Original Article

DOI: 10.5582/ddt.2025.01120

Xuefu Zhuyu Capsule alleviates depression in post-stroke depression model rats *via* modulation of the gut microbiota–gut–brain axis

Bowen Jin[1], Yuqian Wang[1], Zhaowei Zhang[1], Xuejin Fan[1], Yanjun Zhang[1,2,3,*], Pengwei Zhuang[1,2,3,*]

[1] National Key Laboratory of Chinese Medicine Modernization, Haihe Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China;
[2] First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China;
[3] National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China.

SUMMARY: Xuefu Zhuyu Capsule (XFZY) demonstrated potential in alleviating post-stroke depression (PSD), a condition whose underlying mechanisms may involve the gut–brain axis. This study aimed to explore the therapeutic effects of XFZY on PSD and its possible modulation of the gut microbiota–gut–brain axis in a rat model. Wistar rats were randomly assigned to sham, PSD, three XFZY dose (0.216, 0.432, 0.864 g/kg), and fluoxetine (1.80 mg/kg) groups (n = 12 per group). The PSD model was established using transient middle cerebral artery occlusion (t-MCAO) combined with chronic unpredictable mild stress (CUMS), followed by 28 days of XFZY administration. In a separate experiment, gut microbiota was depleted *via* antibiotic cocktails, with rats divided into sham, PSD, XFZY medium Dose (XFM), pseudo-germ-free (PGF) and PGF + XFM (PGFX) groups. Behavioral tests indicated that XFZY ameliorated depressive-like behaviors, with the medium dose (0.432 g/kg) showing the most significant effect. Histological analysis using hematoxylin and eosin (H&E) and Nissl staining revealed that XFZY alleviated colonic and neuronal damage. Furthermore, 16S rRNA sequencing and gas chromatography revealed that XFZY modulated gut microbiota composition, increased species richness, and elevated levels of short-chain fatty acids such as acetic acid, propionic acid, and butyric acid. Enzyme-Linked Immunosorbent Assay (ELISA) results showed that XFZY reduced pro-inflammatory cytokines — interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), while immunohistochemistry indicated enhanced intestinal barrier function and reduced neuroinflammation. Furthermore, after depletion of gut microbiota using antibiotic cocktails, these therapeutic effects of XFZY were abolished. In summary, XFZY may alleviate PSD by modulating the gut microbiota and regulating the gut–brain axis, offering a promising direction for future therapeutic research.

Keywords: Xuefu Zhuyu, post-stroke depression, gut-brain axis, intestinal flora, short-chain fatty acids, neuroinflammatory

1. Introduction

Post-stroke depression (PSD) is a mood disorder marked by symptoms such as low mood, decreased interest in activities, difficulty concentrating, feelings of worthlessness, thoughts of death or suicide, psychomotor changes, and fatigue following a stroke (*1*). As the population ages and the prevalence of cerebrovascular diseases rises, the incidence of post-stroke depression is also increasing (*2*). Research indicates that approximately 33% of stroke patients experience post-stroke depression (*3*), with a cumulative rate of 55% within five years of the stroke event (*4*). While the exact pathogenesis of PSD remains uncertain, numerous studies suggest that its occurrence and progression are influenced by a variety of factors, including biological, functional, social, and psychological elements (*5*). Among these factors, biological components are believed to play a significant role in the advancement of the condition. Research (*6,7*) indicates that PSD is linked to neuroinflammation, disruptions in the hypothalamic-pituitary axis, oxidative stress, abnormalities in brain-derived neurotrophic factor levels, decreased monoaminergic transmission, and genetic predisposition.

The intestinal flora plays a crucial role in the intestinal microenvironment, with research indicating the

significance of gut microbiota in central nervous system function (*8*). The bidirectional communication between the brain and gut can impact internal homeostasis, potentially resulting in the development of diseases, particularly those affecting the nervous system (*9*). The alteration of gut microbiota in the gastrointestinal tract and central nervous system, influenced by changes in the autonomic nervous system and immune system, can result in various consequences including alterations in fat storage and energy balance, dysfunction of the gastrointestinal barrier, low-grade systemic inflammation, stress response, increased anxiety and depression (*10*). These changes in physiological processes have been associated with the pathophysiology of depression (*11*). Numerous studies (*12*) in the literature have indicated a strong correlation between gut microbiota and cerebral ischemia, impacting stroke prognosis *via* mechanisms such as bacterial translocation and gut microbiota metabolites, and contributing to various pathological processes in the onset and progression of cerebral ischemia.

Currently, according to the monoamine neurotransmitter theory, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) are considered the first-line treatment for PSD (*13*) . However, due to long term delay treatment and low response rate, they may not be the most optimal choice. Traditional Chinese medicine theory suggests that depression in PSD patients is caused by liver disorders leading to stagnation of qi and blood. Therefore, treatment should focus on promoting blood circulation to remove stasis and addressing qi imbalances as a priority (*14*).

The Xuefu Zhuyu decoction, originating from Yi Lin Gai Cuo (Corrections on the Errors in Medical Workst) by Wang Qingren during the Qing Dynasty, consists primarily of Angelicae Sinensis Radix, Rehmanniae Radix, Persicae Semen, Carthami Flos, Aurantii Fructus, Paeoniae Radix Rubra, Bupleuri Radix, Glycyrrhizae Radix et Rhizoma, Chuanxiong Rhizoma, Cyathulae Radix, among other ingredients (*15*). This prescription is renowned for its efficacy in promoting the circulation of qi and blood (*16*). Several clinical studies (*17,18*) have demonstrated the efficacy of XFZY decoction in alleviating depression-like symptoms in patients with PSD, as well as in restoring autonomic nerve function and enhancing overall quality of life. The combination of Xuefu Zhuyu capsule with flupentixol and melitracen tablets has also been shown to decrease the severity of depression in PSD patients. However, the precise mechanisms underlying the therapeutic effects of Xuefu Zhuyu treatment for PSD remain unclear.

This study utilized a model of cerebral ischemia-reperfusion (tMCAO) combined with chronic unpredictable stress (CUMS) to replicate the PSD animal model, in order to investigate the pharmacodynamic effects of Xuefu Zhuyu capsule on PSD model rats. Subsequently, 16SrDNA sequencing in conjunction with gas chromatography was employed to analyze the intestinal flora and short-chain fatty acids (SCFAs) in PSD rats treated with Xuefu Zhuyu capsule, facilitating the identification of differential intestinal microorganisms. Finally, utilizing the brain-gut axis as a framework, the study examined the neuroinflammation in the brains and the intestinal barrier function of rats in each experimental group. Furthermore, the potential mechanism by which Xuefu Zhuyu capsule may prevent and treat post-stroke depression in rat models by modulating intestinal flora was preliminarily investigated.

2. Materials and Methods

2.1. Animal grouping and drug administration

Male specific-pathogen-free Wistar rats (Vital River, Beijing, China), aged 8 weeks, were housed in a temperature-controlled room with a 12-hour light-dark cycle and *ad libitum* access to food and water at the Animal Center of Tianjin University of Traditional Chinese Medicine. All experimental procedures were conducted in compliance with the regulations set forth by the Animal Care and Utilization Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2021271).

Experiment 1: Rats were randomly assigned to six groups (n = 12 each): Sham, PSD, XFZY low-dose (XFL, 0.216 g/kg), medium-dose (XFM, 0.432 g/kg), high-dose (XFH, 0.864 g/kg), and fluoxetine (Flu, 1.80 mg/kg). Except for Sham, all groups underwent CUMS for 28 days. Behavioral tests were performed on day 28. XFZY was obtained from Tianjin Hong Ren Tang Pharmaceutical (Tianjin, China). Fluoxetine was obtained from Shanxi QianYuan Pharmaceutical (Shanxi, China).

Experiment 2: Rats were randomly assigned to five groups (n = 12 each): Sham, PSD, XFZY medium (XFM, 0.432 g/kg), pseudo-germ-free (PGF) and PGF + XFM (PGFX, 0.432 g/kg). Except for Sham, all groups underwent CUMS for 28 days. Behavioral tests were performed on day 28. Gut microbiota depletion was achieved by administering a nonabsorbable antibiotic cocktail in drinking water to rats in the PGF and PGFX groups from the onset of CUMS until its cessation (day 1 to day 28). The cocktail consisted of neomycin (5 mg/mL, Solarbio, Beijing, China, N8090), bacitracin (5 mg/mL, Yuanye Bio-Technology, Shanghai, China, S17005), and nystatin (1.25 μg/mL, Solarbio, P9210) — a regimen previously validated to effectively deplete gut microbiota without altering body weight or baseline locomotor activity (*19,20*).

XFZY was administered at low (0.216 g/kg), medium (0.432 g/kg), and high doses (0.864 g/kg) based on clinically equivalent dose calculations (*21*). The medium dose (0.432 g/kg) corresponds to the human

daily dosage (4.8 g/day for a 70 kg adult) adjusted *via* human-to-rat dose conversion formula: rat dose (g/kg) = human dose (g)/70 kg (standard human body weight) × 6.3 (conversion factor). The low and high doses were selected as 0.5× and 2× the clinical equivalent dose, respectively, to assess dose-response relationships. Fluoxetine (1.80 mg/kg), the positive control, was dosed equivalently to the human clinical regimen (20 mg/day) using the same formula (*22*) . All doses were administered orally once daily for 28 days.

