Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Antidepressant-Like Effects of Mongolian Medical Warm Acupuncture via Remodeling the Gut Microbiota–Metabolite–Barrier Axis in CUMS Rats

Riluge Wu*, Qinglin Bao*, Yasula Ba, Chaoketu Saiyin, Lengge Si, Runa A

Mongolian Medical College, Inner Mongolia Medical University, Hohhot, Inner Mongolia, People's Republic of China

*These authors contributed equally to this work

Correspondence: Runa A; Lengge Si, Mongolian Medical College, Inner Mongolia Medical University, Hohhot, Inner Mongolia, People's Republic of China, Email Aruna1987_immu@163.com; Silengge16_immu@163.com

Objective: This study evaluated the antidepressant-like effects of Mongolian medical warm acupuncture (MMWA) in a chronic unpredictable mild stress (CUMS) model and examined its mechanistic involvement in the gut microbiota–metabolite–barrier axis, representing a novel multi-omics investigation of this traditional therapy.

Methods: Control, CUMS, and MMWA rats (n = 9/group) were assessed using sucrose preference, open-field activity, and Morris water maze tasks. Gut microbiota, fecal metabolites, and intestinal barrier markers were measured by 16S rRNA sequencing, UPLC–MS/MS metabolomics, qRT-PCR, and Western blotting.

Results: MMWA improved CUMS-induced deficits, increasing sucrose preference ($p < 0.01$), enhancing locomotor activity ($p < 0.01$), and reducing escape latency ($p < 0.05$). Treatment restored microbial diversity and increased beneficial short-chain fatty acid (SCFA)–producing genera, including Lactobacillus and Prevotella ($p < 0.05$). Metabolomic analysis showed recovery of key neuroactive metabolites such as taurine and arginine (adjusted $p < 0.05$). MMWA also enhanced intestinal barrier integrity by upregulating Occludin, TJP1/ZO-1, and Claudin-4 ($p < 0.001$). Associations across microbiota–metabolite pathways reflected coordinated restoration.

Conclusion: MMWA alleviates depressive-like behaviors by reshaping gut microbiota, normalizing metabolic profiles, and strengthening the intestinal barrier. These findings support its potential as a complementary approach for depressive-like conditions and highlight a mechanistic link involving the microbiota–metabolite–barrier axis.

Keywords: Mongolian medical warm acupuncture, depression, gut microbiota, metabolomics, intestinal barrier

Introduction

Depression is a common yet complex neuropsychiatric disorder that affects more than 300 million individuals globally and contributes significantly to the global disease burden.[1,2] Chronic unpredictable mild stress (CUMS) in rodents is a widely validated experimental paradigm for simulating human depression-like behavior, especially anhedonia and cognitive impairment.[3,4] The CUMS paradigm is widely regarded as a valid model of long-term psychosocial stress in humans because it exposes animals to repeated, low-intensity, and unpredictable stressors over several weeks. This pattern closely mimics the chronic, variable, and uncontrollable nature of psychosocial stress that contributes to the development of major depressive disorder in humans.[3,4]

Accumulating evidence suggests that the pathophysiology of depression extends beyond the central nervous system and is intimately connected to the bidirectional regulatory framework of the gut–brain axis.[5,6]

Dysbiosis of gut microbiota—characterized by altered diversity and abundance of key microbial taxa—has emerged as a crucial contributor to depressive pathology. Microbial imbalance disrupts intestinal barrier integrity, increases systemic inflammation, and perturbs the host's metabolic profile, ultimately affecting brain function and behavior.[7,8] Specifically, microbial-derived metabolites such as short-chain fatty acids (SCFAs), tryptophan derivatives, and bile acids influence

Received: 19 September 2025
Accepted: 14 December 2025
Published: 30 December 2025

neurotransmitter biosynthesis and neuroinflammation through endocrine, neural, and immune signaling pathways.[9,10] Recent studies and systematic reviews have demonstrated that patients with major depressive disorder (MDD) and anxiety disorders exhibit distinct alterations in gut microbiota composition. These changes include increased microbial α-diversity, enrichment of pro-inflammatory and potentially pathogenic taxa such as Enterobacteriaceae, Alistipes, and Desulfovibrio, and a consistent depletion of SCFA-producing genera, notably Faecalibacterium. Such dysbiosis may contribute to the pathogenesis of affective disorders through gut-brain axis signaling and inflammation-mediated mechanisms.[11,12] These findings highlight the microbiota-metabolite-barrier axis as a potential therapeutic target for mood disorders.

Gut microbes influence central nervous system function through several interconnected mechanisms, collectively forming the microbiota–gut–brain axis. These mechanisms include neural signaling via the vagus nerve; immune modulation through systemic cytokines; endocrine interactions involving the hypothalamic–pituitary–adrenal (HPA) axis; and microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), tryptophan derivatives, and bile acids—that regulate neuroinflammation, neurotransmitter biosynthesis, and synaptic plasticity. Disruption of these pathways has been associated with depressive-like behavior and altered emotional regulation in both animal models and clinical studies.[5–12] In addition, acupuncture-based interventions—including MMWA, have been reported to modulate several neurobiological pathways implicated in depression, including inflammatory cytokine signaling, hypothalamic–pituitary–adrenal (HPA) axis regulation, and neurotransmitter synthesis.[13–16] Highlighting these pathways provides a mechanistic bridge linking peripheral microbiota-derived changes to central behavioral outcomes.

