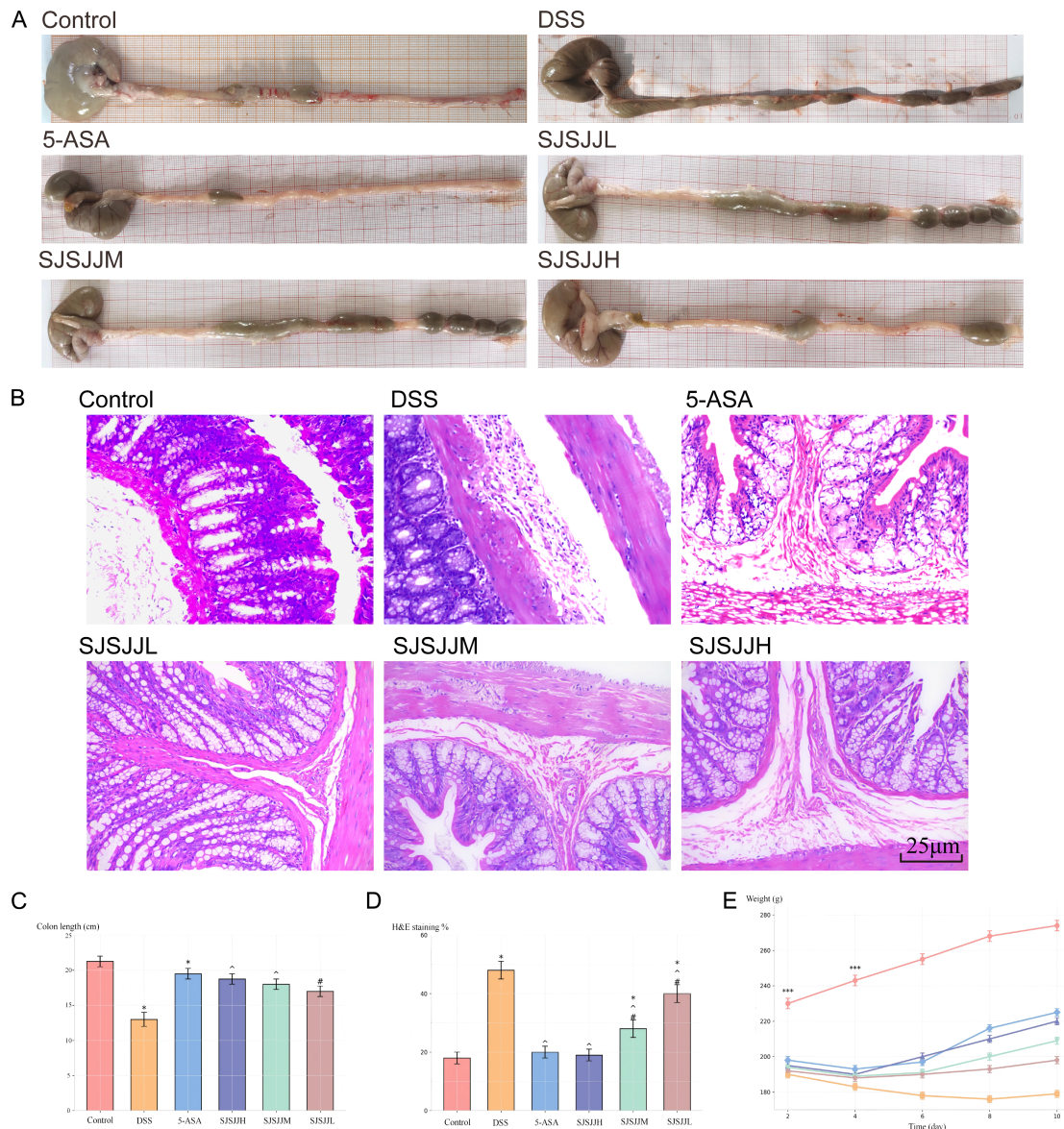


Figure 1. Shengjiang San modification attenuates body weight loss, restores colon morphology, and ameliorates mucosal damage in DSS-induced colitis rats. (A) Representative macroscopic images of colon tissues from each experimental group. Scale bar = 1 cm. (B) Representative hematoxylin and eosin (H&E) staining images of colonic sections showing histopathological changes (magnification: 400 $\times$). Scale bar = 25 μ m. (C) Colon length quantification. Data are presented as mean $\pm$ SD (n = 9 per group). (D) Histopathological score based on H&E staining. Data are presented as mean $\pm$ SD (n = 9 per group). (E) Body weight changes over the experimental period. Data are presented as mean $\pm$ SD (n = 9 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test (C, D), and two-way ANOVA followed by Bonferroni's post hoc test (E). *P < 0.05, **P < 0.01, ***P < 0.001 vs. Control; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. DSS.