2.2. PSD model establishment

PSD animal model was established by tMCAO and CUMS. Focal cerebral ischemia was induced by transient occlusion of the right middle cerebral artery (MCA) for 60 min (*23*). Through the neurological deficit score, rats with impaired neurological function were selected. Then chronic unpredictable mild stress (CUMS) was used to establish the depression model. Rats were subjected to a random allocation of two out of seven distinct stress modalities on a daily basis over a period of 28 days, including fasting, water deprivation, ice water swimming, horizontal shaking, wet cage confinement, oblique cage placement, and day/night reversal (*24*). Each stressor was administered only once every two days.

2.3. Neurological deficit score

Neurological deficit score was used to evaluate neurological status (*25*) . The scores are 0, no observable deficit; 1, forelimb flexion; 2, forelimb flexion and decreased resistance to lateral push; 3, forelimb flexion, decreased resistance to lateral push, and unilateral circling; 4, forelimb flexion and partial or complete lack of ambulation. The score from 1 to 3 included in subsequent analysis.

2.4. Sugar water preference test

The sucrose preference test (*26*) was utilized to evaluate the extent of anhedonia in rats. Prior to the commencement of the experiment, the rats underwent a training period to acclimate to consuming 1% sucrose solution. Following 21 hours fasting period, the experiment was initiated. During the experiment, the quantities of 1% sucrose solution and water were measured. Subsequently, after a one-hour period, the two bottles were weighed again, and the total fluid intake, sucrose consumption, and water consumption of the rats were documented.

2.5. Open field test

Open field testing (*27*) was conducted to assess exploratory behavior and locomotion. Rats were placed in the center (50 × 50 cm) of a 100 × 100 × 40 cm arena and allowed to acclimate for 60 s. Locomotor activity was then recorded for 5 min using a video tracking system (EthoVision XT, Noldus, Wageningen, Netherlands). Total distance traveled (horizontal movement) and number of rearing events (vertical movement) were quantified. Rats were habituated to the testing room for 1 h prior to testing.

2.6. Forced swimming test

The forced swimming test (*28*) was employed to evaluate the level of despair in rats by analyzing the duration of immobility during a 6 min swimming session and determining the presence of desperate behavioral states, typically indicated by the activity of the rat's hind limbs.

2.7. Histological analysis

After sacrifice and dissection, rat brains were fixed in 4% paraformaldehyde (Solarbio, P1110) for 24 h and dehydrated. Coronal brain sections (5 μm thick) were cut using a rotary microtome. For histological analysis, sections were deparaffinized in a 60°C oven for 1 h, cleared in xylene, and rehydrated through a graded ethanol series. Hematoxylin and eosin (H&E) staining (Solarbio, G1126) was performed as described (*29*). For Nissl staining, sections were incubated in 0.1% cresyl violet solution (Solarbio, G1430) at 60°C for 30 min (*30*). Alcian blue–Periodic acid–Schiff (AB-PAS) staining involved sequential incubation in Alcian blue (10 min) and Schiff's reagent (10 min) (Solarbio, G1285). All sections were dehydrated, cleared, and cover-slipped after staining. Images were acquired using a light microscope.

2.8. Immunohistochemical staining

Following deparaffinization and antigen retrieval using sodium citrate buffer (10 mM, pH 6.0, Solarbio, C1010) at 95°C for 10 min, endogenous peroxidase activity was blocked with 3% H_2O_2 (10 min, room temperature). Sections were incubated in 5% goat serum (Solarbio, SL038) for 1 h to block non-specific binding, then incubated overnight at 4°C with primary antibodies: donkey anti- ionized calcium-binding adapter molecule 1 (Iba1, 1:1,000, Novus Biologicals, Colorado, USA, NB100-1028), rabbit anti- mucin 2 (MUC2, 1:200, Bioss, Beijing, China, bs-60331R), rabbit anti-zonula occludens-1 (ZO-1, 1:200, Bioss, bs-34023R), and rabbit anti-Occludin (1:200, Bioss, bs-10011R). After washing with phosphate buffered saline (PBS), sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (30 min, room temperature, Beyotime Biotechnology, Shanghai, China, A0208, A0181), developed with 3,3'-diaminobenzidine (DAB, Solarbio, DA1010) chromogen (5 min), and counterstained with hematoxylin. Finally, sections were

dehydrated, cleared, and cover-slipped. Images were captured using a light microscope.

2.9. Enzyme-linked immunosorbent assay

The levels of interleukin-1β (IL-1β, Genime Biotechnology, Wuhan, China, JYM0419Ra), interleukin-6 (IL-6, Genime Biotechnology, JYM0646Ra), and tumor necrosis factor-α (TNF-α, Genime Biotechnology, JYM0635Ra) in serum were determined using enzyme-linked immunosorbent assay in accordance with the manufacturer's guidelines.

2.10. 16S rRNA sequencing analysis

Genomic DNA was extracted from rat fecal samples using the TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biochemical Technology, Beijing, China, DP812) according to the manufacturer's instructions. The bacterial 16S ribosomal RNA (rRNA) gene V3–V4 hypervariable region was amplified using an ABI GeneAmp 9902 thermal cycler (Applied Biosystems, USA) and specific primers. Polymerase chain reaction (PCR) products were purified with OMEGA DNA columns, confirmed by 1.8% agarose gel electrophoresis (120 V, 40 min), and the target bands excised and recovered. Sequencing was performed on the Illumina NovaSeq 6000 platform. Raw reads were merged using FLASH (v1.2.11), quality-filtered with Trimmomatic (v0.33), and chimeric sequences were removed with UCHIME (v8.1) to generate high-quality tags. Operational taxonomic units (OTUs) were clustered at 97% similarity using USEARCH (v10.0) with a minimum abundance threshold of 0.005% of total sequences. Taxonomic assignment was performed using the Ribosomal Database Project (RDP) Classifier (v2.2) with a confidence threshold of 0.8.

2.11. Gas chromatographic analysis

SCFAs were analyzed by gas chromatography (GC) (*31*). Fecal samples were weighed and homogenized in ultrapure water (1:5, w/v) by vortexing. An aliquot was further diluted 1:15 in a solution containing 1.33% HCl, 75% ethanol, 2-ethylbutyric acid (0.4821 mg/mL), and 2-ethylhexanoic acid (0.0283 mg/mL) as internal standards, vortexed, and sonicated for 2 min. After centrifugation at 17,950× *g* at 4°C for 10 min, the supernatant was collected for GC analysis. GC was performed on an Agilent 6890N gas chromatograph (Agilent, California, USA) equipped with a DB-FFAP column (30 m × 0.25 mm × 0.5 μm) using nitrogen as carrier gas. Injection volume was 3 μL with a split ratio of 3:1. The injector and detector (FID) temperatures were both set to 240°C.

2.12. Statistical analysis

Statistical analyses and graphical representations were conducted utilizing GraphPad Prism 8.0 software. Data sets adhering to a normal distribution were presented as mean ± standard deviation, with comparisons between two groups assessed using *t*-tests. Multiple-group comparisons were evaluated using one-way analysis of variance (ANOVA). Statistical significance was established at a threshold of $P < 0.05$.

3. Results

3.1. The XFZY treatment demonstrated efficacy in managing PSD rats

The PSD rats model was induced using tMCAO and CUMS methods. Following 28 days of drug treatment, behavioral assessments were conducted on the rats. Compared to the Sham group, the PSD group exhibited significantly lower scores in neurological function ($P < 0.05$), sucrose preference rate ($P < 0.05$), spontaneous activity in the open field test ($P < 0.05$), and longer immobility time ($P < 0.05$) in the forced swimming test. These findings indicate the presence of pronounced symptoms of post-stroke depression in the PSD rats. Compared with the PSD group, XFZY treatment significantly decreased the neurological score ($P < 0.05$, Figure 1B), increased the sucrose preference rate ($P < 0.05$, Figure 1C), enhanced spontaneous activity behavior ($P < 0.05$, Figures 1D and 1E), reduced immobility time ($P < 0.05$, Figure 1F), and ameliorated depression-like symptoms in PSD rats. Among the doses, the XFM group exhibited the most significant improvement ($P < 0.05$).

The presence of lesions in the hippocampus is commonly associated with the development of depression (*32*), with abnormalities in both the structure and function of the hippocampus observed in individuals with depression (*33*). In our study, the assessment of hippocampal damage in rats with PSD was conducted using H&E staining and Nissl staining techniques. The findings from Nissl staining revealed that PSD rats exhibited disrupted neuronal organization in the Cornu Ammonis 1 (CA1) regions (Fig. 1H), along with a decrease in the number of Nissl bodies ($P < 0.05$) (Figure 1G) compared to the Sham group. Furthermore, XFZY-treated groups significantly mitigated neuronal damage in the CA1 region and increased the number of Nissl bodies ($P < 0.05$). Among the doses, the XFM group (0.432 g/kg) demonstrated the most pronounced effect ($P < 0.05$).

