Acupuncture and Herbal Medicine

Clinical study on the modulating effects of Jiaotaiwan on the microbiota–SCFAs–neurotransmitter/immune axis in patients with depression

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Abstract

Objective: To investigate the antidepressant mechanism of Jiaotaiwan (JTW), a classic Traditional Chinese Medicine formula, by examining its effects on the gut microbiota short-chain fatty acid (SCFA) neurotransmitter/immune axis in patients with depression.

Methods: In this 8-week multicenter randomized controlled trial, 120 patients with depression were randomized to receive JTW, selective serotonin reuptake inhibitors (SSRIs), or JTW+SSRIs, and 30 healthy volunteers were enrolled as controls without intervention (healthy controls, $n = 30$). Gut microbiota profiling (16S ribosomal RNA [16S rDNA] gene sequencing), fecal SCFA quantification (gas chromatography-mass spectrometry), and plasma levels of neurotransmitters (5-hydroxytryptamine [5-HT], norepinephrine [NE], dopamine [DA]) and gut barrier/inflammatory markers (lipopolysaccharide [LPS], soluble zonula occludens-1 [sZO-1], high mobility group box 1 [HMGB1]) were assessed pre- and post-treatment. Correlations between brain gut peptides, gut flora, SCFAs, and gut barrier/inflammatory markers were analyzed using Spearman correlation analysis.

Results: Treatment with JTW, particularly in combination with SSRIs, significantly modulated gut microbiota composition by reducing Bacteroidetes abundance and increasing Firmicutes. It selectively ameliorated SCFA metabolic disturbances, notably elevating fecal levels of branched-chain fatty acids, including isobutyric and isovaleric acids. These changes were accompanied by increased plasma levels of 5-HT and DA, and reduced levels of LPS and HMGB1, suggesting improved gut barrier integrity and attenuated systemic inflammation. Correlation analysis revealed a positive association between Firmicutes abundance and sZO-1 levels, and overall coordination among microbial shifts, metabolic changes, and neurotransmitter improvements.

Conclusion: JTW may alleviate depressive symptoms through multitarget modulation of the microbiota–SCFA–neurotransmitter/immune axis, potentially involving the restoration of microbial composition, enhanced beneficial SCFA production, improved intestinal barrier function, reduced inflammation, and elevated monoamine neurotransmitters. Synergistic effects were observed when JTW was combined with SSRIs, thereby providing a mechanistic basis for using JTW in microbiota-directed approaches for treating depression.

Keywords: Depression, Jiaotaiwan, Microbiota, Neurotransmitter/immune, SCFAs

Graphical abstract: https://links.lww.com/AHM/A195.

Introduction

Depression refers to a group of mood or affective disorders characterized by depressive symptoms as the primary clinical manifestation[1]. The pathogenesis of depression involves a combination of genetic, environmental, and lifestyle risk factors[2]. Depression is a major public health burden affecting an estimated 350 million people worldwide according to the World Health Organization, ranking among the top 10 severe diseases[3]. In China, the lifetime prevalence of depression is 6.9%, and the 12-month prevalence is 3.6%[4]. Therefore, urgent

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development of more effective antidepressant drugs and elucidation of their therapeutic mechanisms remains a critical need.

Recent studies have highlighted the crucial role of brain–gut interactions in the pathogenesis of depression. The brain–gut axis connects the central nervous system (CNS) and enteric nervous system, improving neuronal function and structure through substances such as brain–gut peptides, reducing negative emotions, and achieving mutual regulation and functional integration between the brain and gut[5]. The gut microbiota, an important component of the brain–gut axis, has garnered increasing attention because its aberrations affect brain function, behavior, neuronal growth, and glial cell development[6–7]. Dysbiosis of the gut microbiota has been observed in both patients with depression and animal models[8–9]. Short-chain fatty acids (SCFAs), the primary intestinal metabolites, are organic fatty acids with 1-6 carbon atoms[10]. SCFAs play a key regulatory role in the microbiota–gut–brain axis by mediating behavioral and gut physiological processes[11]. They cross the blood–brain barrier and influence early brain development by regulating the production of brain–gut peptides, such as 5-hydroxytryptamine (5-HT) and dopamine (DA)[12–13]. Notably, 90% of 5-HT is produced by gastrointestinal epithelial cells, making them a potential link in the microbiota–gut–brain axis[14]. The gut microbiota can produce neurotransmitters directly or *via* host biosynthetic pathways, thereby affecting the function of the microbiota–gut–brain axis[15]. Alterations in gut microbiota may lead to neurotransmitter imbalance and neurodevelopmental disorders of the peripheral system[16].

Recently, extensive research has focused on the use of antidepressant medications. Selective serotonin reuptake inhibitors (SSRIs) selectively inhibit 5-HT reuptake, indirectly elevating the 5-HT concentration in the synaptic cleft. SSRIs are highly selective for 5-HT, and are widely used in clinical practice to treat various types of depression[17–18]. However, monotherapy has limited efficacy due to the complex pathology of depression[19], and its side effects remain a significant concern[20]. Traditional Chinese medicine (TCM), with its advantages of multiple chemical components, multiple target actions, and synergistic effects, has attracted growing attention[21]. TCM theory posits that "heart–kidney disharmony" is the primary etiology and mechanism of depression[22]. Jiaotaiwan (JTW), a classic TCM formula derived from *Han Shi Yi Tong*, is a pill composed of Rhizoma Coptidis (*Coptis chinensis Franch*, Ranunculaceae) and Cortex Cinnamomi (*Cinnamomum cassia*, Lauraceae) in a 10:1 ratio and is officially listed in the Chinese Pharmacopeia[23]. The combination of Coptidis Rhizoma and Cinnamomi Cortex is believed to regulate heart-kidney disharmony[24–25]. Numerous studies have shown that JTW exerts improves symptoms[26] and alleviates depression-like behaviors[27] in individuals with depression. However, the specific mechanism by which JTW regulates the microbiota and intestinal metabolites to achieve antidepressant effects remains unclear, and the relevant reports available are limited to animal experiments[28–29], indicating the need for further clinical studies.

Our randomized clinical trial (RCT) demonstrated the ability of JTW to alleviate depression symptoms and modulate serum SCFAs and the cyclic AMP (cAMP)-protein kinase A (PKA)-cAMP response element-binding protein (CREB)-brain-derived neurotrophic factor (BDNF) pathway[30], which established the clinical and systemic biological foundations of JTW therapy; the RCT included 120 patients with depression with Heart-Kidney Imbalance Syndrome, and demonstrated: (1) robust clinical efficacy: JTW monotherapy and JTW + SSRI combination significantly reduced the Hamilton Depression Scale (HAMD) and Self-rating Depression Scale (SDS) scores and (2) neural pathway activation: cAMP-PKA-CREB-BDNF neurotrophic signaling in serum was upregulated.

Building upon our previous clinical findings, the current mechanistic investigation employed previously unanalyzed multi-omics approaches, including comprehensive gut microbiome profiling, quantitative fecal SCFA analysis, and neurotransmitter-level assessment, to elucidate the upstream microbial drivers underlying the observed therapeutic effects, and aimed to comprehensively characterize the antidepressant mechanisms of JTW by investigating its specific effects on the gut microbiota–SCFA–neurotransmitter axis in patients with depression. However, these studies do not explain how the observed microbial shifts translate into neurological improvements, leaving a critical gap in understanding the intermediate processes involving "microbiota → intestinal barrier disruption → systemic inflammation → neural impairment." Therefore, the present study specifically investigated the key biomarkers in this putative pathway. We quantified the levels of gut barrier/inflammatory markers, including lipopolysaccharide (LPS)[31], a proinflammatory endotoxin derived from Gram-negative bacteria that serves as a marker of microbial translocation; soluble zonula occludens-1 (sZO-1)[32], a sensitive indicator of intestinal epithelial barrier integrity; and high mobility group box 1 (HMGB1)[33], a late-phase inflammatory mediator implicated in neuroinflammation. By examining how JTW modulates these critical biomarkers and their correlations with microbial composition, metabolic products, and neurological improvements, we aimed to validate the proposed "microbiota–SCFAs–neurotransmitters/immune" axis as a fundamental mechanism underlying the antidepressant effects of JTW.