Shengjiang San modulation normalizes gut microbiota diversity and composition

Microbial community structure at different taxonomic levels: Stacked bar charts of species relative abundance were generated at phylum, class, order, family, and genus levels. The top

20 most abundant species were selected from each group (Figure 3G, 3H).

Alpha diversity: Alpha diversity reflects species evenness and richness within samples, including Shannon, ACE, and Chao1 indices. Chao1 and Observed species indices demonstrated

SJS modification blocks LA-Shigella via PKC in UC

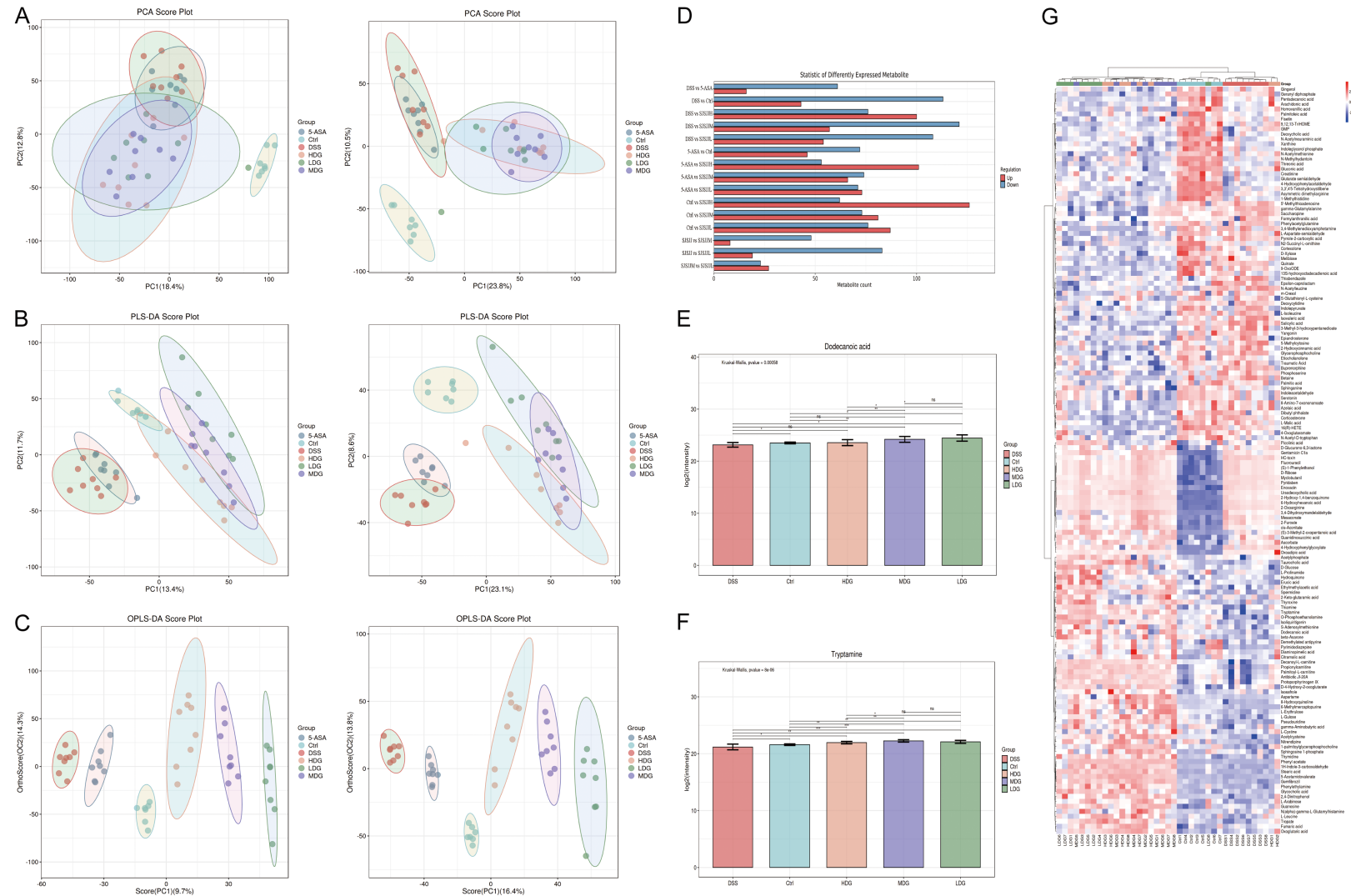


Figure 2. Multivariate analysis score plots in positive and negative ion modes. This picture shows metabolic pattern separation among Control, DSS, 5-ASA, SJSJH, SJSJM, and SJSJL groups. Each point represents an individual sample, with ellipses indicating 95% confidence intervals. (A) PCA score plots. Unsupervised dimensionality reduction was applied to evaluate the overall distribution and natural clustering tendency of the samples. The values in parentheses for PC1 and PC2 represent the variance explained by each principal component. (B) PLS-DA score plots. Supervised modeling was utilized to maximize the separation among the different groups, revealing the metabolic profile differences between groups. (C) OPLS-DA score plots. This model further filters out orthogonal variables (noise) irrelevant to

group classification, thereby enhancing the distinct separation between groups. The x-axis represents the predictive principal component (Score [PC1]), while the y-axis represents the orthogonal component (OrthogonalScore [PC2]). The ellipses surrounding the data points indicate the 95% confidence intervals. (D) Statistics of differentially expressed metabolites across pairwise comparisons. Up-regulated metabolites are shown in red; down-regulated metabolites are shown in blue. (E, F) Box plots showing the relative abundance of representative differential