3.2. The effectiveness of XFZY treatment was diminished as a result of the removal of intestinal flora

To determine whether the therapeutic effect of XFZY on PSD rats depends on gut microbiota, we depleted intestinal flora using antibiotic cocktails and evaluated

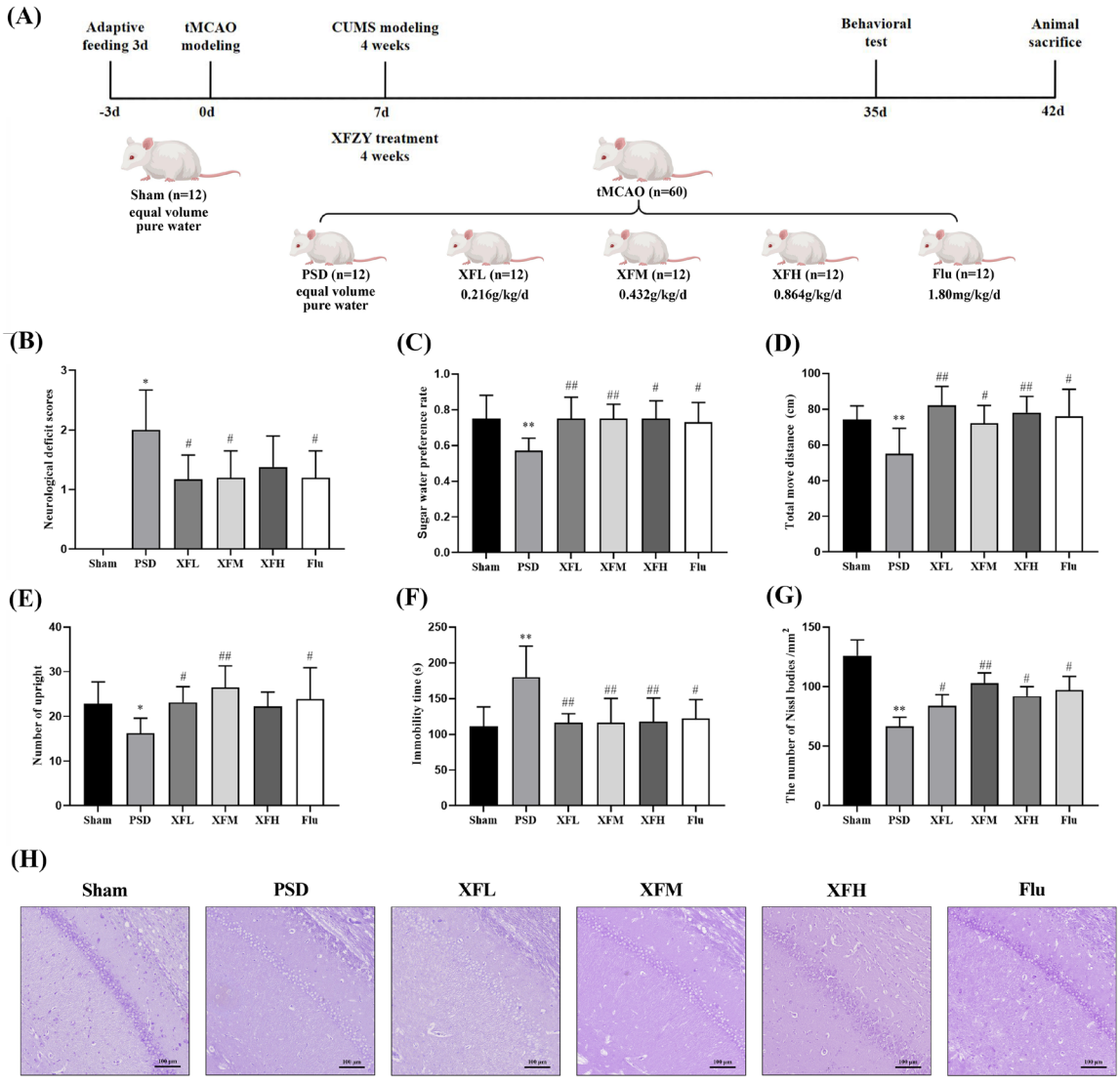

Figure 1. Pharmacodynamics of Xuefu Zhuyu capsule (XFZY) in post-stroke depression (PSD) rats. (**A**) Animals grouping and experimental timeline of the surgery, chronic unpredictable mild stress (CUMS) stimulation, drug treatment and behavioral test. (**B**) Neurological deficit score. (**C**) Sugar water preference rate. (**D**) The total moving distance in the open field test. (**E**) The number of vertical rearings in the open field test. (**F**) The immobility time of the forced swimming test (**G**) The number of Nissl bodies. (**H**) Nissl staining of hippocampus tissues. *$P < 0.05$ compared to Sham; **$P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\#\#}P < 0.01$ compared to PSD.

behavioral and histological outcomes. Behavioral tests revealed that the PGFX group exhibited higher neurological scores ($P < 0.05$) (Figure 2B), a decreased sucrose preference rate ($P < 0.05$) (Figure 2C), reduced spontaneous activity in the open field test ($P < 0.05$) (Figures 2D and 2E), and longer immobility time ($P < 0.05$) (Figure 2F) in the forced swimming test when compared to the XFM group. The findings from Nissl staining analyses revealed a notable decrease in Nissl bodies ($P < 0.05$) (Figure 2G) in the CA1 region of the brain in the PGFX group compared to the XFM group (Figure 2H). These results suggest that the XFM group did not effectively ameliorate neurological deficits and depressive symptoms in PSD rats following intestinal flora depletion, indicating that the therapeutic efficacy of XFZY is potentially dependent on the presence of intestinal flora.

3.3. The effect of XFZY on the intestinal microbiota of rats with PSD

To investigate the impact of XFZY on the gut microbiota in PSD rats, 16S rDNA sequencing was performed. Alpha diversity analysis (Chao1, Observed species, Shannon) revealed reduced microbial richness and diversity in the PSD group compared to Sham ($P < 0.05$), which were restored in the XFM group (Figures 3A–3C). In contrast, PGF and PGFX groups showed significantly lower diversity, indicating severe dysbiosis unmitigated by XFZY. Beta diversity (PCoA, Figures 3E–3F) revealed distinct clustering, separating Sham, PSD, and XFM from PGF and PGFX groups, with clear

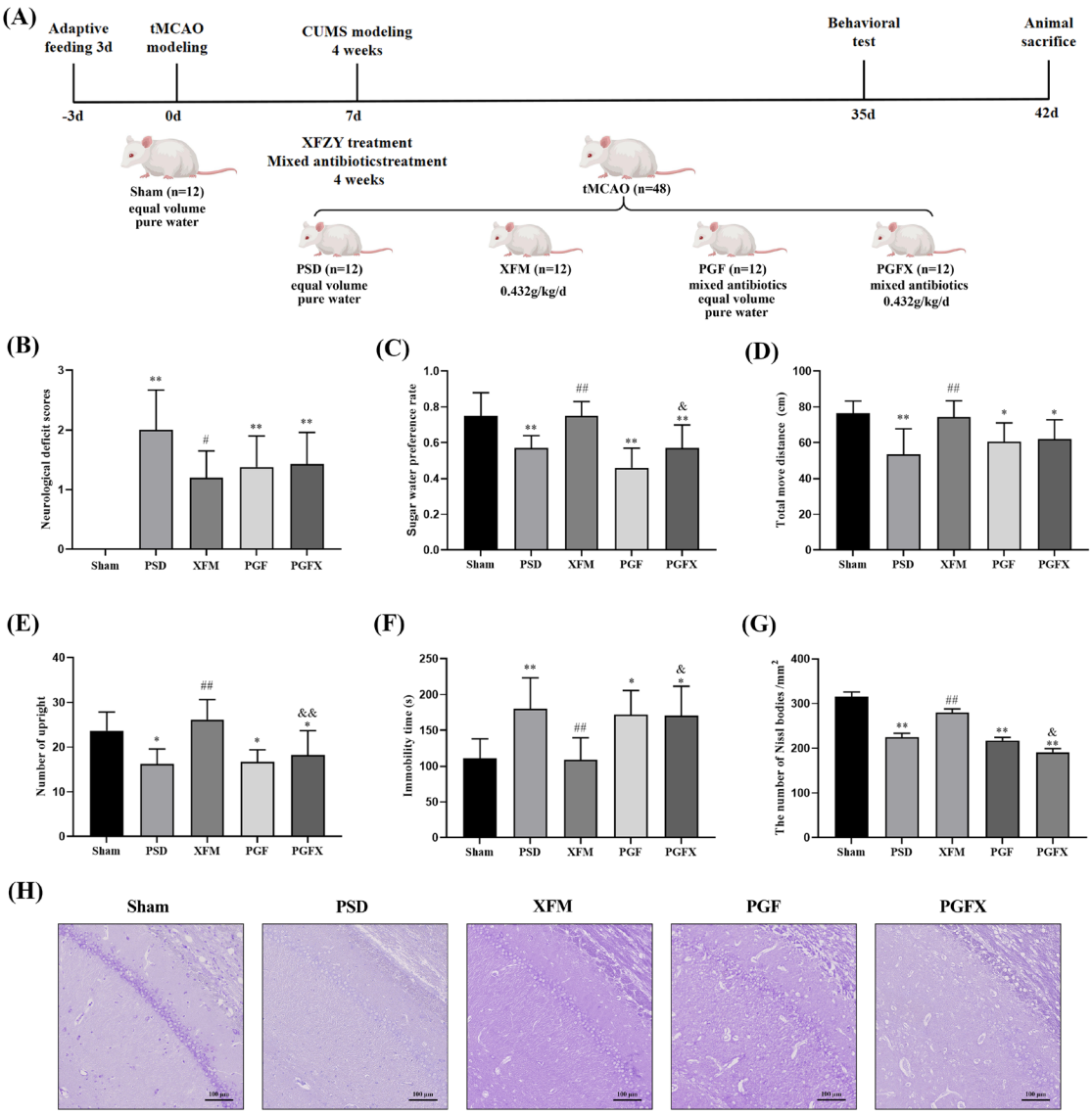

Figure 2. XFZY treatment's effectiveness decreased after removing intestinal flora. (**A**) Animals grouping and experimental timeline of the surgery, cums stimulation, drug treatment and behavioral test. (**B**) Neurological deficit score. (**C**) Sugar water preference rate. (**D**) The total moving distance in the open field test. (**E**) The number of vertical rearings in the open field test. (**F**) The immobility time of the forced swimming test (**G**) The number of Nissl bodies. (**H**) Nissl staining of hippocampus tissues. *$P < 0.05$ compared to Sham; **$P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\#\#}P < 0.01$ compared to PSD; $^{\&}P < 0.05$ compared to XFM; $^{\&\&}P < 0.01$ compared to XFM.

differentiation among all five groups (PCoA1: 20.42%), indicating significant compositional differences.