Methods

Preparation of JTW

The medicinal plants used to prepare the JTW formula granules were *Rhizoma Coptidis* (Huanglian in Chinese, Lot number: 21020067) and *Cinnamomum Cassia* (Rougui in Chinese, Lot number: 21100229), supplied by Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd. (Sichuan, China). Twice a day, one grid each was taken with boiling water. Each grid contained 15 g of *Rhizoma Coptidis* and 1.5 g of *Cinnamomum Cassia*. The quality of JTW was also inspected according to the Tianjin Quality Standard, document number XLS-09-MP-0013-0R01 (05); the

inspection report number of *Rhizoma Coptidis*, containing the main component berberine, is 100221-21001. The inspection report number of *Cinnamomum Cassia*, with the main component of cinnamaldehyde, is 100383-21011 (specific results are shown in Supplementary Figure 1, https://links.lww.com/AHM/A193). The preparation of JTW and the analytical methods of UPLC-Q-TOF/MS are described in previous studies[34–35].

Study design and participants

This study enrolled 120 patients aged 18–65 years with mild-to-moderate depressive disorder, diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria by psychiatrists from four clinical centers in Tianjin, China, between October 2021 and October 2022. Thirty age- and sex-matched healthy volunteers were recruited as controls.

Inclusion criteria for patients were (1) DSM-V diagnosis of depressive disorder, (2) age 18–65 years, (3) Patient Health Questionnaire (PHQ-9) score ≥ 5 and < 15, (4) Generalized Anxiety Disorder (GAD)-7 score ≤ 9, (5) 24-item Hamilton Depression Rating Scale (HAMD) score between 20 and 35, (6) Hamilton Anxiety Rating Scale (HAMA) score ≤ 21, with item 6 (depressive mood) ≥ 2 and item 1 (anxious mood) < 3, (7) standard deviation score between 50 and 70, and (8) provision of informed consent.

Exclusion criteria included (1) secondary depression or severe psychiatric symptoms; (2) significant anxiety (HAMA ≥ 21); (3) allergy to study drugs; (4) suicidal tendency; (5) serious neurological or systemic diseases; (6) severe cardiovascular, hepatic, or renal dysfunction; (7) glaucoma or epilepsy; (8) recent use of antibiotics or probiotics; (9) significant gastrointestinal symptoms or recent gastroscopy; (10) pregnancy or lactation; (11) participation in other clinical trials within 30 days; or (12) other conditions deemed unsuitable by investigators.

Healthy controls were required to: (1) be aged 35–65 years and (2) undergo medical screening to exclude somatic or chronic intestinal diseases through physical examination, medical history, and laboratory tests. A narrower age range (35–65) for healthy controls was chosen to better match the expected age distribution of the patient population while minimizing the baseline metabolic and microbial variability associated with very young adulthood.

The sample size was calculated *a priori* using the G*Power software (version 3.1.9.7). The calculation was based on the primary outcome of the efficacy rate of the TCM syndrome, as reported in a previous clinical trial[36], which showed efficacy rates of 85.71% in the treatment group and 44.12% in the control group. To detect this effect size ($\varphi = 0.425$) using a Chi-square test, with a two-sided alpha (α) error of 0.05 and a statistical power ($1 - \beta$) of 95%, a minimum of 28 participants per group was required. To account for a potential drop-out rate of approximately 20%–25% during the 8-week intervention period, we planned to enroll 40 participants in each of the three patient groups, resulting in a total target enrollment of 120 patients.

Patients who met the inclusion criteria were randomly assigned in a 1:1:1 ratio to one of three treatment groups (JTW, JTW + SSRI, and SSRIs groups; $n = 40$ per group). The randomization sequence was generated using SAS (version 9.4; SAS Institute, Cary, NC, USA) by an independent statistician who was not involved in the participant recruitment or outcome assessment. The allocation sequence was concealed using sequentially numbered opaque sealed envelopes.

Healthy controls ($n = 30$) were not included in the randomization procedure, as they did not receive any intervention. To ensure the comparability of general characteristics between the healthy controls and patient groups, we implemented a proactive matching strategy. Healthy volunteers were recruited to match the expected age and sex distribution of the patient cohort based on the epidemiological data of depressive disorders. The inclusion of healthy controls was staggered throughout the patient recruitment period to allow for real-time matching. The final matching was confirmed by comparing the baseline demographics (age, sex, and body mass index [BMI]) between the healthy control and pooled patient groups using independent t-tests and Chi-square tests, which showed no significant differences (Table 1), thus validating the matching approach.

This study followed the guidelines of the *Declaration of Helsinki* and Tokyo for humans. The study protocol was approved by the Ethics Committee of the Tianjin University of TCM (No. TJUTCMEC20210006). The study protocol was registered in the International Traditional Medicine Clinical Trial Registry (No. ITMCTR2025000151). All participants provided written informed consent before participating in the study procedures. Specific measures to protect participants' rights, including the confidentiality of their data, the voluntary nature of their participation, and their right to withdraw from the study at any time without penalty, were rigorously implemented throughout the trial.

Intervention

The JTW group received one granule packet twice daily. The SSRI group received one of the following: escitalopram oxalate (10 mg/day), citalopram hydrobromide (20 mg/day), sertraline (50 mg/day), or fluvoxamine (100 mg/day). The combination group received both JTW and SSRIs as described earlier. The treatment duration was 8 weeks.

Clinical assessments

Clinical visits were conducted at baseline (week 0) and at weeks 4 and 8. The HAMD and SDS were administered at each visit to evaluate the treatment response.

Sample collection and processing

Fasting blood and fecal samples were collected from the patients at weeks 0 and 8. Fecal samples (5 g) were stored at −80°C for gut microbiota and SCFAs analyses. Blood samples (4 mL) were collected in ethylenediaminetetraacetic acid tubes, centrifuged, and the plasma was stored at −80°C until analysis.

Table 1.

Analysis of participants' general characteristics

Variable/group	JTW group, *n* = 40	JTW + SSRIs group, *n* = 40	SSRIs group, *n* = 40	Heathy people group, *n* = 30	*P* value
Sex, *n* (%)					0.407
Male	13 (32.5)	17 (42.5)	11 (27.5)	13 (43.3)	
Female	27 (67.5)	23 (57.5)	29 (72.5)	17 (56.6)	
Age (year)	38.6 ± 11.6	42.9 ± 13.0	42.0± 14.1	44.7 + 14.6	0.092
Ethnic group, *n* (%)					0.181
Han ethnic group	39 (97.5)	39 (97.5)	40 (100.0)	30 (100.0)	
Other ethnic groups	1 (2.5)	1 (2.5)	0 (0.0)	0 (0.0)	
Blood pressure (mmHg)					
SBP	119.0 ± 8.3	121.9 ± 7.7	121.6 ± 9.1	129.8 ± 20.7	0.002
DBP	80.7 ± 7.5	80.2 ± 6.9	79.6 ± 7.6	83.4 ± 12.1	0.282
BMI, kg/m^2	23.3 ± 3.6	23.6 ± 4.0	23.9 ± 3.8	23.1 ± 3.5	0.819
Marital status, *n* (%)					0.181
Married	28 (70.0)	29 (72.5)	31 (77.5)	17 (56.7)	
Unmarried	11 (27.5)	11 (27.5)	8 (20.0)	11 (36.7)	
Others	1 (2.5)	0 (0.0)	1 (2.5)	2 (6.7)	
Occupation, *n* (%)					0.156
Laborer	2 (5.0)	1 (2.5)	0 (0.0)	0 (0.0)	
Retired	6 (15.0)	8 (20.0)	13 (32.5)	8 (26.7)	
Farmer	0 (0.0)	1 (2.5)	1 (2.5)	3 (10.00)	
Cadre	2 (5.0)	1 (2.5)	3 (7.5)	0 (0.00)	
Employee	19 (47.5)	16 (40.0)	12 (30.0)	4 (13.3)	
Others	11 (27.5)	13 (32.5)	11 (27.5)	15 (50.0)	
PHQ-9 scores	11.4 ± 3.0	10.8 ± 2.7	11.1 ± 2.8	–	0.686
GAD-7 scores	6.8 ± 1.9	6.1 ± 2.3	6.1 ± 2.7	–	0.357
HAMA scores	15.5 ± 4.7	15.0 ± 4.0	14.0 ± 5.0	–	0.335

BMI: Body mass index; DBP: Diastolic blood pressure; GAD-7: Generalized Anxiety Disorder-7; HAMA: Hamilton Anxiety Scale; PHQ-9: Patient Health Questionnaire-9; SBP: Systolic Blood pressure.