metabolites, dodecanoic acid (E) and tryptamine (F), across experimental groups. Data are presented as mean $\pm$ SD ($n = 9$ per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. DSS; ns, not significant. (G) Hierarchical clustering heatmap of differentially expressed metabolites. Rows represent individual metabolites; columns represent individual samples. Red indicates high relative abundance; blue indicates low relative abundance.

that the DSS group median was significantly higher than other groups, indicating increased species richness in UC model rats. Treatment with Shengjiang San modification and mesalazine reduced the median values (**Figure 3A, 3B**).

Beta diversity: PCA, PCoA, and NMDS analyses showed that the DSS group (except one sample) was distributed on the right side of the PC1 axis, clearly separated from other groups. The Ctrl group and treatment groups were not obviously distinguishable. PCoA revealed that the DSS group clustered together and separated from other groups, consistent with PCA results. NMDS analysis, which better reflects non-linear ecological data structures, yielded consistent findings (**Figure 3C-E**).

Shengjiang San modulation reverses DSS-induced gut microbiota dysbiosis

LEfSe analysis identified communities or species with significant differences between Ctrl and DSS groups. In the DSS group, abundances of *Bacteroides*, *Prevotella*, *Allobaculum*, *Bifidobacterium*, *Olsenella*, *Butyrivibrio*, *Parabacteroides*, *Barnesiella*, *Shigella*, *Alistipes*, *Sutterella*, and *Helicobacter* were significantly increased. Treatment restored abundances of some of these bacterial groups (**Figure 3I**).

Genus-level analysis showed genera that were significantly increased in the DSS group but decreased in treatment groups (**Figure 3J**), and genera that were decreased in DSS but increased in treatment groups (**Figure 3K**).

Shengjiang San modulation restores tryptamine-akermansia axis and inhibits lauric acid-Shigella module