Mongolian medical warm acupuncture (MMWA), a traditional external therapy rooted in the concept of "three roots balance" and "Heti regulation," involves applying thermal stimulation to specific acupoints via heated silver needles.[17] Recent high-quality studies and systematic evidence further indicate that acupuncture-related interventions can modulate gut microbiota composition, metabolic signaling, and neuroimmune pathways in depression models, reinforcing the scientific basis for investigating MMWA within a contemporary mechanistic framework.[11,12,18,19] Clinically, MMWA has been widely used to manage insomnia, neurasthenia, and stress-related disorders.[20] Modern experimental studies have demonstrated that Mongolian medical warm acupuncture exerts therapeutic effects by modulating hypothalamic gene and protein expression, suppressing neuroinflammatory pathways, and restoring neuroimmune homeostasis.[13–15] Notably, recent animal studies report that warm acupuncture ameliorates insomnia and anxiety-like behaviors by reshaping gut microbial ecology and modulating serum metabolites.[21]

However, the specific mechanisms through which MMWA influences depression remain insufficiently defined. In particular, whether MMWA can regulate the gut microbiota–metabolite–barrier axis has not been systematically investigated, and no prior studies have employed multi-omics approaches to characterize these integrated pathways. Clearly articulating this gap highlights the novelty and necessity of the present work.

Therefore, this study aimed to evaluate the antidepressant effects of MMWA in CUMS-induced depressive rats and to elucidate its mechanisms using multi-omics approaches. Behavioral outcomes were assessed by the sucrose preference test, open field test, and Morris water maze. Gut microbiota composition was profiled by 16S rRNA sequencing, fecal metabolites were analyzed by untargeted UPLC-MS/MS with KEGG enrichment, and intestinal barrier proteins (Occludin, TJP1, Claudin4) and histidine metabolism enzymes (HAL, HDC) were examined by qRT-PCR and Western blotting. Microbiota-metabolite interactions were further explored using Spearman and sGCCA analyses. These findings provide new insights into the antidepressant mechanisms of MMWA, highlighting its role in modulating the gut microbiota and metabolic pathways within the gut-brain axis framework.

Materials and Methods

Animals and Experimental Design

Twenty-seven specific pathogen-free (SPF) male Sprague–Dawley (SD) rats (170 ± 15 g) were purchased from Beijing Huafukang Biological Technology Co., Ltd. (Beijing, China). Animals were housed individually under controlled temperature (22 ± 2 °C), humidity (50% ± 10%), and a 12-h light/dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee Approval and conducted in accordance with international guidelines. Before grouping, rats underwent an open field test to

exclude individuals with abnormal baseline activity (exploration frequency <30 or >120); no rats were excluded, and all 27 were randomly allocated (random number generator) into three groups (n = 9 each): Control, CUMS, and MMWA. The CUMS protocol, adapted from established methods, included unpredictable stressors such as overnight illumination, cage tilting (45°), cold water swimming (4 °C, 5 min), food or water deprivation (24 h), wet bedding, and noise stimulation, with one to two stressors applied per day for 10 weeks. Body weight, food and water intake, fur condition, and fecal appearance were monitored weekly.

Group allocation was computer-randomized, and all subsequent laboratory procedures, including sequencing, metabolomics, Western blotting, and qRT-PCR, were performed under blinded conditions by investigators unaware of group identity.

Warm Acupuncture Intervention

Warm acupuncture was performed at six acupoints (Dinghui, Heyi, Xin, Badagan, Stomach, and Intestine), selected according to Mongolian medical atlases and their relevance to the brain-gut axis. The anatomical locations of the acupoints were determined according to the Atlas of Standardized Mongolian Medicine Acupoints (Inner Mongolia Medical University Press), which provides detailed surface landmarks and regional anatomical references for Mongolian medical warm acupuncture. One acupoint was stimulated per session in a fixed rotation. Sterile silver needles were inserted to a depth of 5 mm and heated with a temperature-controlled device (MY-I, Inner Mongolia, China) at 40 ± 2 °C for 20 minutes once daily over 10 weeks. Control and CUMS groups were handled similarly without needle insertion or heating. All acupuncture procedures were performed by the same trained operator to minimize inter-operator variability and ensure procedural consistency across the 10-week intervention.

Behavioral Assessments

Behavioral testing was conducted by blinded experimenters. The open field test was performed at baseline and week 10 using a 100×100 × 40 cm arena, where rats were allowed to explore freely for 5 minutes, and horizontal and vertical activity were recorded. The sucrose preference test was conducted at baseline and day 70 following 24 h water deprivation; rats were given access to 1% sucrose and plain water for 12 h, with bottle positions alternated midway. Preference was calculated as sucrose intake/(sucrose + water intake) × 100. Spatial learning and memory were assessed during week 10 using the Morris water maze in a 160 cm diameter pool (water temperature 22 ± 1 °C), with a hidden platform submerged 2 cm below the surface. Rats underwent four training trials daily for 4 days, followed by a probe trial on day 5 with the platform removed, measuring escape latency, path length, time in the target quadrant, and proximity ratio.