Owing to constraints in sample collection, processing, and quality, not all collected samples were analyzed for all assays. The final sample sizes for each assay and group were as follows.

16S rRNA gene sequencing: JTW group (*n* = 13), JTW + SSRIs group (*n* = 11), SSRIs group (*n* = 13), and healthy control group (*n* = 14).

SCFAs analysis: JTW group (*n* = 25), JTW + SSRIs group (*n* = 25), SSRIs group (*n* = 29), and healthy control group (*n* = 30).

Enzyme-linked immunosorbent assay of brain gut peptide biomarkers: JTW group (*n* = 31), JTW + SSRIs group (*n* = 30), SSRIs group (*n* = 30), and healthy control group (*n* = 30).

Validation assays of gut barrier/inflammatory markers: To maintain direct comparability with the gut microbiota profiles, validation analyses were performed only on a subset of samples with available identical sample sizes for 16S rRNA sequencing.

The final sample size for gut microbiota analysis was smaller than that of the clinical cohort, primarily due to stringent quality control measures. Samples with insufficient biomass, low DNA yield, or poor DNA quality (as assessed by spectrophotometry and gel electrophoresis) were excluded to ensure reliability of the sequencing data.

To ensure a direct and unbiased correlation between gut microbial profiles and downstream host validation assays, all subsequent biochemical and molecular analyses of inflammatory markers were strictly performed on an identical subset of samples that passed quality control for 16S rRNA sequencing.

This approach, although reducing the overall N for validation, maximizes the internal validity of the brain–gut axis correlations investigated in this study. Baseline characteristics were compared between the full cohort and the subset with gut microbiota data (as shown in Supplementary Table 1, https://links.lww.com/AHM/A193) to demonstrate the representativeness of the gut microbiota data. Crucially, all 120 randomized patients were included in the primary clinical efficacy analysis based on the intention-to-treat principle.

Laboratory analyses

The plasma levels of 5-HT, norepinephrine (NE), and DA were determined using kits purchased from Genomics Biotechnology Co., Ltd. (Wuhan, China) according to the manufacturer's protocols. The levels of LPS, sZO-1, and HMGB1 in plasma were determined using kits purchased from Colorful Gene Biological Technology Co., Ltd. (Wuhan, China), according to the manufacturer's protocols.

Fecal microbial DNA was extracted, and the V3–V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina NextSeq 2000 platform (Illumina, San Diego, USA).

The SCFAs were quantified using an Agilent 8890B-7000D (Agilent Technologies Inc., CA, USA) GC/MS system with a high-polarity free fatty acid phase (HP-FFAP) column under optimized chromatographic and mass spectrometric conditions.

Statistical analysis

Data were analyzed using SPSS Statistics (version 25.0) and R software (version 4.4.3; www.rproject.org). To handle missing data, multiple imputations were performed using the Multivariate Imputation by Chained Equations algorithm.

The normality of continuous variables was assessed using the Shapiro-Wilk test. The primary analyses focused on within-group changes over time and the between-group differences in these changes. To account for repeated measurements from the same subjects and optimally utilize all available data, we fitted linear mixed models (LMMs). Subjects were included as a random effect (random intercept) to model within-subject correlation. The fixed effects included Group, Time, and the Group × Time interaction term. For *post hoc* analyses of significant effects, the estimated marginal means were compared. The false discovery rate (FDR) was controlled for all *post hoc* pairwise comparisons using the Benjamini–Hochberg procedure. The results of the FDR-corrected comparisons are presented in Supplementary Material 2 (https://links.lww.com/AHM/A194). For clarity in interpreting the primary within-group effects, uncorrected *P* values from the LMM *post hoc* tests are reported in the main text.

The final sample sizes for analysis varied by assay for reasons stated in Sample collection and processing section. This resulted in an imbalanced study design. The LMM approach was employed for its robustness in handling unbalanced data and missing at random mechanisms. Furthermore, the multiple imputation technique was applied to potential baseline covariates to maximize statistical power and minimize bias arising from missingness, ensuring the reliability of our inferences.

Between-group differences in baseline characteristics were compared using independent-sample t-tests (for normal data) or Mann-Whitney U tests (for non-normal data) for continuous variables, and Chi-square tests for categorical variables.

Correlations among brain–gut peptides, gut flora, SCFAs, and gut barrier/inflammatory markers were assessed using Spearman's rank correlation analysis.

A two-sided *P* value < 0.05 was considered statistically significant for all analyses, unless otherwise specified such as "after FDR correction."

Results

General information

Of the 150 subjects, 36% were male with a mean age of (42.8 ± 13.4) years and mean BMI of (23.5 ± 3.7); the 120 patients with depression had mean PHQ-9 scores of (11.4 ± 3.0), GAD-7 scores of (6.3 ± 2.3), and HAMA scores of (14.8 ± 4.8). The ethnicity, marital status, and occupational distribution of patients in the four groups were not statistically different (Table 1). As previously reported[30], all three treatment groups showed significant reductions in HAMD and SDS scores from baseline to week 8 ($P < 0.01$), with the JTW + SSRIs group demonstrating the most pronounced improvement.

Comparison of intestinal flora in the four groups

Alpha diversity analysis

Alpha diversity analysis primarily aims to evaluate the richness and diversity of the microbial communities in clinical samples using multiple diversity indices. As depicted in Figure 1A–C, the Sobs, Chao, and Ace curves were employed to reflect community richness, while Figure 1D–F illustrates the Shannon, Simpson, and coverage curves for assessing community diversity. The dilution curves of all groups showed a flattening trend, indicating that the sequencing data, depth, and sample size were adequate to ensure data reliability. To observe intergroup differences in intestinal flora diversity, we analyzed Sobs, Chao, Ace, Shannon, Simpson, and Coverage indices. Specifically, Sobs, Chao, and Ace were used to characterize community richness, whereas Shannon, Simpson, and Coverage reflected community diversity. Figure 2 demonstrates the statistically significant differences in community diversity among groups for the Sobs, Chao, Ace, and Coverage indices ($P < 0.01$). These results suggest that administration of JTW and/or SSRIs induces significant changes in the alpha diversity of the gut flora in patients with depression.

Beta diversity analysis

Beta diversity involves comparing microbial community compositions across different samples to assess inter-community differences. Principal Coordinate Analysis (PCoA) at the Operational Taxonomic Unit level was used to explore between-group variations (Figure 3A), where dots of distinct colors/shapes represent different groups, and closer dots indicate more similar communities. PCoA explained 23.19% of the total variation in the gut microbial communities. Adonis permutational multivariate analysis of variance revealed significant between-group variability in gut microbes ($R^2 = 0.087$, $P = 0.031$), whereas ANOSIM analysis by sample grouping showed significant differences in the intestinal flora among groups ($R^2 = 0.050$, $P = 0.010$) (Figure 3B), confirming the rationality of the grouping strategy.

Species composition and differences analysis

Community structure component maps can more intuitively show the community structure (species with higher relative abundances and proportions) of each group at different taxonomic levels. Based on the results of species annotation, the top 10 species in each group at each taxonomic level (phylum and genus) were selected to