At the phylum level, Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia dominated the microbiota (Figure 4A). The PSD group showed decreased Firmicutes and increased Proteobacteria *vs.* Sham, which were reversed in the XFM group. The PGF and PGFX groups exhibited further reductions in Firmicutes and increases in Proteobacteria and Bacteroidetes. At the genus level, PSD was characterized by decreased beneficial genera (*e.g.*, *Lactobacillus*, *Ruminococcus*, *Clostridium*) and increased pathobionts (*e.g.*, *Escherichia-Shigella*, *Enterococcus*, *Klebsiella*), which were partially reversed in the XFM group. In contrast, PGF and PGFX groups showed enrichment of potential pathogens and loss of commensals.

Linear discriminant analysis effect size (LEfSe) analysis (Figures 4E–4F) identified *Romboutsia* and *Dorea* as discriminant taxa in PSD, while *Lactobacillus*, *Ruminococcus*, and *Clostridia*_UCG-014 were enriched in the XFM group. The PGF and PGFX groups were dominated by Enterobacteriaceae members (*e.g.*, *Escherichia*, *Klebsiella*), suggesting distinct microbial dysbiosis patterns.

3.4. The effect of XFZY on the short-chain fatty acids of rats with PSD

Short-chain fatty acids—including acetic, propionic, and butyric acid—are gut microbial metabolites implicated

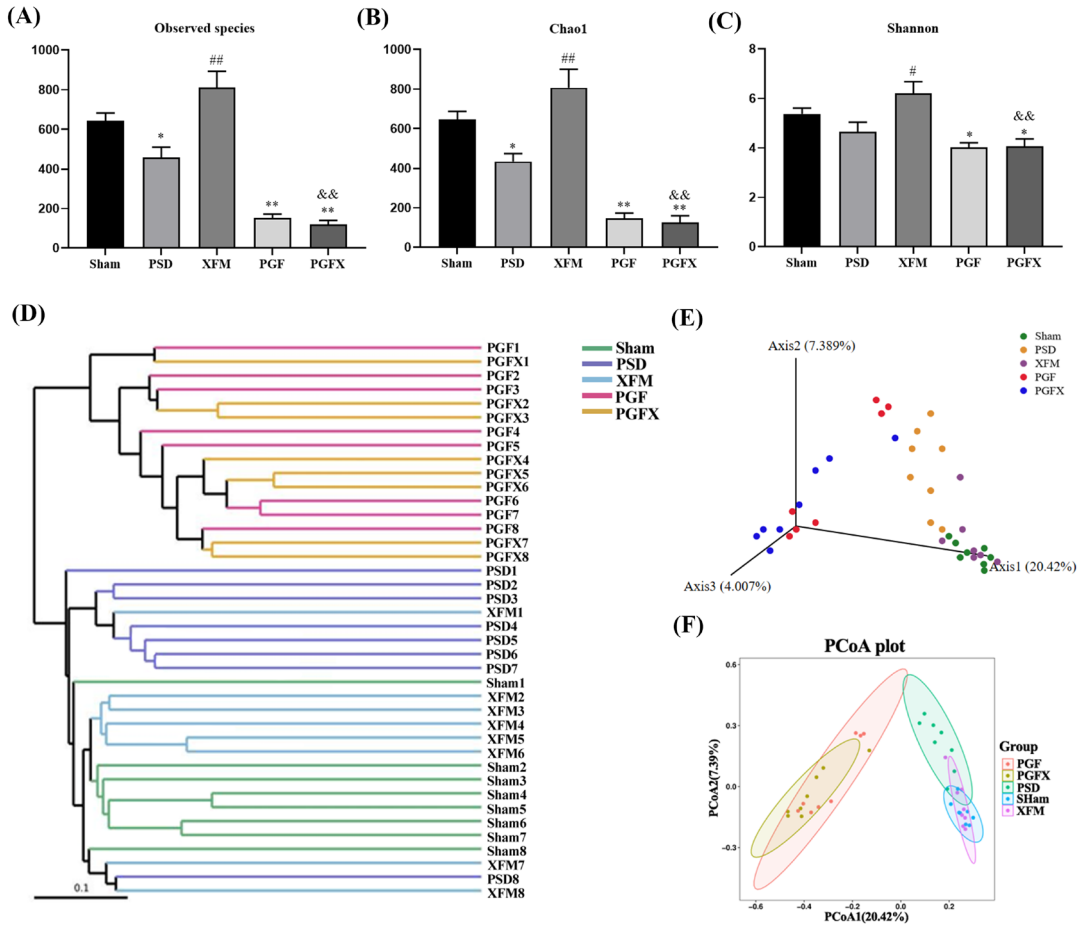

Figure 3. Analysis of the alpha diversity and beta diversity of intestinal flora. (**A**) Observed species. (**B**) Chao1 index. (**C**) Shannon index. (**D**) Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering plot. (**E**) PCoA plot in 3D. (**F**) Principal Coordinates Analysis (PCoA) plot in 3D. *$P < 0.05$ compared to Sham; **$P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\#\#}P < 0.01$ compared to PSD; $^{\&\&}P < 0.01$ compared to XFM.

in the gut-brain axis, influencing emotional and cognitive functions (*34*). In this study, serum and fecal SCFAs were analyzed by gas chromatography. Both compartments showed similar trends: the PSD group exhibited significantly lower levels of all three major SCFAs compared to Sham group ($P < 0.05$), while the XFM group reversed these reductions (Figures 5A–5F). These results suggest that XFZY modulates SCFA production, particularly acetic and butyric acid, likely through remodeling of the gut microbiota.

3.5. XFZY demonstrates efficacy in improving the intestinal barrier function in rats with PSD

The gut microbiota influences intestinal barrier integrity, a factor implicated in neuropsychiatric disorders such as depression and anxiety (*35,36*). In this study, intestinal barrier function and inflammation were evaluated using H&E, AB-PAS, and immunohistochemical (IHC) staining. H&E staining revealed well-organized colonic glands in the sham group, whereas the PSD group exhibited inflammatory cell infiltration and structural damage to the epithelium and glands—pathology ameliorated in the XFM group. In contrast, PGF and PGFX groups showed more severe inflammation. AB-PAS staining showed reduced goblet cell numbers in the PSD group ($P < 0.05$), which were restored in the XFM group (Figures 6A and 6B). IHC analysis confirmed downregulation of mucosal and tight junction proteins—MUC2, ZO-1, and Occludin in PSD rats ($P < 0.05$; Figures 6A, 6C–6E), all of which were significantly upregulated following intervention with the XFM group ($P < 0.05$). These results indicate that PSD impairs intestinal barrier function, and XFZY exerts protective effects by reducing inflammation and restoring key barrier components.

3.6. XFZY demonstrates efficacy in suppressing neuroinflammation in rats with PSD

Microglia, central nervous system innate immune cells, contribute to depression *via* neuroinflammation (*37*). IHC analysis showed increased microglial activation in the PSD and PGF groups compared to Sham ($P < 0.05$), which was attenuated in the XFM group ($P < 0.05$; Figures 7A and 7B). Serum levels of pro-inflammatory