Gut Microbiota Profiling

Fresh fecal samples were collected and stored at −80 °C. DNA was extracted with a commercial stool DNA kit (Qiagen, Germany) and the V3-V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform. Reads were processed in QIIME2, and OTUs clustered at 97% similarity. Reads were rarefied to 20,000 sequences per sample before calculating alpha diversity metrics to ensure consistent sequencing depth across samples. Operational taxonomic units (OTUs) were assigned using the SILVA database. For statistical comparisons, abundance tables were normalized using total-sum scaling and log-transformed where appropriate. Beta diversity analyses were performed using UniFrac distance matrices generated from rarefied data. Alpha diversity indices (Shannon, Simpson, Chao1, ACE, Observed OTUs) and beta diversity (UniFrac PCA) were calculated. Differential taxa were identified by LEfSe (Kruskal–Wallis and Wilcoxon tests, LDA >2.0). Functional prediction was conducted using PICRUSt2 with KEGG ortholog annotation.

Untargeted Fecal Metabolomics

Fecal metabolites were extracted with methanol–acetonitrile (2:1), centrifuged, and analyzed using UPLC-MS/MS (Q Exactive, Thermo Fisher). Data were processed with Compound Discoverer (Thermo) and metaX (R package). Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed, with model validity tested by 200-permutation analysis. Differential metabolites were identified using $|\log_2 FC| > 1$ and adjusted $p < 0.05$ (Benjamini–Hochberg correction). KEGG-based pathway enrichment was performed in MetaboAnalyst.

Correlation Analysis

Differentially abundant microbial genera and metabolites were selected for correlation analysis. Spearman correlation coefficients (adjusted $p < 0.05$, FDR-corrected) were calculated, and a microbiota–metabolite interaction network was constructed using sparse generalized canonical correlation analysis (sGCCA) with the mixOmics R package (v6.20).

Assessment of Intestinal Barrier Integrity and Histidine Metabolism

Colon tissues were collected post-mortem. Total RNA was extracted (TRIzol reagent, Invitrogen) and subjected to RT-qPCR for Tjp1, Claudin4, HAL, and HDC using SYBR Green Master Mix (Applied Biosystems). GAPDH was used as an internal control. Primer sequences (designed via Primer3 software) are listed in Table 1.

For protein analysis, colon tissues were lysed in RIPA buffer. Protein concentrations were quantified by BCA assay. Protein molecular weight markers were obtained from Abcam (Prestained Protein Ladder, 10–245 kDa, Cat# ab116028). Equal amounts of protein (30 μg) were separated by SDS–PAGE and transferred to PVDF membranes. Membranes were blocked and incubated overnight at 4 °C with primary antibodies against Occludin (1:1000, Abcam, ab216327), TJP1 (ZO-1, 1:1000, CST, #13663), Claudin4 (1:1000, Abcam, ab15104), HAL (1:500, Proteintech, 15756-1-AP), HDC (1:500, Santa Cruz, sc-271213), and β-actin (1:2000, Abcam, ab8226). After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000). Bands were visualized by ECL (Bio-Rad) and quantified using ImageJ. All experiments were performed in biological triplicates.

Statistical Analysis

All statistical analyses were performed using SPSS 26.0, GraphPad Prism 9.0, and R software. Data distributions were assessed for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. For behavioural and molecular assays that met parametric assumptions, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple-comparison test was applied. When assumptions were not met, the Kruskal–Wallis test with Dunn's post-hoc correction was used. Effect sizes for ANOVA results were calculated using partial eta-squared (η^2), and 95% confidence intervals were reported where appropriate.

For microbiota analyses, α-diversity indices were compared using the Kruskal–Wallis test, while β-diversity was assessed using PERMANOVA based on UniFrac distance matrices. Taxonomic differential abundance was evaluated using LEfSe with a linear discriminant analysis (LDA) threshold of 2.0. For microbiota differential abundance analyses beyond LEfSe, false discovery rate (FDR) correction (Benjamini–Hochberg) was also applied to control for type I error. For metabolomics, differential metabolites were identified using orthogonal partial least squares discriminant analysis (OPLS-DA) and univariate analysis with Benjamini–Hochberg false discovery rate (FDR) correction (adjusted $p < 0.05$). For correlation analyses, Spearman coefficients were computed and corrected for multiple testing using FDR.

All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant unless otherwise specified.

Table 1 Primer Sequences Used for RT-qPCR Analysis of Target Genes in Rat Colon Tissues

Gene	Primer	Sequence (5′→3′)	Tm (°C)	Product Size (bp)
Tjp1	Tjp1-F	TGGAGTCGAGACTTTCTCTG	58.1	20
	Tjp1-R	TAGCTCCACAGGCTTCAGG	59.9	19
Claudin4	Claudin4-F	ATACGCACTTAGGAGTCATCC	58.4	21
	Claudin4-R	TACACAGGCACCATAATCAGC	58.4	21
HAL	HAL-F	TCATGATAGCCCACTGTACC	58.1	20
	HAL-R	TTCCGGAATGTGCTCCATTC	58.1	20
HDC	HDC-F	ACCACCAAGGATGACATCCTG	60.3	21
	HDC-R	CTGAACAGAAAGGACGACAG	58.1	20
GAPDH	GAPDH-F	ACTCCCTCAAGATTGTCAGC	58.1	20
	GAPDH-R	AGTTGCTGTTGAAGTCACAGG	58.4	21

Results

Warm Acupuncture Ameliorates Depression-Like Behaviors and Cognitive Deficits in CUMS Rats

CUMS rats exhibited attenuated body weight gain (Figure 1A) and significantly reduced sucrose preference compared with controls ($p < 0.01$, Figure 1B), both of which were improved by MMWA treatment ($p < 0.05$). In the open field test, locomotor activity and rearing frequency were decreased in CUMS rats, whereas MMWA partially restored these behaviors (Figure 1C–E). In the Morris water maze, CUMS rats showed prolonged escape latency and fewer platform crossings, indicating impaired spatial learning and memory; these deficits were significantly alleviated by MMWA ($p < 0.05$, Figure 1F–H). Representative effect sizes were substantial (partial $\eta^2 = 0.41–0.58$), supporting the robustness of these behavioral differences despite the modest sample size.