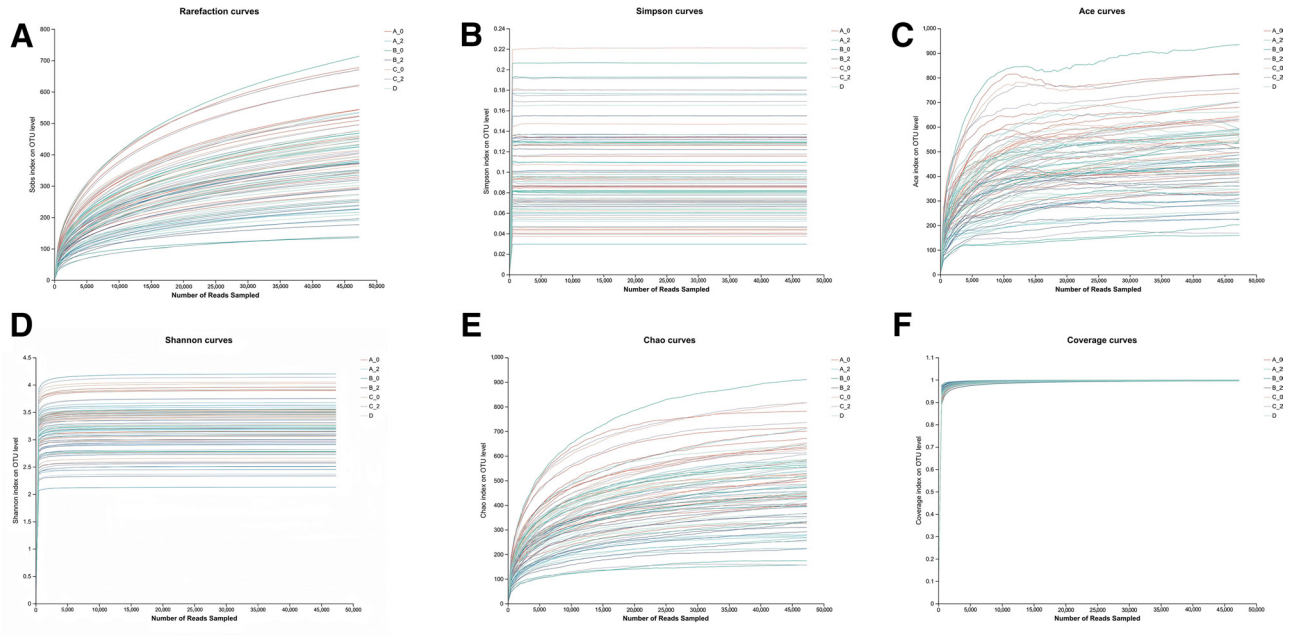

Figure 1. Curve graph of species diversity and richness. (A) Rarefraction curves. (B) Simpson curves. (C) Ace curves. (D) Shannon curves. (E) Chao curves. (F) Coverage curves. Note: A_0: JTW group at 0 weeks; A_2: JTW group at 8 weeks; B_0: JTW + SSRIs at 0 weeks; B_2: JTW + SSRIs at 8 weeks; C_0: SSRIs group at 0 weeks; C_2: SSRIs group at 8 weeks; D: Healthy control group. JTW: Jiaotaiwan; SSRI: selective serotonin reuptake inhibitor.

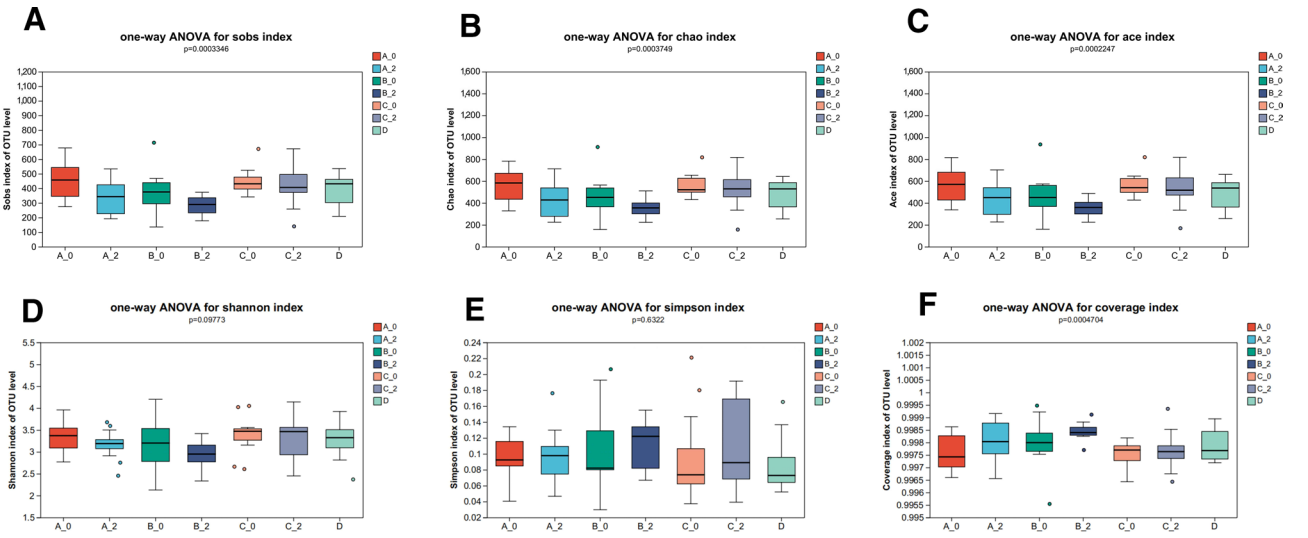

Figure 2. Alpha diversity index of inter-group difference. (A) One-way ANOVA for sobs index. (B) One-way ANOVA for chao index. (C) One-way ANOVA for ace index. (D) One-way ANOVA for Shannon index. (E) One-way ANOVA for simpson index. (F) One-way ANOVA for coverage index. Note: A_0: JTW group at 0 weeks; A_2: JTW group at 8 weeks; B_0: JTW + SSRIs at 0 weeks; B_2: JTW + SSRIs at 8 weeks; C_0: SSRIs group at 0 weeks; C_2: SSRIs group at 8 weeks; D: Healthy control group. JTW: Jiaotaiwan; SSRI: selective serotonin reuptake inhibitor.

generate a bar-cumulative plot of the relative abundance of species.

At the phylum level, the top 10 species in relative abundance for each group were Firmicutes, Actinobacteria, Bacteroidota, Proteobacteria, Verrucomicrobia, Desulfobacterota, Patescibacteria, Cyanobacteria, unclassified_k_norank_d_Bacteria, and Fusobacteria, with Firmicutes, Actinobacteria, Bacteroidota, and Proteobacteria being the most abundant; among them the proportion of Firmicutes was the most abundant. Firmicutes accounted for more than 50% of the abundance (Figure 4A).

At the genus level, the top 10 species in terms of relative abundance in each group were *Faecalibacterium*, *Blautia*, *Bifidobacterium*, *Bacteroides*, *Subdoligranulum*, *Agathobacter*, *Eubacterium_hallii_group*, *Anaerostipes*, *Escherichia-Shigella*, *Dorea*, with *Faecalibacterium*, *Blautia*, and *Bifidobacterium* being the most abundant (Figure 4B).

The composition of superior and inferior bacteria within each group was represented using a species community heat map, which shows the grouping horizontally and the clustering of the different species in each sample vertically, with shades of colors indicating the magnitude and differences in the abundance of the species in the two groups of samples. At the genus level, *Faecalibacterium*, *Bacteroides*, *Roseburia*, *Fusicatenibacter*, *Lactobacillus*, *Parabacteroides*, and Eubacterium_ventriosum_group in the depression group, and *Phascolarctobacterium*, *Klebsiella*, and *Lachnoclostridium* were in higher