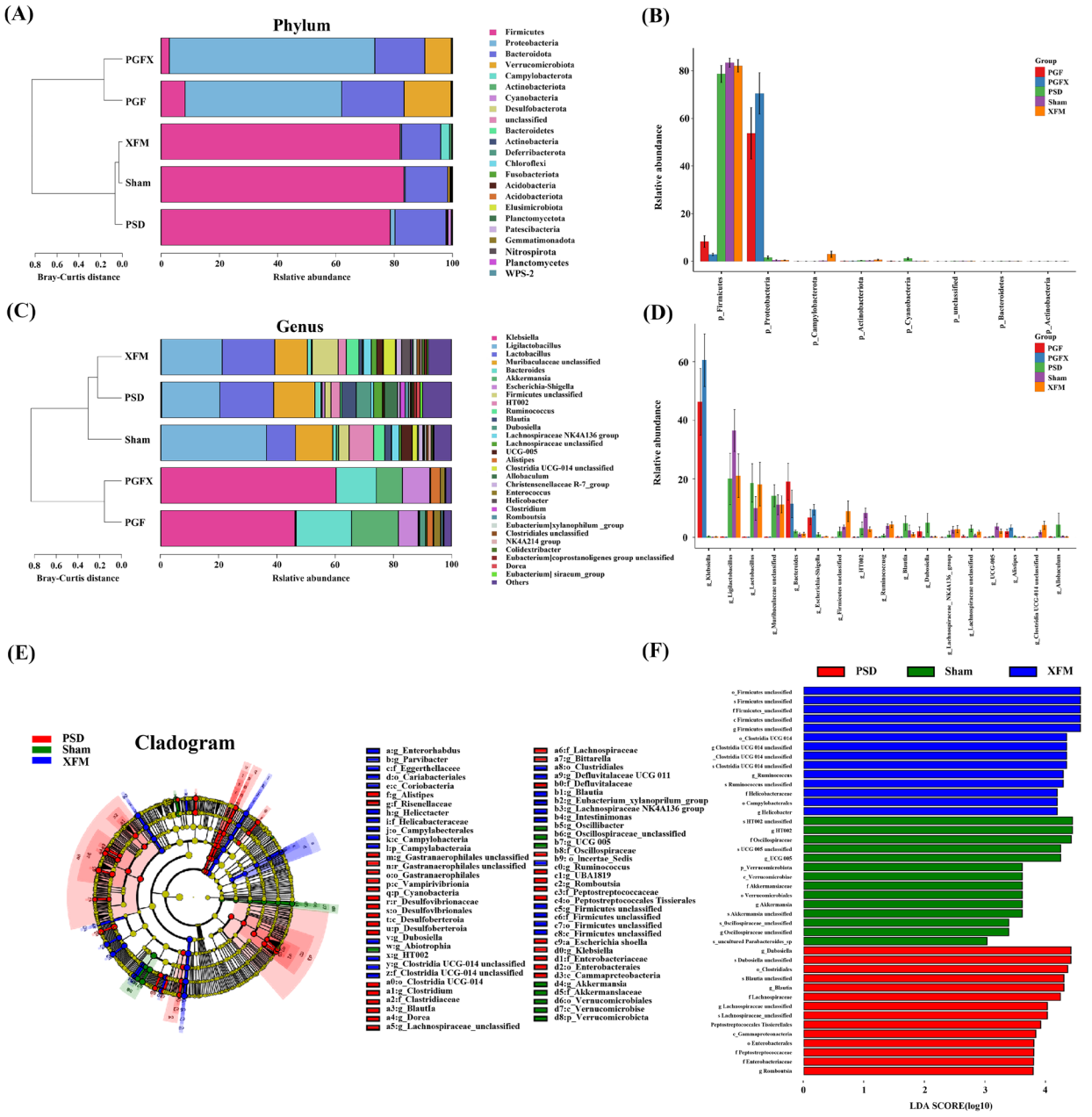

Figure 4. Analysis of relative abundance and LEfSE analysis of intestinal flora in various groups. (**A**) Cluster analysis at the gate level. (**B**) Analysis of differences at the gate level. (**C**) Cluster analysis at the genus level. (**D**) Analysis of differences at the genus level. (**E**) Cladogram from linear discriminant analysis effect size (LEfSe) analysis. (**F**) Linear discriminant analysis (LDA) scores from LEfSe analysis.

cytokines (TNF-α, IL-1β, IL-6) were significantly elevated in PSD rats ($P < 0.05$), and reduced in the XFM group ($P < 0.05$; Figures 7C–7E). No significant difference was observed between PGF and PGFX groups ($P > 0.05$). These findings indicate that systemic and central neuroinflammation is attenuated in the XFM group.

4. Discussion

Post-stroke depression is a prevalent complication of neuropsychiatric disorders following a stroke, with a reported prevalence rate of approximately 29% that has shown minimal variation over time (*38*). Research (*39*) indicates that the gut microbiota may play a significant role in the pathogenesis of neurological conditions *via* the brain-gut axis, highlighting its importance as a key factor in susceptibility to such disorders. There exists a relationship between stroke and dysbiosis of the intestinal flora, potentially impacting the prognosis of stroke through mechanisms including bacterial translocation, intestinal metabolites, and immune regulation (*40*). Additionally, the gut microbiota is implicated in the pathogenesis of depression, potentially exacerbating depressive symptoms through inflammatory responses, hypothalamic-pituitary-adrenal axis dysregulation

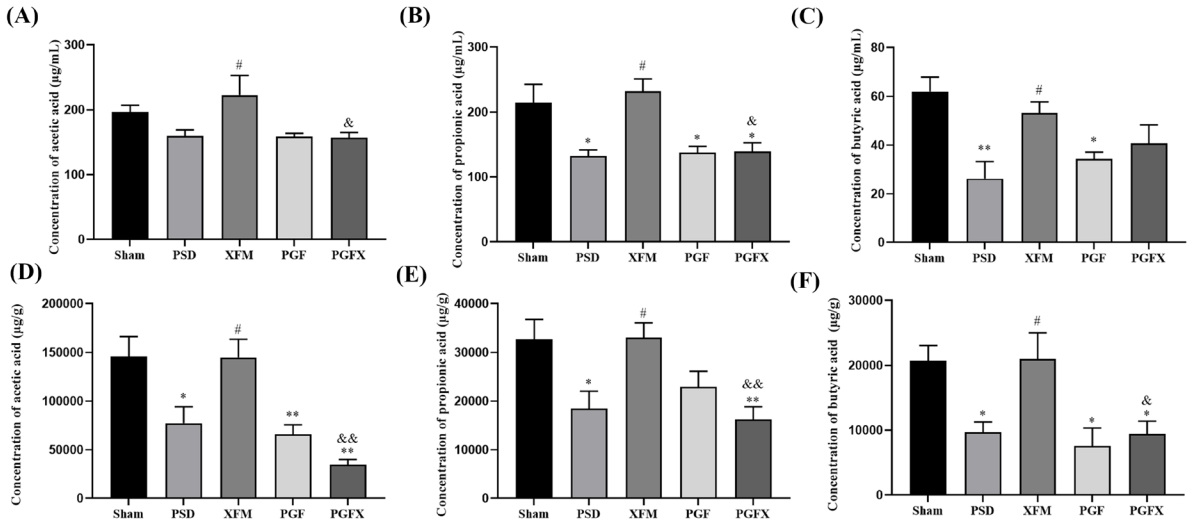

Figure 5. Short chain fatty acids in serum and feces. (**A**) Concentration of acetic acid in serum. (**B**) Concentration of propionic acid in serum. (**C**) Concentration of butyric acid in serum. (**D**) Concentration of acetic acid in feces. (**E**) Concentration of propionic acid in feces. (**F**) Concentration of butyric acid in feces. *$P < 0.05$ compared to Sham; **$P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\&}P < 0.05$ compared to XFM; $^{\&\&}P < 0.01$ compared to XFM.