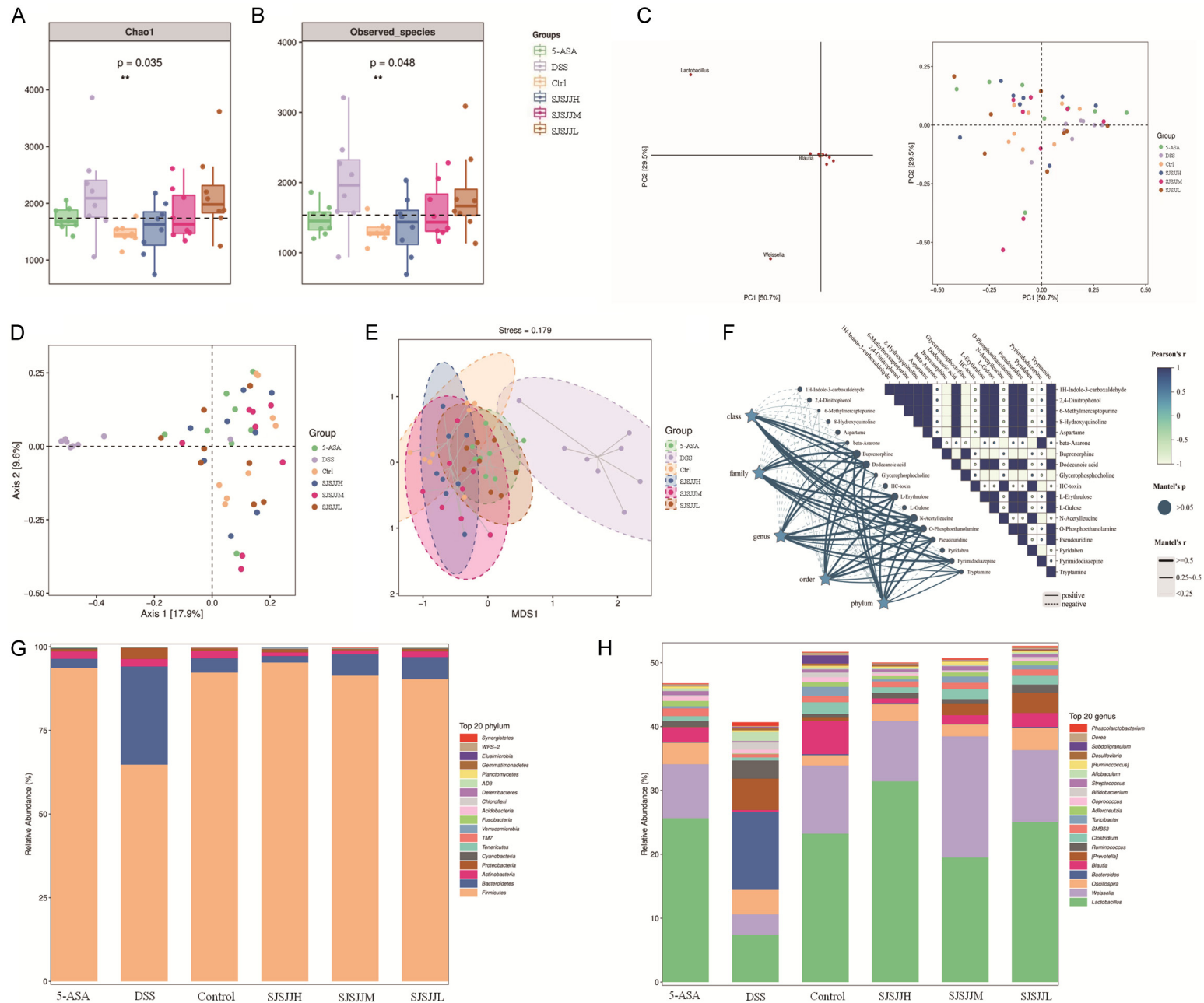
Spearman correlation analysis revealed a significant positive correlation between lauric acid and L-erythrulose, while glycerophosphocholine and tryptamine showed significant negative correlation. Buprenorphine exhibited high correlations with microbiota at phylum and class levels ($r > 0.97$), though P -values were not significant, possibly due to insufficient sample size. Aspartame was significantly positively correlated with Firmicutes (**Figure 3F**). Comparative analysis showed that the DSS group was characterized by enrichment of pathogenic bacteria such as Enterobacteriaceae, along with significantly elevated lipopolysaccharide and prostaglandin E2 levels ($P < 0.01$). Pathogenic bacteria and inflammatory mediators showed strong positive correlation, suggesting that microbiota dysbiosis drives inflammatory metabolic imbalance. In the Ctrl group, a balanced Firmicutes/Bacteroidetes ratio ($F/B = 1.2$) was maintained with stable short-chain fatty acid levels. The high-dose group exhibited intervention-specific recovery, with Akkermansia relative abundance increased 4.8-fold compared with DSS group ($P = 0.004$), concomitant with increases in tryptamine and butyrate (1.9-fold and 2.5-fold, respectively).