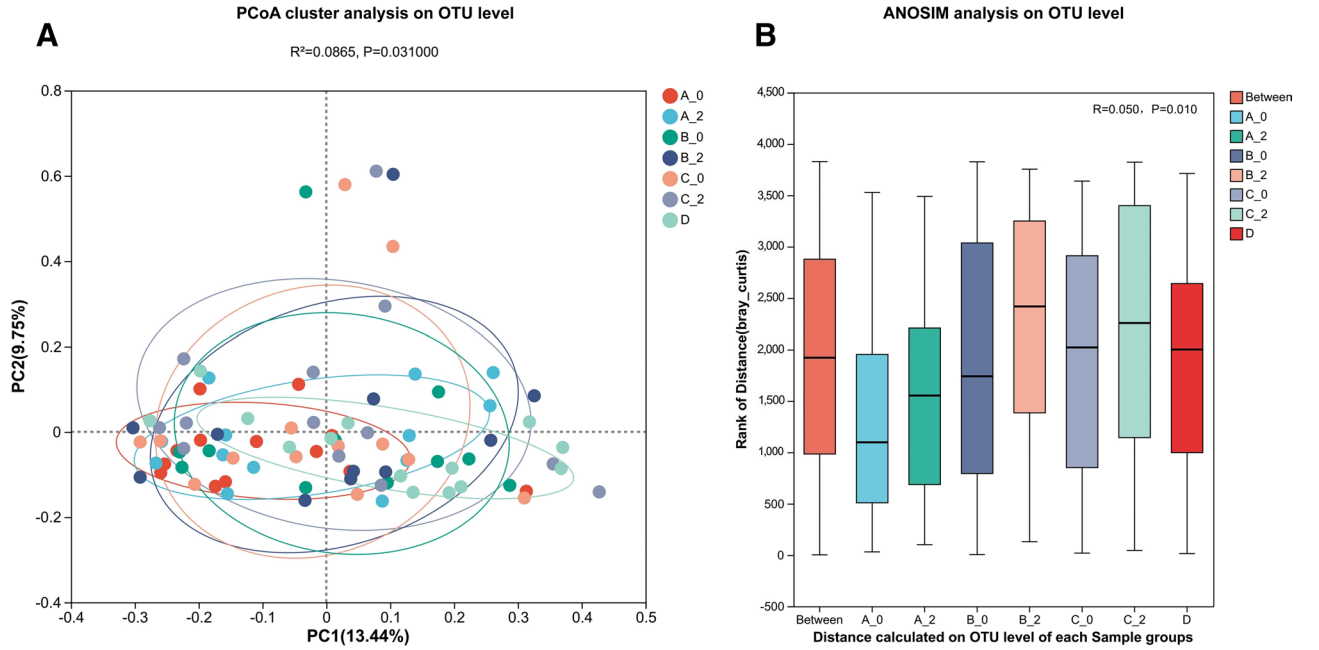

Figure 3. PCoA cluster analysis and ANOSIM analysis on the OTU level. (A) PCoA cluster analysis on the OTU level and (B) ANOSIM analysis on the OTU level, X-axis is the distance value within or between groups; Between Corresponding bins represent distance values for differences between groups; and the rest of the boxes represent the distance value of the difference within groups; the Y-axis scale indicates the size of the distance value. A_0: JTW group at 0 weeks; A_2: JTW group at 8 weeks; B_0: JTW + SSRIs at 0 weeks; B_2: JTW + SSRIs at 8 weeks; C_0: SSRIs group at 0 weeks; C_2: SSRIs group at 8 weeks; D: Healthy control group. JTW: Jiaotaiwan; OUT: Operational Taxonomic Unit; PCoA: principal coordinate analysis; SSRI: selective serotonin reuptake inhibitor.

abundance and may be associated with the development of depression (Figure 4C).

At the phylum level, compared with the healthy human group, the abundance of Bacteroidetes was significantly increased in the JTW and JTW + SSRIs groups before drug administration, and the difference was statistically significant ($P < 0.01$); after drug administration, the abundance of Bacteroidetes in both the JTW and JTW + SSRIs groups was significantly reduced compared to that before drug administration ($P < 0.01$). Compared to the healthy group, Firmicutes was significantly less abundant in the JTW + SSRI group before drug administration ($P < 0.01$); after drug administration, Firmicutes was significantly more abundant in the JTW + SSRI group ($P < 0.05$).

At the genus level, the abundance of *Bacteroides* significantly increased in the JTW and JTW + SSRIs groups compared to the healthy group before drug administration, and the difference was statistically significant ($P < 0.01$); after administration, the abundance of *Bacteroides* decreased in the JTW and JTW + SSRIs groups, and the difference was statistically significant ($P < 0.05$) (Figure 4D).

Comparison of SCFAs in the four groups

Before treatment, there was no statistically significant difference in SCFA levels between the three groups of patients with depression. Fecal isobutyric acid, butyric acid, isovaleric acid, valeric acid, isohexanoic acid, and hexanoic acid levels tended to decrease, whereas acetic acid levels tended to increase in patients with depression compared to healthy individuals ($P < 0.05$ or $P < 0.01$).

Compared with pre-treatment levels, butyric acid and isohexanoic acid levels were significantly higher in the JTW group; acetic acid levels were significantly lower; isobutyric, isovaleric, and isohexanoic acid levels were significantly higher in the JTW + SSRI group; and isobutyric and isovaleric acid levels were significantly higher in the SSRIs group ($P < 0.05$ or $P < 0.01$) (Figure 5).

Comparison of brain gut peptides in the four groups

Before treatment, 5-HT and NE tended to decrease in the three groups of patients with depression compared to the healthy control group. After treatment, 5-HT and NE levels were significantly higher in the three groups of patients with depression than in the pre-treatment group ($P < 0.05$ or $P < 0.01$). (Figure 6).

Comparison of gut barrier/inflammatory markers in the four groups

Prior to treatment, LPS and HMGB1 levels were significantly higher in all three groups than in the healthy control group ($P < 0.05$ or $P < 0.01$). Following treatment, LPS, sZO-1, and HMGB1 levels in the JTW + SSRIs group were significantly lower than the pre-treatment levels, whereas sZO-1 levels in both the JTW and JTW + SSRIs groups were lower than the pre-treatment levels ($P < 0.05$ or $P < 0.01$) (Figure 7).

Correlation analysis of treatment-induced changes

To comprehensively characterize the impact of JTW + SSRIs treatment on the gut–brain axis, we conducted correlation analyses of longitudinal changes (Δ) to evaluate co-varying shifts in these parameters induced by the 8-week treatment (Figure 8). Several significant correlations were observed; a strong positive

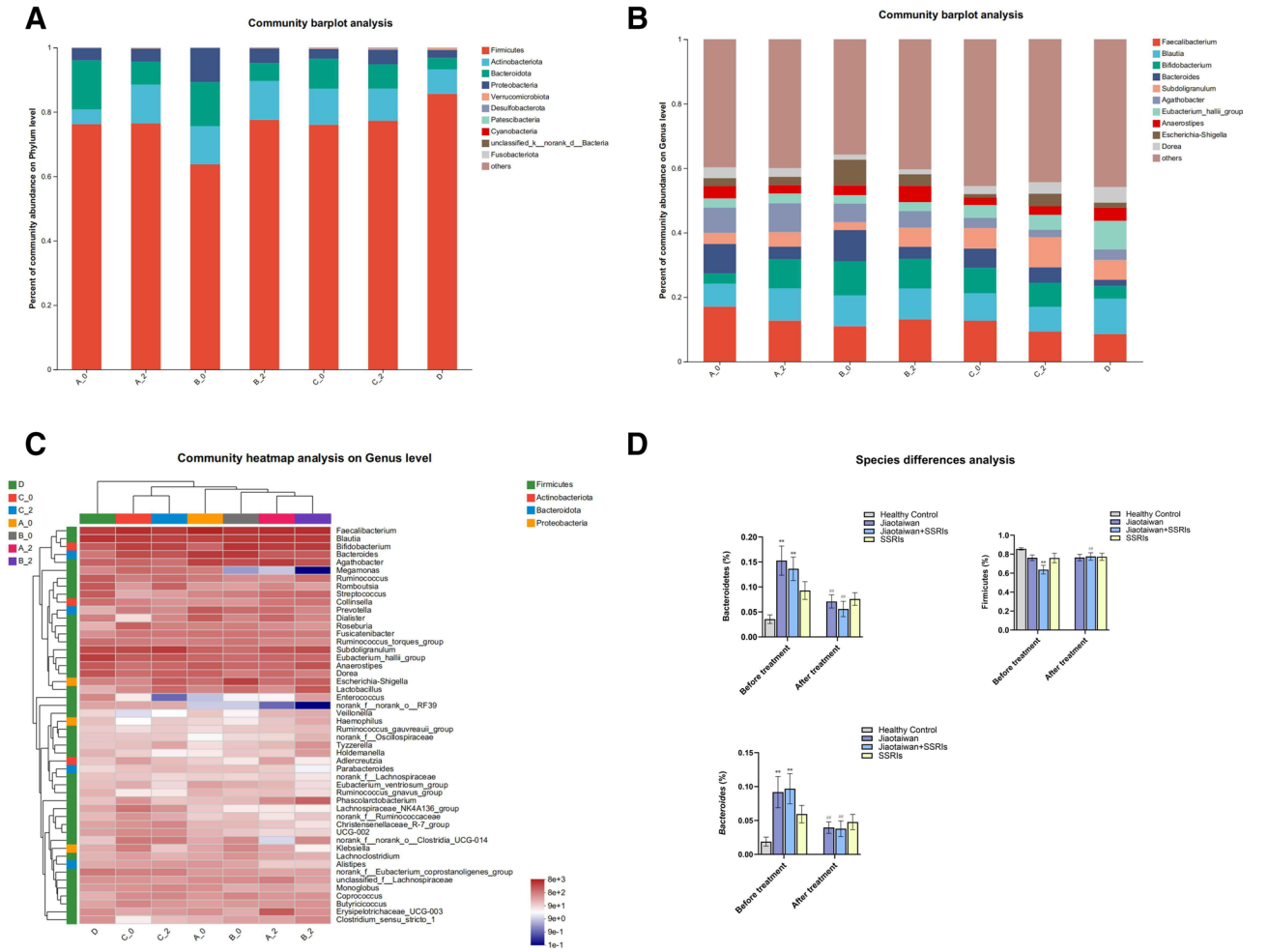

Figure 4. Species composition and differences analysis. (A) Community bar plot analysis at the phylum level; (B) community bar plot analysis at the genus level; (C) community heatmap analysis at the genus level; and (D) analysis of species differences. A_0: JTW group at 0 weeks; A_2: JTW group at 8 weeks; B_0: JTW + SSRIs at 0 weeks; B_2: JTW + SSRIs at 8 weeks; C_0: SSRIs group at 0 weeks; C_2: SSRIs group at 8 weeks; and D: Healthy control group. Before treatment, compared with the healthy group, $^{**}P < 0.01$; The group after treatment compared to before treatment, $^{\#\#}P < 0.01$. JTW: Jiaotaiwan; SSRIs: Selective Serotonin Reuptake Inhibitors.