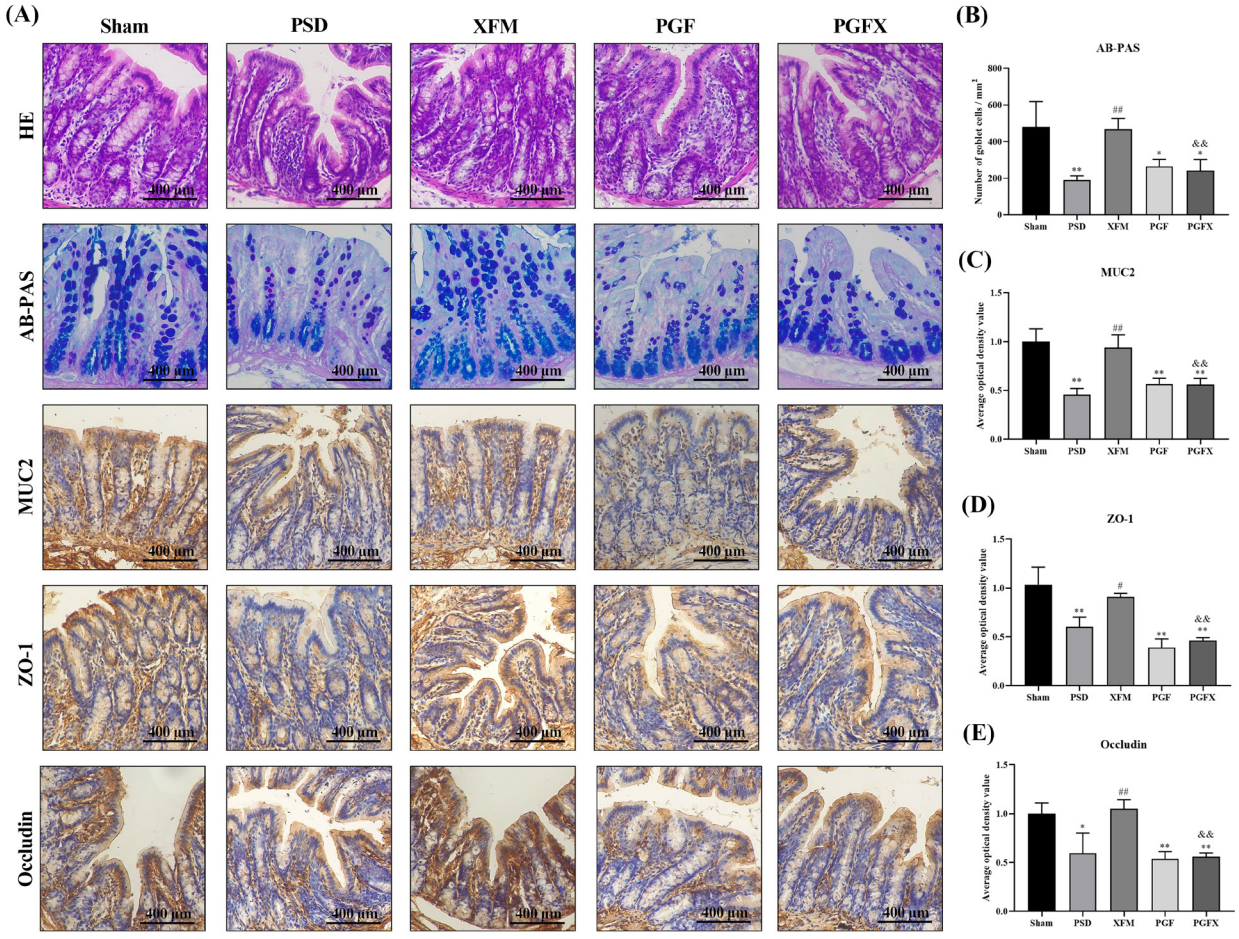

Figure 6. XFZY effectively enhances intestinal barrier function in rats with PSD. (**A**) Representative images of hematoxylin and eosin (H&E) staining, Alcian blue-periodic acid–Schiff (AB-PAS) staining, and immunohistochemistry (IHC) staining for mucin 2 (MUC2), zonula occludens-1 (ZO-1), and Occludin in the colon. (**B**) Goblet cell counts. (**C**) Average optical density value of MUC2. (**D**) Average optical density value of ZO-1. (**E**) Average optical density value of Occludin. *$P < 0.05$ compared to Sham; **$P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\#\#}P < 0.01$ compared to PSD; $^{\&\&}P < 0.01$ compared to XFM.

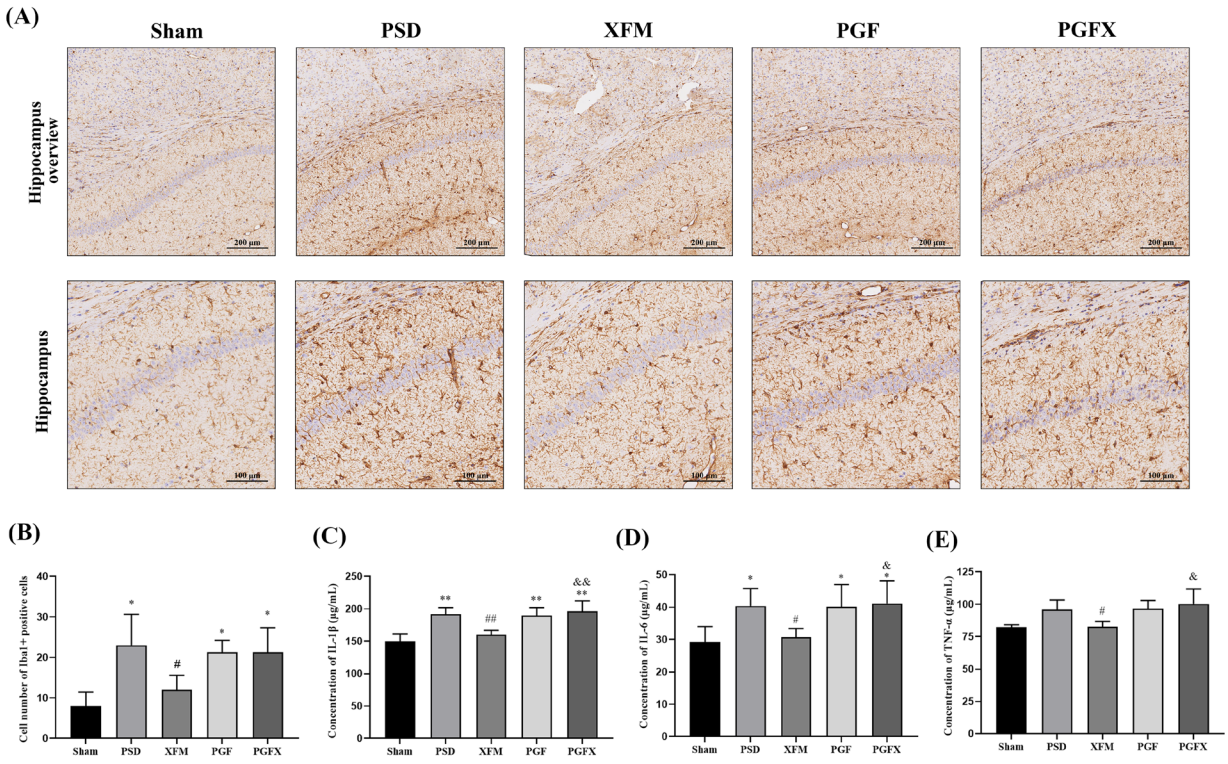

Figure 7. XFZY shows effectiveness in inhibiting neuroinflammation in rats with post-stroke depression. (**A**) IHC staining of ionized calcium-binding adapter molecule 1 (Iba1) in hippocampal. (**B**) Total number of Iba1-positive microglia. (**C**) Concentration of interleukin-1β (IL-1β) in serum. (**D**) Concentration of interleukin-6 (IL-6) in serum. (**E**) Concentration of tumor necrosis factor-α (TNF-α) in serum. $^{*}P < 0.05$ compared to Sham; $^{**}P < 0.01$ compared to Sham; $^{\#}P < 0.05$ compared to PSD; $^{\#\#}P < 0.01$ compared to PSD; $^{\&}P < 0.05$ compared to XFM; $^{\&\&}P < 0.01$ compared to XFM.

(*41,42*). Thus, the restoration of intestinal flora homeostasis is crucial for the prevention and treatment of PSD.

Existing pharmacological studies provide support for the microbiota-modulating potential of XFZY. The individual herbal components of XFZY have been reported to beneficially influence the gut microbiota. For instance, Bupleuri Radix polysaccharides can ameliorate colitis by modulating gut microbial composition (*43*). *Angelica sinensis* has been shown to reshape the gut microbiota and improve intestinal barrier function (*44*). *Rehmannia glutinosa* polysaccharides can promote the production of beneficial SCFAs by gut bacteria (*45*), while *Paeonia lactiflora* extracts alleviate intestinal inflammation partly through microbiota regulation (*46*). More directly, a study on the whole formula of XFZY demonstrated its ability to attenuate atherosclerosis in mice by reversing gut microbiota dysbiosis, highlighting the integral role of microbiota modulation in its therapeutic mechanism (*47*). These reports align with the core findings of the present study, wherein XFZY intervention significantly altered the gut microbial structure and increased SCFA levels in PSD rats.

Our findings indicate that XFZY has a significant impact on increasing Alpha diversity in rats with post-stroke depression. At the phylum level, there was a significant increase in the relative abundance of *Bacteroides* and Proteobacteria in the PSD group compared to the Sham group, while the abundance of Firmicutes and Campylobacterota decreased. Conversely, following intervention with XFZY, these microbiota exhibited a contrasting trend. This suggests that regulating intestinal flora to restore intestinal microecology may offer an effective treatment for PSD. The relative abundance of Proteobacteria and Ruminococcaceae shows a negative correlation with the increase in intestinal permeability (*48*). A decrease in the abundance of rumen bacteria in the gut may lead to intestinal inflammation and damage to the intestinal mucosal barrier, allowing harmful factors such as TNF-α to enter the bloodstream (*49*). Experimental findings indicate a reduction in *Ruminococcus* levels in the PSD, PGF, and PGFX groups, suggesting a potential increase in intestinal permeability and inflammatory factors. Most members of the *Desulfurococcus* genus are producers of lipopolysaccharides (LPS) (*50*). Research (*51*) has demonstrated that the peripheral administration of LPS can stimulate the immune system *via* Toll-like receptors, leading to the production of pro-inflammatory cytokines including IL-6, IL-1β, and TNF-α. Our study found that XFZY treatment reduced the presence of *Desulfovibrio* and *Vibrio* and mitigated the inflammatory reaction.