Warm Acupuncture Restores Gut Microbiota Diversity and Composition in CUMS Rats

Rarefaction curve analysis confirmed that sequencing depth was sufficient to capture most of the microbial diversity in all samples, ensuring reliable data quality (Figure S1). At the α-diversity level (Figure 2A–E), CUMS rats showed significantly reduced richness and evenness, as indicated by lower Chao1, Shannon, ACE, and Observed OTUs indices and a higher Simpson index ($p < 0.05$). These alterations were partially reversed in the MMWA group, suggesting restored microbial diversity. At the taxonomic level (Figure 2F and G), CUMS rats exhibited a notable decrease in Firmicutes and enrichment of Proteobacteria and Escherichia-Shigella, consistent with stress-induced dysbiosis. In contrast, MMWA intervention partially normalized these phylum- and genus-level imbalances and promoted the recovery of beneficial taxa such as Prevotella. These results indicate that warm acupuncture not only improves microbial diversity but also helps re-establish a more balanced gut microbiota community disrupted by chronic stress.

Warm Acupuncture Modulates Gut Microbiota Composition in CUMS Rats

Chronic stress induced marked shifts in gut microbiota across multiple taxonomic levels. At the phylum level, CUMS rats showed an increased abundance of Proteobacteria and a decrease in Firmicutes compared with controls, whereas MMWA treatment partially restored this imbalance (Figure S2A and Figure 3A). At finer taxonomic levels, CUMS promoted the enrichment of potentially harmful taxa such as Enterobacteriaceae and Escherichia-Shigella, while beneficial genera including Lactobacillus, Prevotella, and Ruminococcus were depleted. These alterations were partially reversed by MMWA, resulting in a microbial profile closer to that of healthy controls (Figure S2B–F and Figure 3B–F).

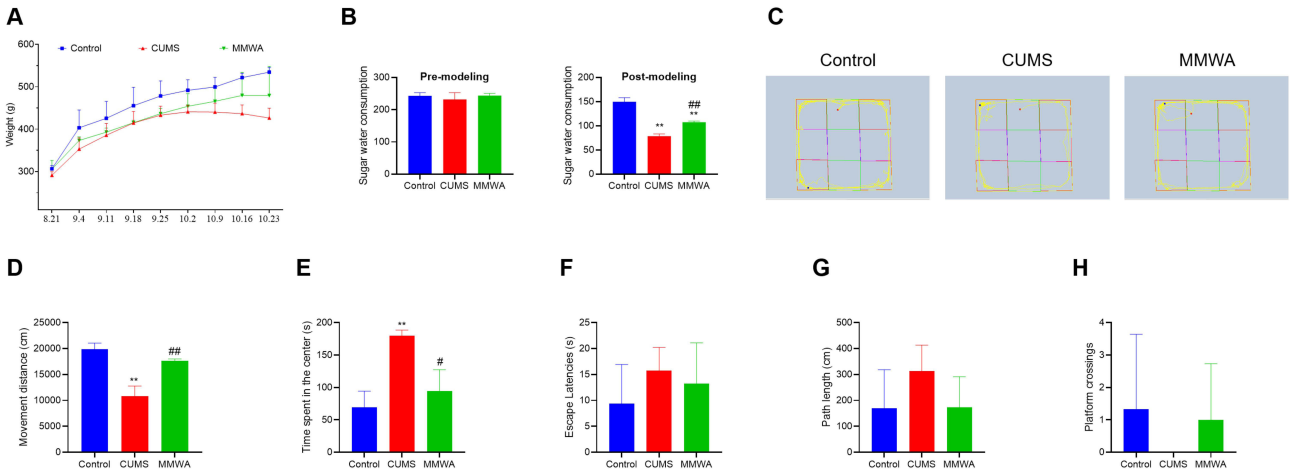

Figure 1 Warm acupuncture improves body weight, anhedonia, locomotion, and cognitive function in CUMS rats. (**A**) Body weight gain during the 10-week modeling period. (**B**) Sucrose preference before and after modeling. (**C**) Representative open field trajectories. (**D** and **E**) Total distance traveled and rearing frequency in the open field test. (**F** and **G**) Escape latency and path length during Morris water maze training. (**H**) Platform crossings during the probe trial. Data are presented as mean ± SD (n = 9/group). $^{**}p < 0.01$ vs Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs CUMS.