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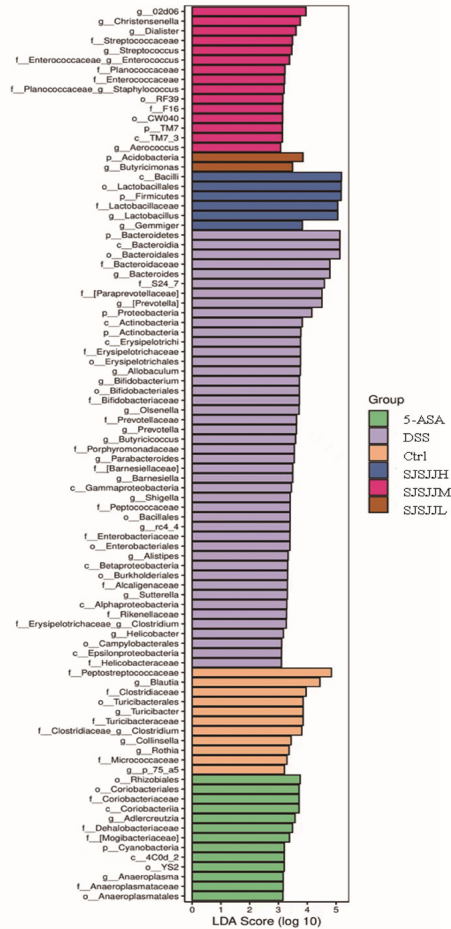
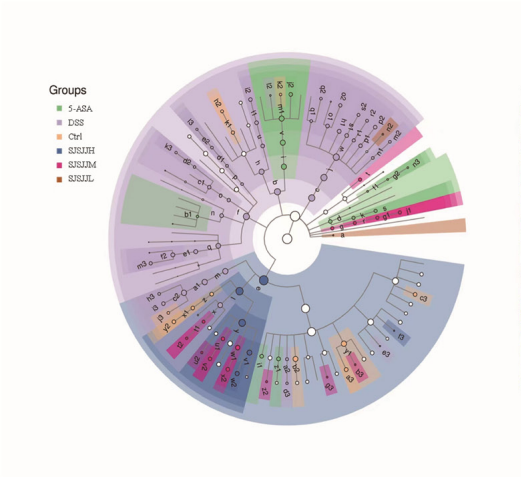
Discussion

According to Wen Bing Tiao Bian (Treatise on Febrile Diseases), the section on "Lower Burner Disorders" provides a theoretical basis for understanding ulcerative colitis (UC) etiology and treatment [8]. Shengjiang San originated from Yang Lishan's Shang Han Wen Yi Tiao Bian (Qing Dynasty) and comprises four herbs: Jiangcan (*Bombyx Batryticatus*), Chantui (*Periostracum Cicadae*), Jianghuang (*Curcuma longa*), and Dahuang (*Rheum palmatum*). Jiangcan and Chantui ascend the clear and relieve the exterior, while Jianghuang and Dahuang descend the turbid and purge the heat. This combination balances ascending and descending functions, harmonizing the interior and exterior, hence the name "Shengjiang San" (Ascending and Descending Powder). Originally formulated