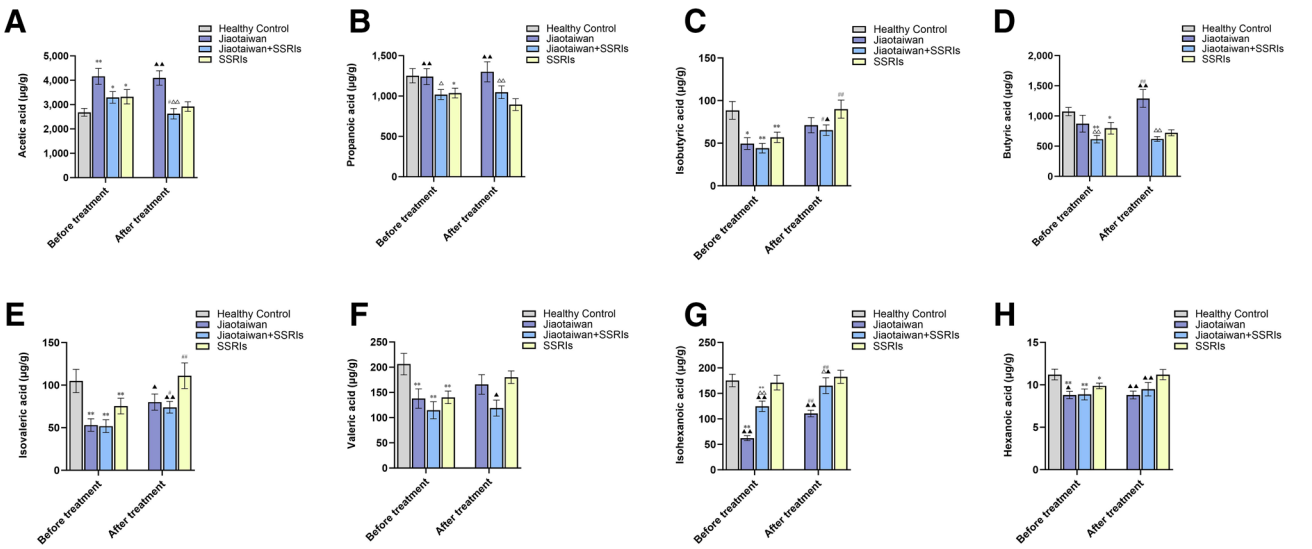

Figure 5. Comparison of SCFAs before and after treatment. (A) comparison of acetic acid level before and after treatment; (B) comparison of propanoic acid level before and after treatment; (C) comparison of isobutyric acid level before and after treatment; (D) comparison of butyric acid level before and after treatment; (E) comparison of isovaleric acid level before and after treatment; (F) comparison of valeric acid level before and after treatment; (G) comparison of isohexanoic acid level before and after treatment; (H) comparison of hexanoic acid level before and after treatment. Note: Before treatment, compared with the healthy group, $^{*}P < 0.05$, $^{**}P < 0.01$; The group after treatment compared to before treatment, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; Compared with JTW group, $^{\triangle\triangle}P < 0.01$; Compared with the SSRIs group, $^{\blacktriangle\blacktriangle}P < 0.01$. JTW: Jiaotaiwan; SSRI: selective serotonin reuptake inhibitor.

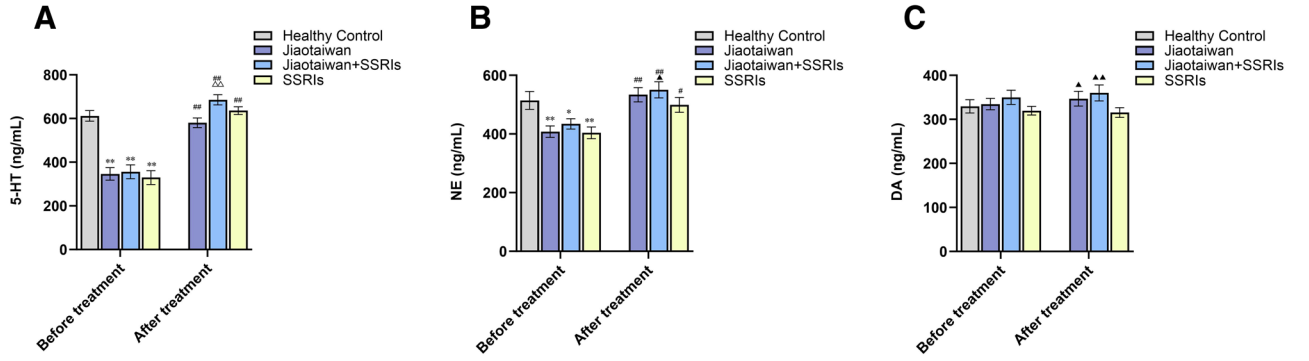

Figure 6. Comparison of brain–gut peptides before and after treatment. (A) comparison of 5-HT level before and after treatment; (B) comparison of NE level before and after treatment; (C) comparison of DA level before and after treatment. Note: Before treatment, compared with the healthy group, $^{*}P < 0.05$, $^{**}P < 0.01$; The group after treatment compared to before treatment, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared with the SSRIs group, $\blacktriangle P < 0.05$. 5-HT: 5-Hydroxytryptamine; DA: Dopamine; JTW: Jiaotaiwan; NE: Norepinephrine; SSRIs: Selective serotonin reuptake inhibitors.

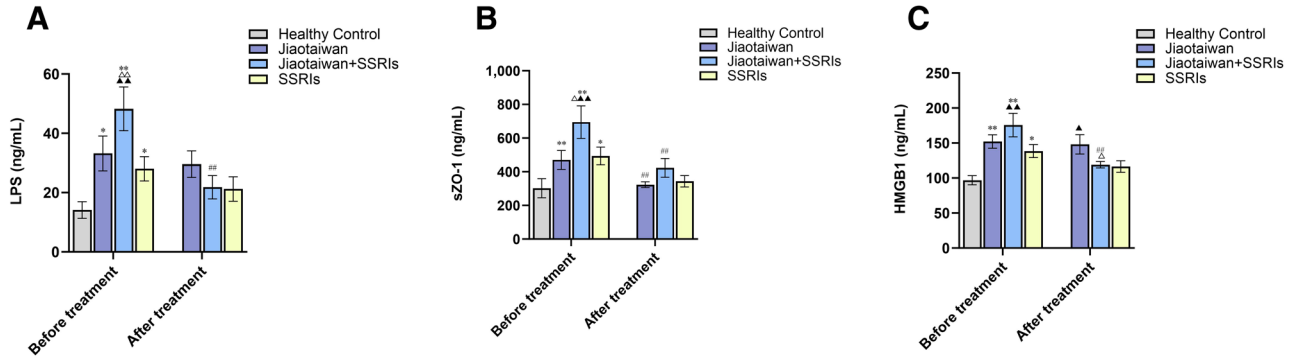

Figure 7. Comparison of gut barrier/inflammatory markers before and after treatment. (A) comparison of LPS level before and after treatment; (B) comparison of sZO-1 level before and after treatment; (C) comparison of HMGB1 level before and after treatment. Note: Before treatment, compared with the healthy group, $^{*}P < 0.05$, $^{**}P < 0.01$; the group after treatment compared to before treatment, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the JTW group, $^{\triangle}P < 0.05$, compared with the SSRIs group, $\blacktriangle P < 0.05$. HMGB1: High mobility group box 1; JTW: Jiaotaiwan; LPS: Lipopolysaccharide; SSRIs: Selective serotonin reuptake inhibitors; sZO-1: Soluble zonula occludens-1.

correlation was found between isobutyric acid and isovaleric acid ($r = 0.78$, $P = 0.0045$). The abundance of Firmicutes was positively correlated with sZO-1 ($r = 0.64$, $P = 0.035$), a key tight junction protein, and isohexanoic acid ($r = 0.66$, $P = 0.026$). Similarly, the LPS and HMGB1 levels were significantly correlated ($r = 0.78$, $P = 0.0045$). Bacteroidetes abundance positively correlated with *Bacteroides* ($r = 0.69$, $P = 0.019$), isobutyric acid ($r = 0.75$, $P = 0.007$), and isovaleric acid ($r = 0.64$, $P = 0.035$). Additionally, NE and 5-HT levels were positively correlated ($r = 0.70$, $P = 0.017$). Scatter plots visualizing these significant Spearman correlations are shown in Supplementary Figure 2 (https://links.lww.com/AHM/A193).