https://doi.org/10.2147/NDT.S566386

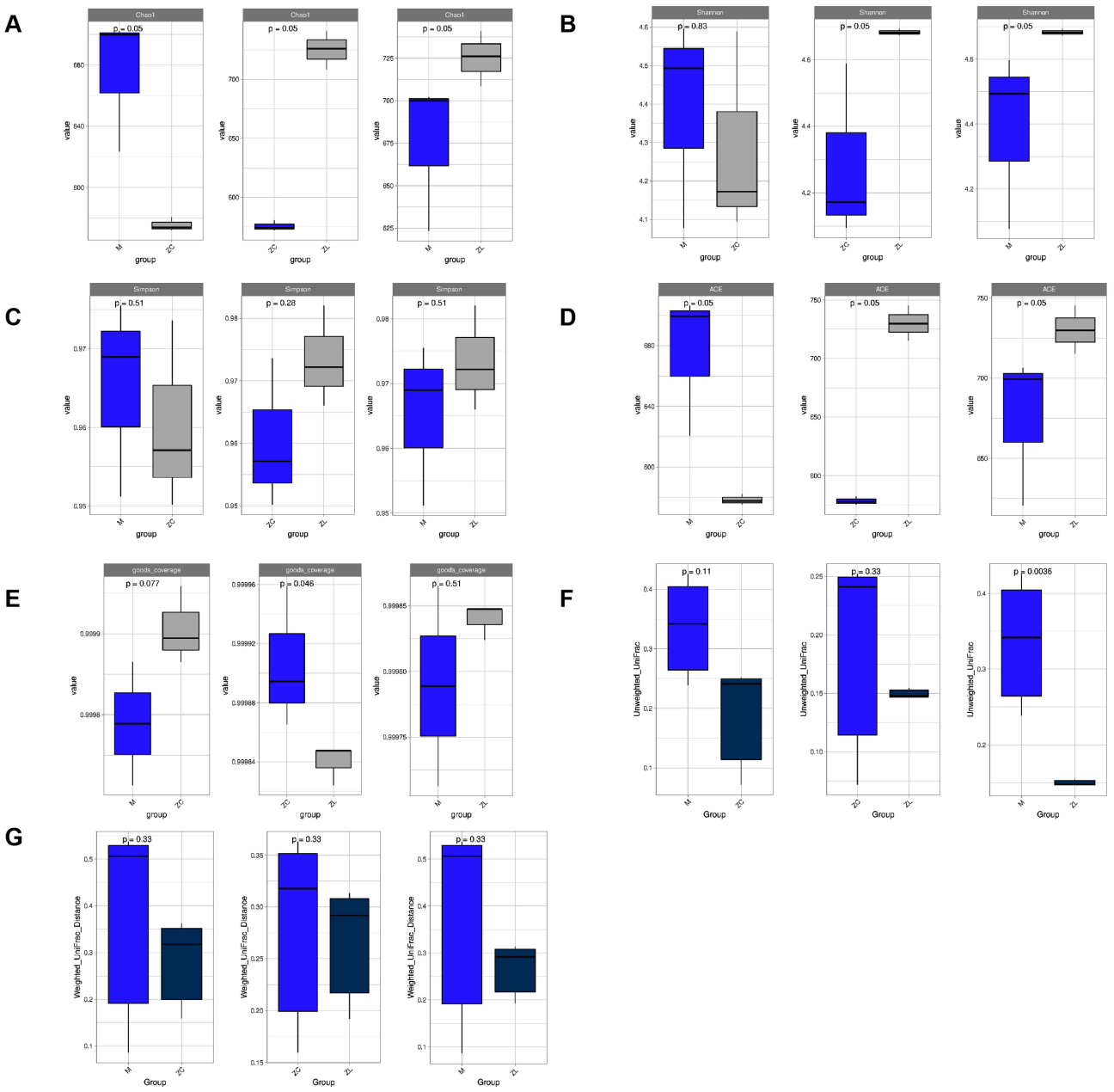

Figure 2 Effects of warm acupuncture on gut microbial diversity in CUMS rats. (**A**–**E**) α-diversity indices of gut microbiota among Control, CUMS, and MMWA groups, including Chao1, Shannon, Simpson, ACE, and Observed OTUs. CUMS rats exhibited reduced richness and evenness, which were partially restored by MMWA treatment. (**F** and **G**) Relative abundance of major bacterial taxa at the phylum and genus levels. CUMS rats showed reduced Firmicutes and increased Proteobacteria and Escherichia-Shigella, whereas MMWA intervention partially normalized these changes and enriched beneficial genera such as Prevotella.

Collectively, these findings indicate that warm acupuncture helps re-establish a more balanced gut microbial community disrupted by chronic stress.

Warm Acupuncture Alters Key Microbial Taxa and Restores Functional Potential

LEfSe analysis revealed distinct bacterial signatures between groups. CUMS rats were enriched in pathogenic taxa such as Campylobacteraceae, Streptococcaceae, and Mycoplasmataceae, whereas MMWA increased beneficial commensals including Prevotellaceae and Rikenellaceae (Figure 4A and B). Functional prediction with PICRUSt2 further showed that CUMS favored pathways related to fermentation, chemoheterotrophy, and pathogen-associated metabolism, while MMWA shifted functional potential toward sulfur compound respiration, acetogenesis, and symbiosis-associated