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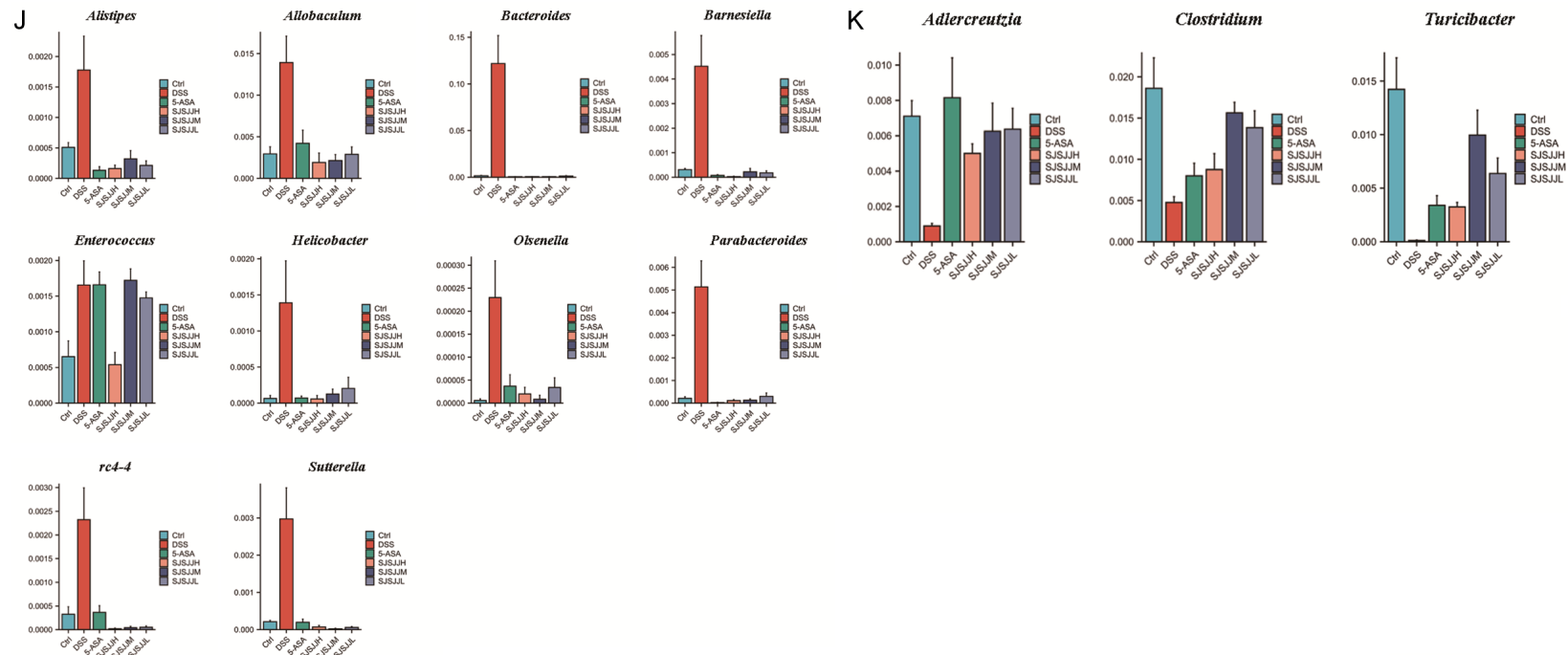


Figure 3. Gut microbiota composition and diversity analysis. (A, B) Alpha diversity indices (Chao1 and Observed species) of gut microbiota across experimental groups. Data are presented as box plots with individual data points ($n = 9$ per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. $*P < 0.05$, $**P < 0.01$ vs. indicated group. (C-E) Principal Coordinate Analysis (PCoA) plots based on Bray-Curtis distance (C), Weighted UniFrac distance (D), and Non-metric Multidimensional Scaling (NMDS) analysis (E) showing beta diversity patterns among groups. Each point represents an individual sample, with ellipses indicating 95% confidence intervals. (F) Correlation network analysis between bacterial taxa at different levels (class, family, order, phylum). Edge width and color intensity represent the strength of correlation (Pearson's r), with blue indicating positive correlation and red indicating negative correlation. Node size represents relative abundance. (G, H) Relative abundance of top 20 phyla (G) and top 20 genera (H) presented as stacked bar charts. (I) Left panel: Taxonomic cladogram generated by Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis showing differentially abundant taxa across groups. Circles represent taxa from phylum (innermost) to species (outermost) levels; node size indicates relative abundance, and color indicates the group with highest abundance. Right panel: LDA scores (log 10) of differentially abundant taxa with LDA score > 2.0 . (J, K) Relative abundance of specific bacterial genera showing significant alterations among groups. Data are presented as mean $\pm$ SD ($n = 9$ per group).

for warm diseases, it regulates qi mechanism of the exterior, interior, and triple energizer, relieving stagnation and clearing heat [9].