To assess the robustness of the significant correlations identified, we performed a sensitivity analysis using the interquartile range (IQR) criterion to identify and remove potential outliers. The results demonstrated varying degrees of sensitivity among the correlations: the correlations between 5-HT and NE ($\Delta r = 0.000$, outliers removed = 0) and between Firmicutes and isohexanoic acid ($\Delta r = 0.000$, outliers removed = 0) remained consistent after outlier removal, indicating that these relationships were highly robust. Three correlations showed moderate sensitivity to outlier removal: isobutyric acid *versus* isovaleric acid ($\Delta r = -0.165$, P value change: 0.004 to 0.077), HMGB1 *versus* LPS ($\Delta r = -0.116$, P value change: 0.004 to 0.071), and Firmicutes *versus* sZO-1 ($\Delta r = -0.109$, P value change: 0.035 to 0.117). While the correlation coefficients were attenuated, the overall positive relationships were maintained. However, three correlations were identified as sensitive to outliers: Bacteroidetes *versus Bacteroides* ($\Delta r = -0.241$, P value change: 0.019 to 0.224), Bacteroidetes *versus* isobutyric acid ($\Delta r = -0.205$, P value change: 0.007 to 0.125), and Bacteroidetes *versus* isovaleric acid ($\Delta r = -0.286$, P value change: 0.035 to 0.356). The strength of these correlations decreased substantially after removing the two outliers in each case, and they lost statistical significance at the $P < 0.05$ level. These findings suggest that while several key relationships in our gut–brain axis analysis are robust, some correlations involving Bacteroidetes may be particularly influenced by extreme values and should be interpreted with caution (Supplementary Table 2, https://links.lww.com/AHM/A193).

Discussion

Depression is a highly prevalent and debilitating disorder that imposes significant economic and psychological burden on patients and their families[37]. SSRIs are widely used as first-line antidepressants because of their relative safety and tolerability[38]. However, they are often associated with incomplete response, slow onset of action, and

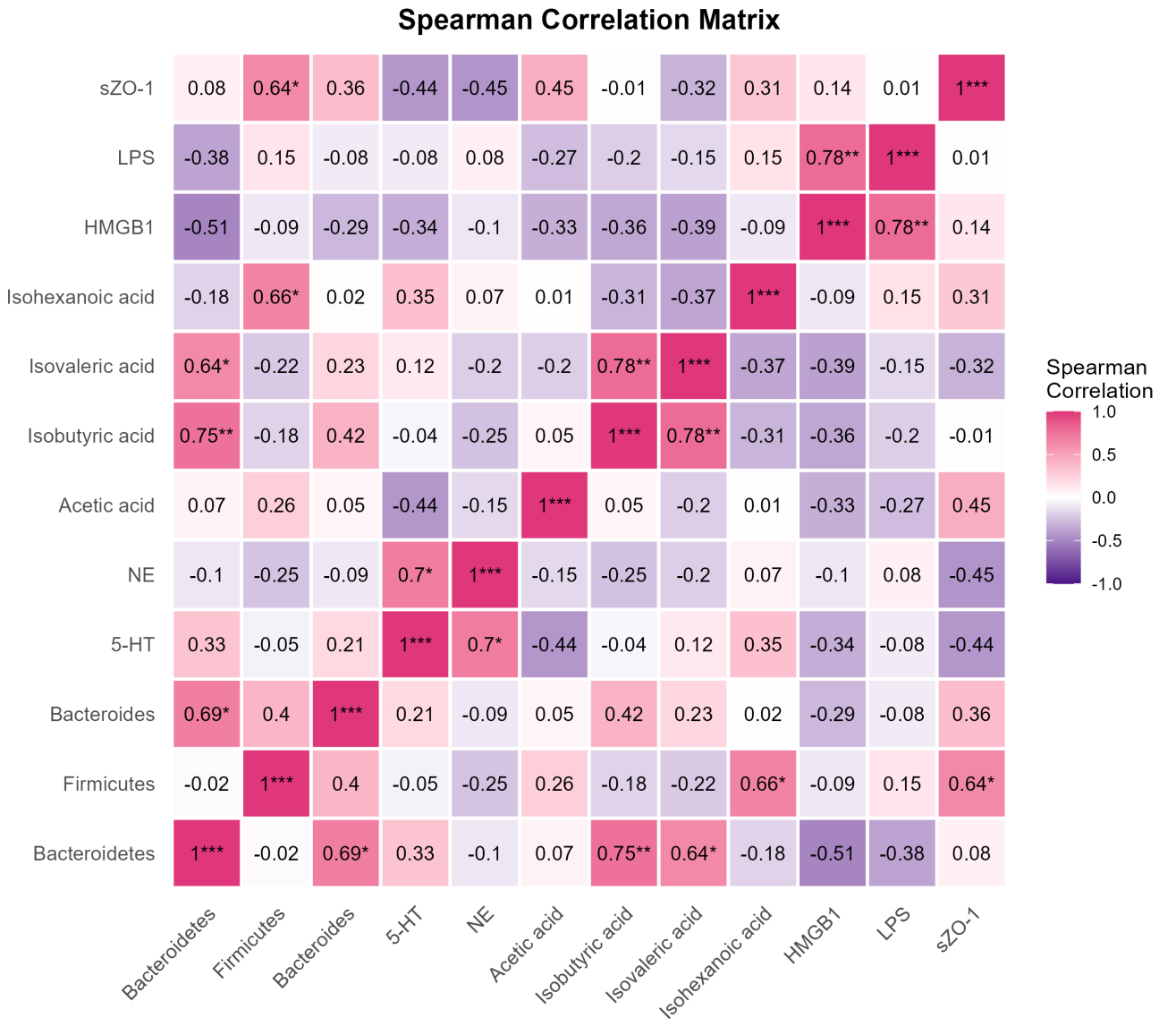

Figure 8. Correlation analysis of JTW + SSRIs treatment-induced changes. *$P < 0.05$, **$P < 0.01$, 5-HT: 5-Hydroxytryptamine; NE: Norepinephrine; LPS: Lipopolysaccharide; sZO-1: soluble Zonula Occludens-1; HMGB1: High mobility group box 1.

risk of relapse after discontinuation[39–40]. TCM offers a holistic approach with potential advantages in terms of personalized treatment and reduced side effects. JTW, a classic TCM formula, has previously demonstrated efficacy in improving depressive symptoms in animal models[27,35]. This study provides a comprehensive analysis of the mechanisms through which JTW, both as a monotherapy and in combination with SSRIs, may alleviate depression by modulating the gut microbiota–SCFA–neurotransmitter axis.

Consistent with previous reports linking gut dysbiosis to depression, the patients in this study had significantly increased abundance of Bacteroidetes and decreased Firmicutes compared to healthy controls. Elevated levels of Bacteroidetes may contribute to inflammation by increasing LPS production[41–42]. Following treatment, both the JTW and JTW + SSRIs groups showed reduced Bacteroidetes and increased Firmicutes, particularly in the combination group, suggesting a rebalancing effect on the gut microbiota. Because Firmicutes are important producers of SCFAs, their recovery may support the integrity of the gut barrier and anti-inflammatory responses[43]. At the genus level, reductions in *Bacteroides* further supported targeted modulation by JTW-containing treatments. These findings suggest that the antidepressant effects of JTW may partially originate from its multi-targeted regulation of the gut microbial ecosystem, potentially suppressing opportunistic pathogens while promoting beneficial bacteria.