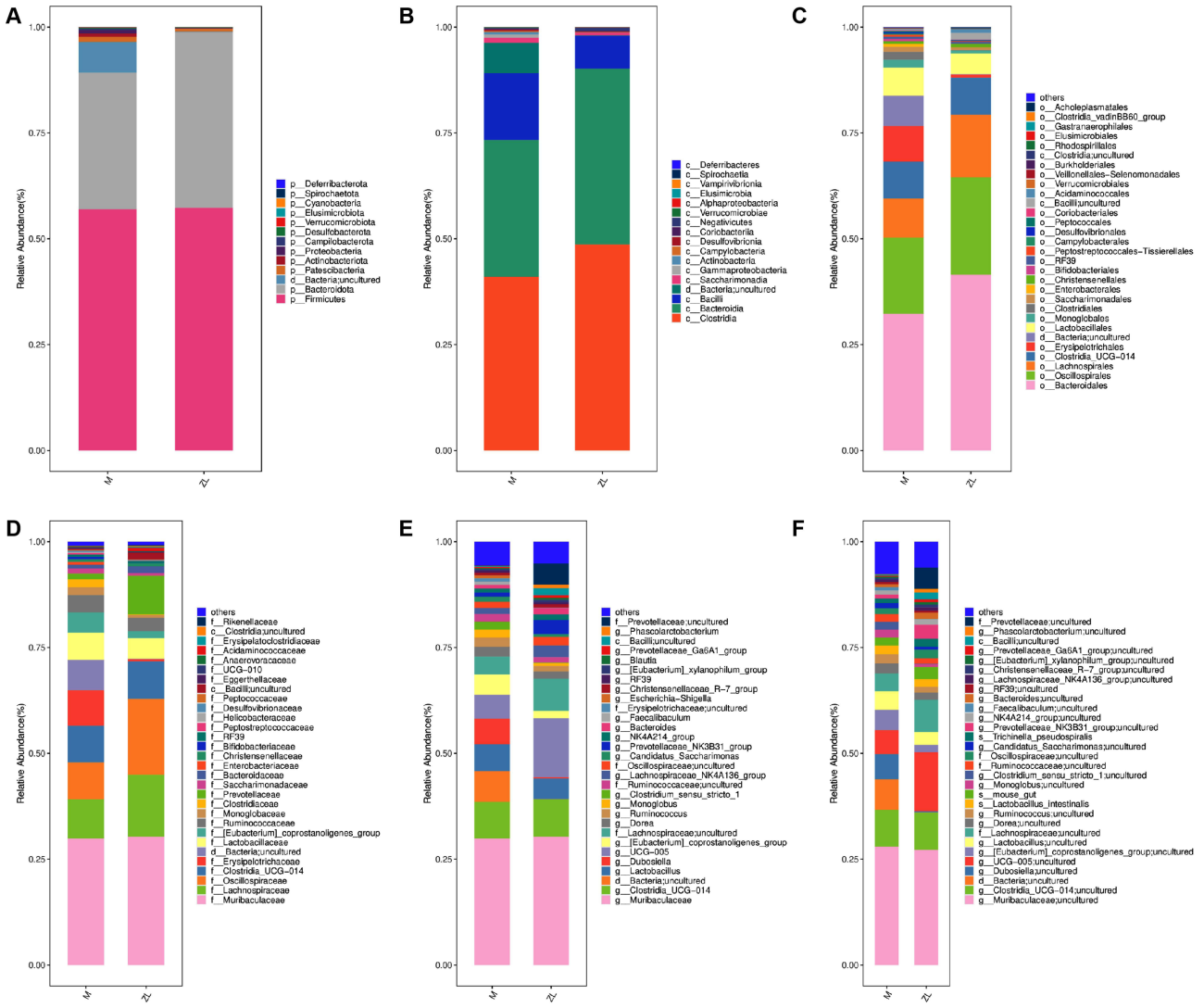

Figure 3 Warm acupuncture reshapes gut microbiota composition in CUMS rats. (**A**–**F**) Relative abundance of gut microbiota at the phylum (**A**), class (**B**), order (**C**), family (**D**), genus (**E**), and species (**F**) levels in the CUMS and MMWA groups. Chronic stress induced dysbiosis characterized by increased Proteobacteria and Escherichia-Shigella and decreased beneficial taxa such as Firmicutes and Lactobacillus. Warm acupuncture partially corrected these alterations, promoting a shift toward a more balanced microbial community.

processes (Figure 4C and D). Together, these results indicate that warm acupuncture mitigates stress-induced dysbiosis at both the taxonomic and functional levels.

Warm Acupuncture Reverses Fecal Metabolic Disorders in CUMS Rats

Untargeted metabolomic profiling revealed distinct group-specific clustering. PCA showed that CUMS rats exhibited clear separation from controls, whereas MMWA treatment shifted the metabolic profile closer to that of controls, indicating partial restoration of homeostasis (Figure 5A and B). Volcano plot analysis identified 209 differential metabolites between the Control and CUMS groups (189 downregulated, 20 upregulated), while 26 metabolites were altered between the CUMS and MMWA groups, with 17 downregulated in response to treatment (Figure 5C and D). These metabolites were mainly associated with lipid metabolism, amino acid pathways, and bile acid-related functions, suggesting that warm acupuncture alleviates CUMS-induced fecal metabolic disturbances.