The modified formula contains 11 herbs: Jiangcan, Chantui, Jianghuang, Jiudahuang (processed *Rheum palmatum*), Fuling (*Poria*), Baishao (*Paeonia lactiflora*), Huangqi (*Astragalus membranaceus*), Hongjingtian (*Rhodiola rosea*), Diyu (*Sanguisorba officinalis*), Fengweicao (*Pteris multifida*), and Muxiang (*Aucklandia lappa*). The formula combines ascending and descending actions, astringent and dispersing effects, cold and warm properties, tonifying and purging approaches, and simultaneous regulation of qi and blood. It eliminates turbid toxins, harmonizes qi and blood, and restores intestinal health.

Modern pharmacological studies suggest that Shengjiang San modification acts through multiple targets and pathways, including regulation of NF- κ B signaling to inhibit excessive fibrosis, inflammatory responses, and immune reactions [10]. The PKC signaling pathway plays a broad role in UC treatment by various drug components [11-13]. Our previous research demonstrated that Shengjiang San modification downregulates CXC chemokine ligand 5 (CXCL5) expression, inhibits CXC chemokine receptor 4 (CXCR4) activity, blocks PKC signaling pathway activation, and suppresses inflammation [7]. The metabolite-microbiota interplay in our Shengjiang San modification model operates through specific microbial-metabolite signaling axes. We found that restored *Akkermansia* abundance correlated with elevated tryptamine, while the lauric acid-*Escherichia-Shigella* association was disrupted after treatment. This aligns with multi-omics evidence that UC disease activity involves characteristic taxonomic, functional, and biochemical shifts where integrative analysis identified microbial, biochemical, and host factors central to this dysregulation [14]. The microbial source of these metabolites is critical - Mills et al. linked *Bacteroides vulgatus* protease overabundance to barrier dysfunction through multi-omics integration, demonstrating that these results, stemming from a multi-omics approach, improve understanding of functional microbiota alterations that drive UC [15]. Similarly, Gonzalez et al. showed that colonic Crohn's disease (CD) and UC share strikingly similar fea-

ture enrichments including *B. vulgatus* involvement when analyzed by metabolomics and metaproteomics [16]. The therapeutic relevance is underscored by fecal microbiota transplantation (FMT) studies: Nusbaum et al. observed that the mean relative abundance of bacteria in the class *Clostridia* shifted toward donor levels alongside metabolomic shifts, with butyric acid lower at baseline and increased toward donor levels after FMT [17]. Kurilshikov et al. further suggested that the microbiome might have causal effects in ulcerative colitis [18], supporting that microbiota-driven metabolite changes are mechanistically relevant. These metabolite changes connect to PKC signaling through tryptamine, which activates PKC signaling via tryptamine receptors, influencing intracellular signal transduction [7]. PKC thus serves as the intracellular convergence point for microbial metabolite signals, transducing both the positive effects of *Akkermansia*-derived tryptamine and the relief from pathogen-associated metabolic disruption.

Herbs in Shengjiang San modification such as Fengweicao, Diyu, Huangqi, and Baishao contain flavonoids. Our previous in vitro study identified the top three effective chemical components by degree value: apigenin, luteolin, and kaempferol. These flavonoids possess antioxidant and anti-inflammatory activities. Apigenin alleviates DSS-induced UC by improving intestinal mucosal immune, mechanical, and biological barrier function [19]. Luteolin ameliorates colitis by inhibiting pathogenic bacteria growth (e.g., *Shigella*) and increasing beneficial bacteria abundance (including *Muribaculaceae*, *Rikenella*, and *Prevotellaceae*), thereby suppressing colonic and systemic inflammatory responses and promoting colonic barrier repair [20]. Comparative studies showed that luteolin had particularly significant effects on UC-related somatic symptoms, while apigenin had limited microbiota effects, probably due to mesentery accumulation from intraperitoneal administration [21]. Kaempferol remodels the gut microbiome by increasing the F/B ratio, enhancing probiotic LDA scores, and reducing Proteobacteria abundance in DSS-challenged mice [22]. Furthermore, metabolomic analyses indicate that kaempferol reaches higher levels in intestinal contents, potentially explaining its pronounced effects on intestinal metabolism

[23]. These three compounds represent the main active constituents of Shengjiang San modification in UC treatment.