Short-chain fatty acids, the principal metabolites produced by gut microbiota, have been shown to

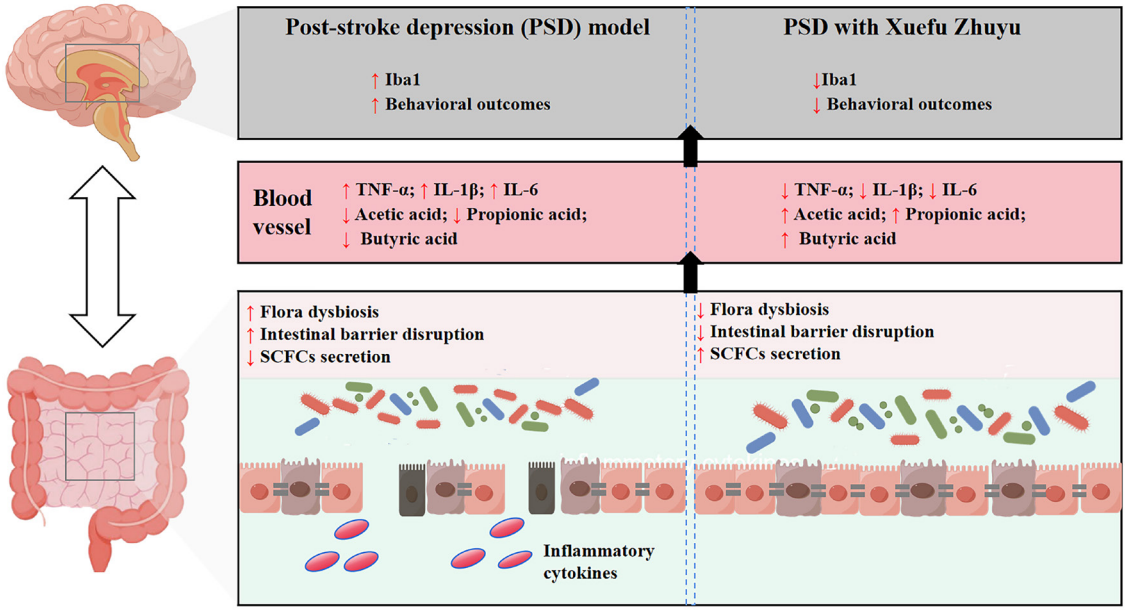

Figure 8. The diagram of the mechanism of XFZY anti-PSD. Brain injury after stroke induces gut dysbiosis and intestinal barrier damage, promoting systemic translocation of microbial metabolites (*e.g.*, short-chain fatty acids, SCFAs), which exacerbate neuroinflammation and hippocampal injury. XFZY restores gut homeostasis, reduces SCFA levels, and alleviates neuroinflammation and depressive-like behaviors.

have various beneficial effects on gut health (*52*). For instance, SCFAs can enhance intestinal barrier function and decrease inflammation. Butyrate, for instance, has been found to increase the expression of tight junction proteins, thereby reducing intestinal permeability (*53*). Additionally, SCFAs have been implicated in modulating immune responses and impacting the central nervous system (*54*). Increased levels of SCFAs have been associated with alleviation of depressive symptoms in animal experiment (*55*). Furthermore, studies (*56*) have indicated that individuals with major depression have reported consuming higher amounts of butyrate, acetate, and propionate. SCFAs can stimulate nerve pathways and induce behavioral changes through the activation of G protein-coupled receptors free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3) (*57*). Additionally, research suggests (*58*) that germ-free mice treated with SCFAs show improvements in microglial function, indicating a potential role for SCFAs in reversing microglial deficiencies. Our experiments demonstrate that PSD rats exhibit dysbiosis in their gut microbiota, characterized by a reduction in the presence of SCFA-producing bacterial flora and a subsequent decrease in SCFA content. Following intervention with XFZY, there was an improvement in the abundance and composition of gut microbiota, leading to an increase in the relative abundance of SCFA-producing bacteria in the gut. Notably, our results revealed an inverted U-shaped dose-response relationship, where the high dose of XFZY (0.864 g/kg) showed inferior effects compared to the medium dose (0.432 g/kg). This can be attributed to excessive modulation of the gut microbiota and overproduction of SCFAs at the high dose, which may disrupt the homeostasis of the gut-brain axis. As supported by previous research, both microbial balance and SCFA levels require maintenance within an optimal physiological range for their beneficial effects; deviations beyond this range can impair therapeutic outcomes (*59,60*).

Of particular significance is that this study provides the first systematic evidence that XFZY ameliorates PSD by modulating the gut microbiota–gut–brain axis. To our knowledge, this is the first report demonstrating that XFZY alleviates depressive-like behaviors in a PSD model through restoration of gut microbial diversity and activation of the SCFAs pathway. These findings highlight the unique microbiota-modulating characteristics of XFZY and establish a novel link between this herbal formulation and PSD treatment.

Given the regulatory impacts of gut microbiota and SCFAs on the gut barrier and neuroinflammation, our study investigates the potential of XFZY in mitigating brain neuroinflammation and safeguarding the intestinal mucosal barrier. The findings of our study indicate that activated microglial cells in the PSD group exhibited an increase and a significant rise in the expression of proinflammatory cytokines in the serum. Following treatment with XFZY, there was a reduction in microglial activation and a significant decrease in the expression of inflammatory factors, suggesting a mitigation of neuroinflammation in PSD rats. However, the number of activated microglia and the expression of inflammatory factors increased in the PGF and PGFX groups, which further proved that XFZY can alleviate brain neuroinflammation in PSD rats by regulating intestinal flora.

The results of histological examination using HE staining revealed the presence of inflammatory infiltrates in the colon tissue of rats with PSD, while IHC demonstrated an increase in intestinal barrier permeability in these rats. Following treatment with XFZY, a significant reduction in colon tissue inflammation and improvement in intestinal barrier function were observed. These findings provide further evidence of the efficacy of XFZY in enhancing intestinal barrier function in PSD rats.

Hence, it is hypothesized that dysbiosis of gut microbiota disrupts intestinal barrier integrity, triggering activation of the peripheral immune system and subsequent synthesis and release of pro-inflammatory cytokines. This interaction between peripheral and central immunity may activate microglia in the brain, resulting in neuroinflammation and ultimately manifesting as symptoms depression. Under the intervention of XFZY, it can improve the intestinal barrier function and alleviate the inflammatory response by increasing the relative abundance of intestinal flora, thus reducing the depressive symptoms (Figure 8).

Despite the novel findings, several limitations of this study should be acknowledged. First, while our data indicate that gut microbiota modulation mediates the antidepressant effects of XFZY, causality has not been definitively proven through interventional approaches such as fecal microbiota transplantation. Second, the findings from this rodent model of PSD require validation in humans due to species-specific differences in physiology and microbiota. Third, the specific active component(s) within the XFZY formulation responsible for the observed effects remain unknown. Future studies should therefore employ FMT experiments, pursue clinical translation, and identify the key active constituents to fully elucidate the therapeutic mechanism.

XFZY alleviates depressive-like behaviors in PSD rats through modulation of the gut microbiota and restoration of intestinal barrier integrity, leading to reduced secretion of short-chain fatty acids, which in turn attenuates hippocampal neuronal damage and neuroinflammation.

Funding: This study was supported by the Science and Technology Project of Haihe Laboratory of Modern Chinese Medicine (22HHZYSS00015).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Zhou H, Wei YJ, Xie GY. Research progress on post-stroke depression. Exp Neurol. 2024; 373:114660.
2. Chen J, Wu Y, Chen Z, Yi B, Zhang L, Yin C, Feng H. High incidence of stroke in COVID-19 patients. Aging (Albany NY). 2020; 12:22390-22398.
3. Hackett ML, Yapa C, Parag V, Anderson CS. Frequency of depression after stroke: a systematic review of observational studies. Stroke. 2005; 36:1330-1340.
4. Ayerbe L, Ayis S, Crichton S, Wolfe CD, Rudd AG. The natural history of depression up to 15 years after stroke: the South London Stroke Register. Stroke. 2013; 44:1105-1110.
5. De Ryck A, Brouns R, Geurden M, Elseviers M, De Deyn PP, Engelborghs S. Risk factors for poststroke depression: Identification of inconsistencies based on a systematic review. J Geriatr Psychiatry Neurol. 2014; 27:147-158.
6. Medeiros GC, Roy D, Kontos N, Beach SR. Post-stroke depression: A 2020 updated review. Gen Hosp Psychiatry. 2020; 66:70-80.
7. Paolucci S. Advances in antidepressants for treating post-stroke depression. Expert Opin Pharmacother. 2017; 18:1011-1017.
8. Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology. 2012; 37:1369-1378.
9. Elangovan A, Dahiya B, Kirola L, *et al.* Does gut brain axis has an impact on Parkinson's disease (PD)? Ageing Res Rev. 2024; 94:102171.
10. Abdellatif AM, Sarvetnick NE. Current understanding of the role of gut dysbiosis in type 1 diabetes. J Diabetes. 2019; 11:632-644.
11. Morais LH, Schreiber HLt, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol. 2021; 19:241-255.
12. Pu J, Liu Y, Zhang H, *et al*. An integrated meta-analysis of peripheral blood metabolites and biological functions in major depressive disorder. Mol Psychiatry. 2021; 26:4265-4276.
13. Mortensen JK, Andersen G. Pharmacological management of post-stroke depression: an update of the evidence and clinical guidance. Expert Opin Pharmacother. 2021; 22:1157-1166.
14. Zhu L, Han R, He L, Pan B, Zhong W, Li Y, Liu X. Innovative strategies for post-stroke depression: integrating traditional Chinese medicine with neurobiological insights, including the gut-brain axis. Front Pharmacol. 2025; 16:1539357.
15. Yuan J, Yan F, Li W, Yuan G. Network pharmacological analysis of Xuefu Zhuyu decoction in the treatment of atherosclerosis. Front Pharmacol. 2022; 13:1069704.
16. Wang X, Xing X, Huang P, Zhang Z, Zhou Z, Liang L, Yao R, Wu X, Yang L. A Chinese classical prescription Xuefu Zhuyu decoction in the treatment of coronary heart disease: An overview. Heliyon. 2024; 10:e28919.
17. Han H, Wu LM, Yang WM, Wang MX, Tang JJ, Wang H, Liu ZX, Liu RZ, Dong T, Zhang J, Yang B, Han MX. Characteristics of traditional Chinese medicine syndromes in post-stroke depression. Zhong Xi Yi Jie He Xue Bao. 2010; 8:427-431.
18. Lu LL, Shen XH, Chen JX. Xuefu zhuyu oral liquid intervened stress-stimulated depression model rats. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013; 33:638-640.
19. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, Collins SM. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011; 141:599-609, 609.e591-593.
20. O'Mahony SM, Felice VD, Nally K, Savignac HM, Claesson MJ, Scully P, Woznicki J, Hyland NP, Shanahan F, Quigley EM, Marchesi JR, O'Toole PW, Dinan TG,