https://doi.org/10.2147/NDT.S566386

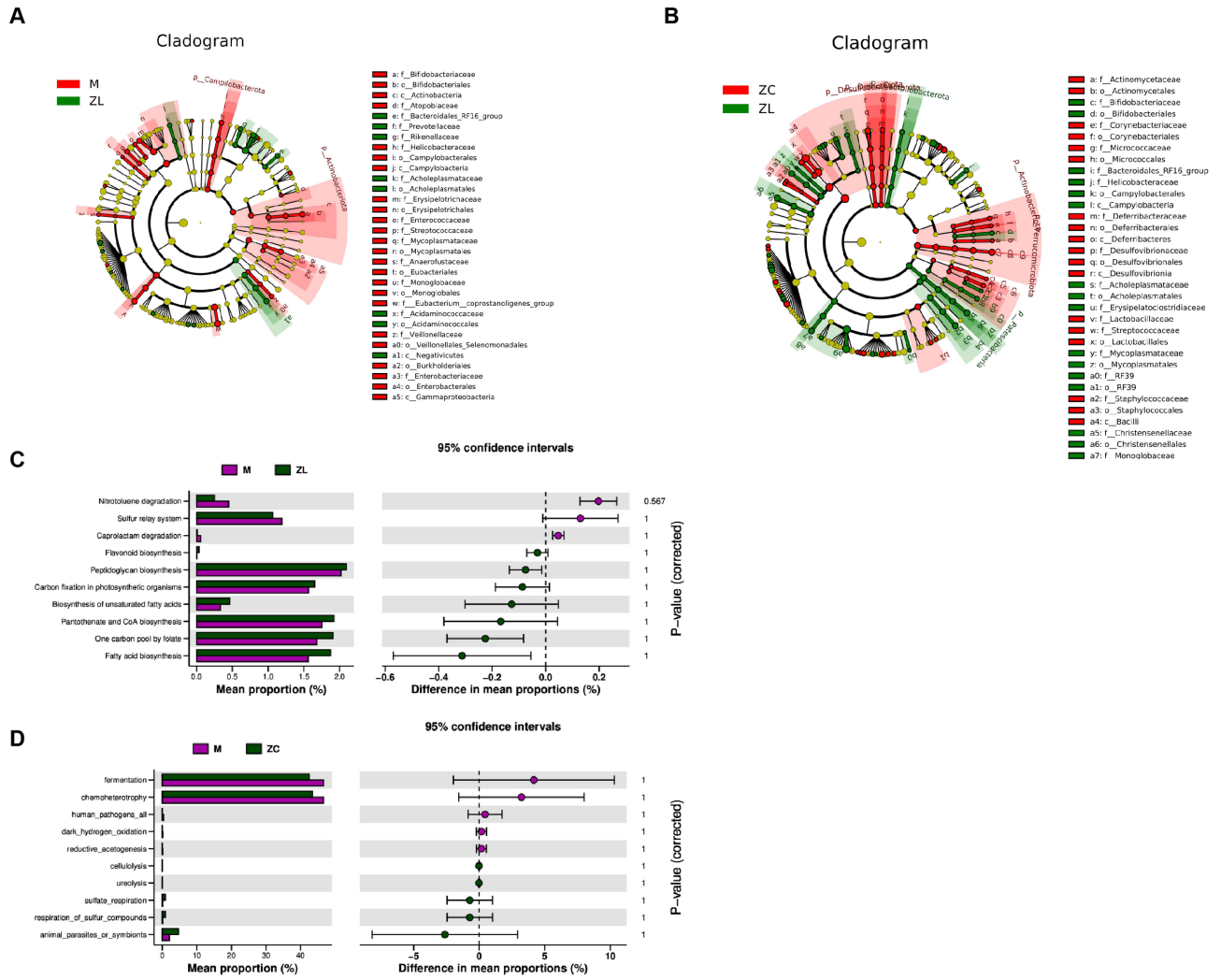

Figure 4 Differential microbial taxa and predicted functions between groups based on LEfSe and PICRUSt2 analyses. (**A**) Comparison between Control and CUMS groups showing enrichment of pathogenic taxa including Campylobacteraceae, Streptococcaceae, and Mycoplasmataceae in CUMS rats. (**B**) Comparison between CUMS and MMWA groups showing increased abundance of commensal taxa such as Prevotellaceae and Rikenellaceae after MMWA treatment. (**C**) Predicted microbial functions (Control vs CUMS) highlighting enrichment of fermentation, chemoheterotrophy, and pathogen-associated pathways in CUMS rats. (**D**) Predicted microbial functions (CUMS vs MMWA) indicating restoration of health-associated pathways such as sulfur compound respiration, acetogenesis, and symbiosis-related metabolism by MMWA.

KEGG Enrichment Analysis Reveals Key Pathways Involved in Therapeutic Effects

KEGG pathway enrichment was conducted to elucidate the biological significance of fecal metabolite alterations. Compared with controls, CUMS rats exhibited enrichment of pathways related to amino acid and neurotransmitter metabolism, including tyrosine metabolism, tryptophan metabolism, and glutathione metabolism, reflecting stress-induced metabolic dysregulation (Figure 6A). In contrast, MMWA treatment shifted the enrichment profile toward pathways associated with amino acid biosynthesis (eg, arginine, valine, leucine, and isoleucine biosynthesis), taurine and hypotaurine metabolism, and thiamine metabolism, indicating restoration of neuromodulatory and energy-related functions (Figure 6B). These results suggest that warm acupuncture alleviates depression-like symptoms partly by modulating amino acid–related metabolic pathways along the gut–brain axis.