Our study demonstrated that Shengjiang San modification intervention restored abundances of *Bacteroides*, *Allobaculum*, *Olsenella*, and *Parabacteroides*, and reversed 18 differential metabolites including 1-methylindole-3-carboxaldehyde, tryptamine, lauric acid, and HC toxin. Strong positive correlation between tryptamine and *Akkermansia* was observed. In the SJSJJH group, *Akkermansia* abundance increased 4.8-fold, promoting tryptamine synthesis and synergistically repairing the intestinal barrier.

Tryptamine activates PKC signaling via tryptamine receptors, influencing intracellular signal transduction and regulating neurotransmitter release, cell proliferation, and differentiation [24]. Due to its involvement in multiple signal transduction systems controlling cell proliferation, differentiation, survival, invasion, migration, and apoptosis, PKC is considered an important therapeutic target for various diseases including infections, cancer, neurological disorders, and cardiovascular diseases [25, 26].

In the DSS group, lauric acid showed positive correlation with *Escherichia-Shigella*, which was disrupted after Shengjiang San modification treatment. Proteobacteria abundance decreased by 37%, and plasma LPS levels were significantly reduced ($P < 0.01$). Gut microbiota dysbiosis, particularly Proteobacteria-dominated communities, may predispose genetically susceptible individuals to colitis [27]. Previous studies demonstrated that Gancao Xiexin Decoction reduced *Escherichia-Shigella* abundance in UC [28], while Banxia Xiexin Decoction increased *Akkermansia* abundance in mice [29], consistent with our findings.

Increased *Akkermansia* abundance elevates intestinal tryptamine levels. By activating the AhR pathway, these metabolites play key roles in protecting the intestinal mucosal barrier, exerting anti-inflammatory and neuroprotective effects. Therefore, *Akkermansia* is considered a potential probiotic for improving metabolic and neurological diseases through tryptamine metabolism regulation [30-32]. Lauric acid exerts potent inhibitory effects against *Clostridium difficile* (also belonging to Proteobacteria) in vitro [33]. *Clostridium difficile* infec-

tion (CDI) is particularly challenging in clinical settings, especially in UC patients who face higher risk for CDI with more severe disease courses and higher recurrence rates [34].

In this study, Shengjiang San modification increased *Akkermansia* abundance and activated tryptamine, consistent with previous studies. The intestinal mucosal barrier integrity depends on mitochondrial function in intestinal epithelial cells, which generate energy through oxidative phosphorylation (OXPHOS). The effects of saturated fatty acids on mitochondrial function and intestinal barrier vary with chain length [35]. Lauric acid, as a medium-chain fatty acid and mitochondrial substrate with anti-inflammatory properties, improves mitochondrial function in intestinal epithelial cells. Studies show that lauric acid significantly upregulates tight junction proteins (ZO-1, Occludin, Claudin-1) and mucin (MUC2) expression in the colon, repairing intestinal barrier damage. It regulates gut microbiota structure by increasing *Bacteroides* abundance, reducing pathogenic bacteria, and inhibiting inflammatory factor expression (TNF- α , IL-6) [36]. At high concentrations, lauric acid induces reduced cell viability, lipid accumulation, inhibited proliferation, and apoptosis in porcine intestinal epithelial cells [37, 38].

In the DSS group, lauric acid may disrupt intestinal epithelial barrier function under high concentration or specific conditions, providing favorable environment for *Shigella* colonization and proliferation. *Shigella* rapidly proliferates by exploiting the damaged colonic mucosal barrier, leading to severe mucosal damage and inflammatory responses. Following Shengjiang San modification treatment, the lauric acid-*Shigella* module was disrupted, consistent with our hypothesis. This provides supporting evidence for the therapeutic mechanism of Shengjiang San modification in UC, involving disruption of the lauric acid-*Shigella* module. Apigenin, luteolin, and kaempferol represent the top three effective chemical components of Shengjiang San modification. Additionally, tryptamine activates the PKC signaling pathway through tryptamine receptors.

Conclusions

Through multi-omics combined analysis, our study suggests that Shengjiang San modification may exert anti-inflammatory effects and

promote colonic mucosal repair in rats with ulcerative colitis by blocking the synergistic damage between lauric acid and Shigella via the PKC signaling pathway. Among the different dosage formulations, the high-dose formulation demonstrated optimal therapeutic efficacy.

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Disclosure of conflict of interest

None.

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