The observed SCFA metabolic disturbances may be associated with the activation of inflammatory pathways, elevated proinflammatory cytokines, and microglial proliferation in the CNS[44]. Simultaneously, such disruptions may impair the normal biosynthesis of neuroprotective factors such as BDNF[45]. Ultimately, these processes may lead to reduced 5-HT levels due to neuronal apoptosis and metabolic dysfunction, potentially contributing to depression[46]. In this study, pretreatment SCFA deficits (isobutyric, butyric, isovaleric, valeric, isohexanoic, and hexanoic acids) along with elevated acetic acid aligned with impaired gut–brain axis signaling in depression[47]. Following treatment, JTW specifically

increased isovaleric and isohexanoic acids, whereas JTW + SSRIs elevated branched-chain SCFAs (isobutyric/isovaleric/isohexanoic) but reduced acetic acid. SSRIs alone increased isobutyric and valeric acid levels. Since branched-chain SCFAs are primarily produced by protein fermentation, their abnormal variations may reflect disruptions in microbial substrate utilization and could influence mitochondrial function and gamma-aminobutyric acid (GABA)ergic activity[48]. These alterations suggest that JTW may not simply elevate all SCFAs indiscriminately but rather selectively correct abnormal metabolic patterns, particularly deficiencies in branched-chain fatty acids.

Pretreatment reductions in 5-HT and NE reflect the monoaminergic dysfunction characteristically observed in depression[49]. All treatments elevated 5-HT and NE levels, but JTW + SSRIs group showed superior efficacy: higher 5-HT than that of JTW alone and higher NE than that of SSRIs alone. This synergy suggests that JTW potentiates the effects of SSRIs on monoamine transmission, possibly *via* gut-mediated pathways. The DA increase in the JTW + SSRIs group is particularly notable, as DA deficiency has been implicated in anhedonia, a core symptom of depression[50]. This suggests that JTW might enhance the classical 5-HT reuptake inhibitory effect of SSRIs, potentially offering a new combination strategy for patients with poor response to SSRIs alone, particularly those with DA-ergic dysfunction.

Endotoxin LPS and the late inflammatory mediator HMGB1 serve as key indicators of intestinal barrier function and systemic low-grade inflammation[51–53]. Before treatment, the patients showed evidence of intestinal permeability and immune activation, manifested by elevated LPS and HMGB1 levels. The positive correlation between LPS and HMGB1 suggests a potential pathological pathway involving increased intestinal permeability → endotoxin translocation → immune activation → inflammatory mediator release. Following treatment with JTW + SSRIs, the levels of these inflammatory markers were significantly reduced, suggesting anti-inflammatory and gut barrier repair effects. Furthermore, the positive correlation between Firmicutes and sZO-1 (a tight junction protein) indicates a potential mechanism: "JTW treatment → Firmicutes recovery → beneficial metabolite production → intestinal barrier repair"[54].

Our integrated analysis revealed several significant correlations that helped elucidate the potential antidepressant mechanisms of JTW. The positive association between Bacteroidetes abundance and branched-chain SCFA levels (isobutyric and isovaleric acids) appears counterintuitive, as Bacteroidetes are primarily known for polysaccharide fermentation[55], whereas branched-chain SCFAs are typically derived from protein fermentation by Firmicutes and Proteobacteria[56]. This discrepancy might be explained by microbial cross-feeding, wherein metabolites derived from Bacteroidetes' polysaccharide fermentation serve as substrates for coexisting bacteria that are efficient producers of branched-chain fatty acids[57].

Interestingly, although we found an expected positive correlation between 5-HT and NE, we did not observe significant direct correlations between SCFAs and neurotransmitters or inflammatory markers. This suggests that the effects of JTW on neurotransmitters may be achieved through holistic restoration of the gut ecosystem and reduction of systemic inflammation, rather than through direct linear relationships with single metabolites. Sensitivity analysis confirmed the robustness of several key correlations such as 5-HT/NE and Firmicutes/isohexanoic acid. However, some relationships, particularly those centered on Bacteroidetes, were sensitive to outliers, indicating that while a biological signal is likely to be present, its strength may vary.

This gut–brain axis investigation, utilizing the same patient cohort as in our prior RCT[30], provides crucial mechanistic insights. While a previous study identified peripheral and central signaling changes, the current multi-omics analysis defines the potential site of action within the gut. We demonstrated that JTW, particularly in combination with SSRIs, may modulate the gut microbiota composition, enhance SCFA production, and subsequently elevate monoamine neurotransmitters. Taken together, these studies offer an integrated view of JTW's potential antidepressant mechanism: initial gut microbiota → metabolite modulation → systemic changes → neuroimmune regulation → final neurotrophic pathway activation → neurotransmitter elevation.

The bioactive compounds of JTW modulate gut microbiota and SCFA profiles through various mechanisms. Berberine, a key alkaloid in Rhizoma Coptidis, has documented prebiotic-like effects, inhibiting pathogenic bacteria and promoting beneficial SCFA producers such as Faecalibacterium and Roseburia[58–59]. Its anti-inflammatory properties may further contribute to a favorable gut environment[60]. Components from Cortex Cinnamomi, such as cinnamaldehyde, may also influence microbial composition through antimicrobial and anti-inflammatory activities[61–62]. This combination likely acts synergistically to restore microbial ecology and metabolic function[35].

This study had several limitations. First, the sample size for the gut microbiome and validation analyses was reduced due to stringent quality control, although the analyzed subsets were representative of the overall cohort. Second, the observational design could not establish causality, and future intervention studies, including fecal microbiota transplantation, would be valuable. Additionally, potential confounding factors such as participants' diet were not recorded or controlled. Second, the specific type of SSRI was not randomized but chosen based on clinical judgment, which could introduce confounding factors; however, the overall random allocation of patients is expected to mitigate systematic bias between groups. Future research should focus on the functional validation of specific bacterial strains, the roles of SCFA receptors in the CNS, and the influence of JTW's active components on microbial metabolism.

Conclusion

This study suggests that JTW, both as monotherapy and in combination with SSRIs, may alleviate depressive symptoms by modulating the microbiota–SCFA–neurotransmitter/immune axis. Specifically, JTW

treatment reshaped the gut microbiota structure in patients with depression, reducing the relative abundance of Bacteroidetes and *Bacteroides* while promoting Firmicutes restoration. It also selectively corrected SCFA metabolic dysregulation and increased fecal levels of branched-chain SCFAs. These microbial and metabolic changes were associated with improved neural function: the serum levels of 5-HT and DA were elevated after treatment, with the JTW + SSRIs combination showing potential synergistic effects. Treatment also reduced circulating LPS and HMGB1 levels, suggesting improved gut barrier integrity and attenuated systemic inflammation. Correlation analyses indicated a temporal relationship between microbial restoration, metabolic changes, and improvements in neural function. This clinical study provides evidence that JTW may exert antidepressant effects through multi-target modulation of the microbiota–SCFAs–neurotransmitter/immune axis, supporting the TCM concept of "multi-component, multi-target, holistic regulation." Further interventional studies are warranted to validate the potential causal mechanisms.

Conflict of interest statement

Chunquan Yu is an editorial board members member of this journal. The other authors declare no conflict of interest.

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Author contributions

Yang Tong, Yuanyuan He, Mengnan Huang: wrote the paper draft, participated in research design, the writing of the paper, data analysis and performance of the research; Yijia Liu, Fengmin Liu, Yuting Li, Shan Gao: participated in the performance of the research; Li Shen, Qiang Xu, Chunquan Yu: corrected the draft, supervised the experimentators, participated in funding acquisition, project administration, writing – review & editing. All authors read and approved the final manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Ethical approval of studies and informed consent

This research followed guidelines of the *Declaration of Helsinki* and Tokyo for humans. The study protocol was approved by the Ethics Committee of Tianjin University of Traditional Chinese Medicine (No. TJUTCMEC20210006). This study protocol has been registered in the International Traditional Medicine Clinical Trial Registry (No. ITMCTR2025000151). All participants provided written informed consent before any study procedures were performed. Specific measures to protect participant rights, including the confidentiality of their data, the voluntary nature of their participation, and their right to withdraw from the study at any time without penalty, were rigorously implemented throughout the trial.

Acknowledgments

None

Data availability

This research data includes sensitive information such as patient data so the research data is unavailable to access.

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