Cryan JF. Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. Neuroscience. 2014; 277:885-901.
21. Duan J, Lin J, Zhang N, Wang Q, Li N, Yao K. Effect of Xuefu Zhuyu Capsule on Myocardial Infarction: Network Pharmacology and Experimental Verification. Evid Based Complement Alternat Med. 2023; 2023:5652276.
22. Bai L, Zhang D, Cui TT, Li JF, Gao YY, Wang NY, Jia PL, Zhang HY, Sun ZR, Zou W, Wang L. Mechanisms Underlying the Antidepressant Effect of Acupuncture *via* the CaMK Signaling Pathway. Front Behav Neurosci. 2020; 14:563698.
23. Li J, Sha L, Xu Q. An early increase in glutamate is critical for the development of depression-like behavior in a chronic restraint stress (CRS) model. Brain Res Bull. 2020; 162:59-66.
24. Mourão AM, Vicente LCC, Abreu MNS, Vale Sant'Anna R, Vieira ELM, de Souza LC, de Miranda AS, Rachid MA, Teixeira AL. Plasma Levels of Brain-Derived Neurotrophic Factor are Associated with Prognosis in the Acute Phase of Ischemic Stroke. J Stroke Cerebrovasc Dis. 2019; 28:735-740.
25. Sicard KM, Fisher M. Animal models of focal brain ischemia. Exp Transl Stroke Med. 2009; 1:7.
26. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. Physiol Behav. 2013; 118:227-239.
27. Hamon M, Blier P. Monoamine neurocircuitry in depression and strategies for new treatments. Prog Neuropsychopharmacol Biol Psychiatry. 2013; 45:54-63.
28. Davey CG, Yücel M, Allen NB. The emergence of depression in adolescence: development of the prefrontal cortex and the representation of reward. Neurosci Biobehav Rev. 2008; 32:1-19.
29. Bai F, Hu N, Yang R, Qu LY, Ma S, Huang J, Wang JH, Yang BF, Li CL. Tongmai granules improve rat hippocampal injury by regulating TLR4/MyD88/AP-1 signaling pathway. J Ethnopharmacol. 2022; 285:114874.
30. Wu Q, Chen J, Yue J, Ying X, Zhou Y, Chen X, Tu W, Lou X, Yang G, Zhou K, Jiang S. Electroacupuncture improves neuronal plasticity through the A2AR/cAMP/PKA signaling pathway in SNL rats. Neurochem Int. 2021; 145:104983.
31. Micalizzi G, Buzzanca C, Chiaia V, Mondello M, Cacciola F, Caccamo D, Mondello L. Measurement of short-chain fatty acids in human plasma by means of fast gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2024; 1235:124044.
32. Liu W, Ge T, Leng Y, Pan Z, Fan J, Yang W, Cui R. The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. Neural Plast. 2017; 2017:6871089.
33. Han S, Li XX, Wei S, Zhao D, Ding J, Xu Y, Yu C, Chen Z, Zhou DS, Yuan TF. Orbitofrontal cortex-hippocampus potentiation mediates relief for depression: A randomized double-blind trial and TMS-EEG study. Cell Rep Med. 2023; 4:101060.
34. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol. 2019; 16:461-478.
35. Aherne CM, Collins CB, Masterson JC, Tizzano M, Boyle TA, Westrich JA, Parnes JA, Furuta GT, Rivera-Nieves J, Eltzschig HK. Neuronal guidance molecule netrin-1 attenuates inflammatory cell trafficking during acute experimental colitis. Gut. 2012; 61:695-705.
36. Wang Y, Shi Y, Li W, Wang S, Zheng J, Xu G, Li G, Shen X, Yang J. Gut microbiota imbalance mediates intestinal barrier damage in high-altitude exposed mice. FEBS J. 2022; 289:4850-4868.
37. Li B, Yang W, Ge T, Wang Y, Cui R. Stress induced microglial activation contributes to depression. Pharmacol Res. 2022; 179:106145.
38. Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW, Ho RC. Prevalence of Depression in the Community from 30 Countries between 1994 and 2014. Sci Rep. 2018; 8:2861.
39. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, Poleszak E, Fichna J, Wlaź P. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol Res. 2021; 172:105840.
40. Hu W, Kong X, Wang H, Li Y, Luo Y. Ischemic stroke and intestinal flora: an insight into brain-gut axis. Eur J Med Res. 2022; 27:73.
41. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression - A systematic review. Clin Psychol Rev. 2021; 83:101943.
42. Ding W, Wang L, Li L, Li H, Wu J, Zhang J, Wang J. Pathogenesis of depression and the potential for traditional Chinese medicine treatment. Front Pharmacol. 2024; 15:1407869.
43. Li P, Wu M, Xiong W, Li J, An Y, Ren J, Xie Y, Xue H, Yan D, Li M, Zhong G. Saikosaponin-d ameliorates dextran sulfate sodium-induced colitis by suppressing NF-κB activation and modulating the gut microbiota in mice. Int Immunopharmacol. 2020; 81:106288.
44. Liu C, Wu Y, Wang Y, Yang F, Ren L, Wu H, Yu Y. Integrating 16 S rRNA gene sequencing and metabolomics analysis to reveal the mechanism of Angelica sinensis oil in alleviating ulcerative colitis in mice. J Pharm Biomed Anal. 2024; 249:116367.
45. Lv H, Jia H, Cai W, Cao R, Xue C, Dong N. Rehmannia glutinosa polysaccharides attenuates colitis *via* reshaping gut microbiota and short-chain fatty acid production. J Sci Food Agric. 2023; 103:3926-3938.
46. Yan BF, Chen X, Chen YF, Liu SJ, Xu CX, Chen L, Wang WB, Wen TT, Zheng X, Liu J. Aqueous extract of Paeoniae Radix Alba (Paeonia lactiflora Pall.) ameliorates DSS-induced colitis in mice by tunning the intestinal physical barrier, immune responses, and microbiota. J Ethnopharmacol. 2022; 294:115365.
47. Ji W, Jiang T, Sun Z, Teng F, Ma C, Huang S, Yan S. The Enhanced Pharmacological Effects of Modified Traditional Chinese Medicine in Attenuation of Atherosclerosis Is Driven by Modulation of Gut Microbiota. Front Pharmacol. 2020; 11:546589.
48. Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, Windey K, Tremaroli V, Bäckhed F, Verbeke K, de Timary P, Delzenne NM. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A. 2014; 111:E4485-4493.
49. Voltolini C, Battersby S, Etherington SL, Petraglia F, Norman JE, Jabbour HN. A novel antiinflammatory role for the short-chain fatty acids in human labor. Endocrinology. 2012; 153:395-403.
50. Huang G, Zheng Y, Zhang N, Huang G, Zhang W, Li Q, Ren X. *Desulfovibrio vulgaris* caused gut inflammation

and aggravated DSS-induced colitis in C57BL/6 mice model. Gut Pathog. 2024; 16:39.
51. O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, Kelley KW, Dantzer R. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry. 2009; 14:511-522.
52. Liu G, Tang J, Zhou J, Dong M. Short-chain fatty acids play a positive role in colorectal cancer. Discov Oncol. 2024; 15:425.
53. Mohamed Elfadil O, Mundi MS, Abdelmagid MG, Patel A, Patel N, Martindale R. Butyrate: More Than a Short Chain Fatty Acid. Curr Nutr Rep. 2023; 12:255-262.
54. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunology. 2016; 5:e73.
55. Kelly JR, Borre Y, C OB, *et al*. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res. 2016; 82:109-118.
56. Schroeder FA, Lin CL, Crusio WE, Akbarian S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry. 2007; 62:55-64.
57. Brown AJ, Goldsworthy SM, Barnes AA, *et al*. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003; 278:11312-11319.
58. Erny D, Hrabě de Angelis AL, Jaitin D, *et al*. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015; 18:965-977.
59. Huang Y, Wu J, Zhang H, *et al*. The gut microbiome modulates the transformation of microglial subtypes. Mol Psychiatry. 2023; 28:1611-1621.
60. Xu K, Wang G, Gong J, Yang X, Cheng Y, Li D, Sheng S, Zhang F. Akkermansia muciniphila protects against dopamine neurotoxicity by modulating butyrate to inhibit microglia-mediated neuroinflammation. Int Immunopharmacol. 2025; 152:114374.

Received November 13, 2026; Revised January 20, 2026; Accepted February 5, 2026.

**Address correspondence to:*
Yanjun Zhang, National Key Laboratory of Chinese Medicine Modernization, Haihe Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, 88 Changling Road, Xiqing District, Tianjin, China.
E-mail: zyjsunye@163.com

Pengwei Zhuang, National Key Laboratory of Chinese Medicine Modernization, Haihe Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, 88 Changling Road, Xiqing District, Tianjin, China.
E-mail: zhuangpengwei@163.com

Released online in J-STAGE as advance publication February 13, 2026.