Identification of Key Metabolites with an "Abnormal-Recovery" Trend

Venn diagram analysis identified eight metabolites consistently altered in CUMS rats relative to controls and reversed by MMWA treatment (Figure 7A). Heatmap visualization showed that CUMS rats had significant reductions in taurine, nicotinamide, arginine, and aspartic acid-metabolites linked to neurotransmitter synthesis, antioxidant defense, and amino

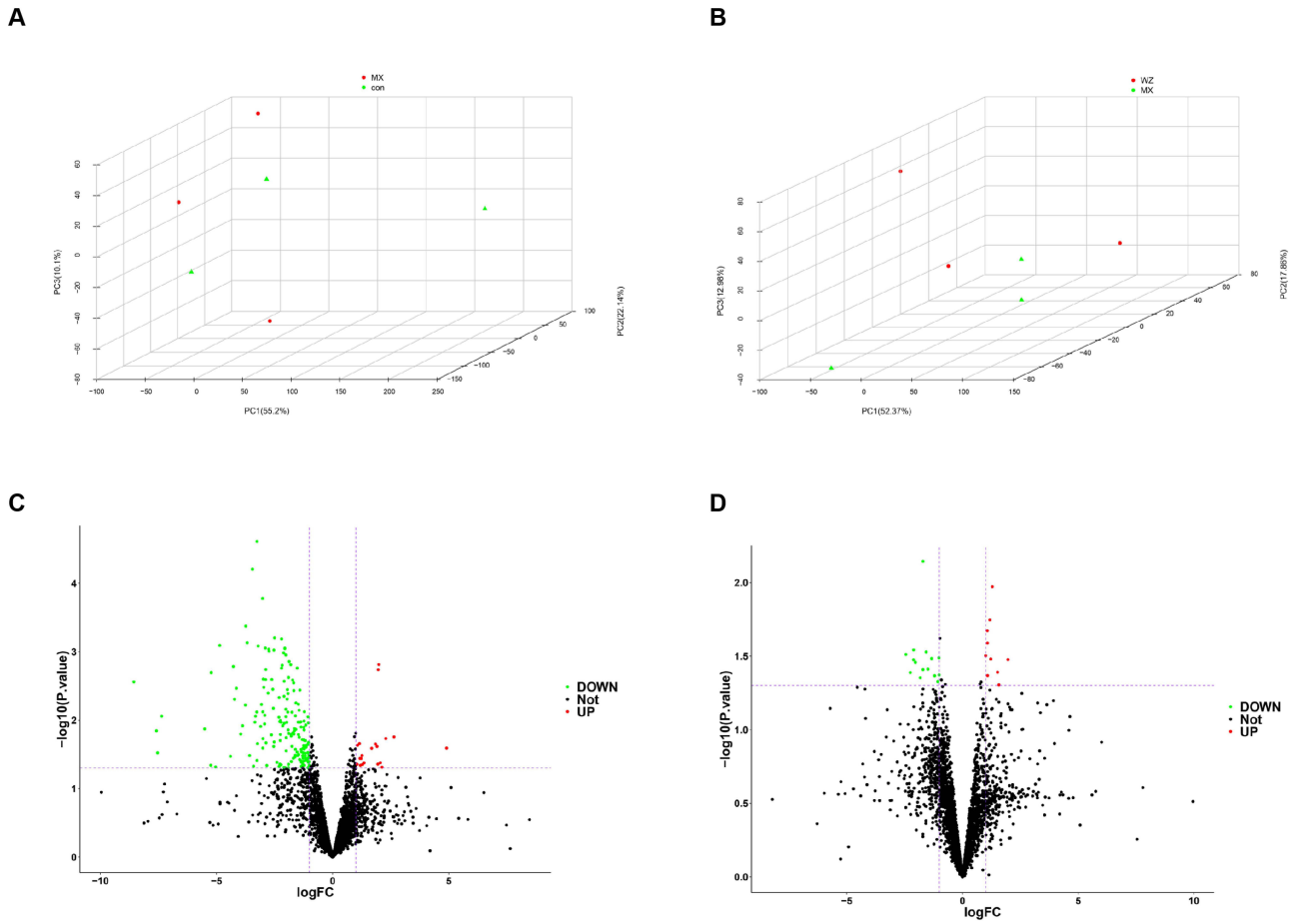

Figure 5 Warm acupuncture ameliorates fecal metabolic disturbances in CUMS rats. (**A**) PCA score plot of fecal metabolites in the Control and CUMS groups, indicating distinct separation of metabolic profiles due to chronic stress. (**B**) PCA score plot comparing the CUMS and MMWA groups, showing partial restoration of metabolic signatures after warm acupuncture treatment. (**C**) Volcano plot of differentially expressed fecal metabolites between the Control and CUMS groups. (**D**) Volcano plot of differentially expressed metabolites between the CUMS and MMWA groups. Green dots represent significantly downregulated metabolites, red dots represent significantly upregulated metabolites, and black dots indicate non-significant features (VIP > 1.0, $p < 0.05$).

acid metabolism-while MMWA restored their levels toward control values (Figure 7B and C). These findings highlight a core set of gut-derived metabolites that may mediate the antidepressant effects of warm acupuncture via the microbiota-metabolite-brain axis.

Correlation Analysis Between Gut Microbiota and Key Metabolites

At the genus level, CUMS rats showed enrichment of Streptococcus, Mycoplasma, and unclassified_Muribaculaceae, alongside a depletion of beneficial taxa such as Lactobacillus and Prevotella (Figure S3A). MMWA treatment partially restored this imbalance, increasing symbiotic genera such as Prevotella and Rikenella while reducing potential pathogens (Figure S3B). Spearman correlation analysis revealed that beneficial genera, including Lactobacillus and Prevotella, were positively associated with neuroprotective metabolites such as taurine, nicotinamide, and arginine, whereas Streptococcus and Mycoplasma showed negative correlations (Figure 8A). Similar associations were observed between CUMS and MMWA groups (Figure 8B), suggesting that the antidepressant effects of warm acupuncture are mediated, at least in part, through the restoration of specific microbiota–metabolite interaction networks. The observed correlations between microbial genera, metabolite levels, and behavioural changes indicate concurrent alterations across biological layers but do not establish directional causality.

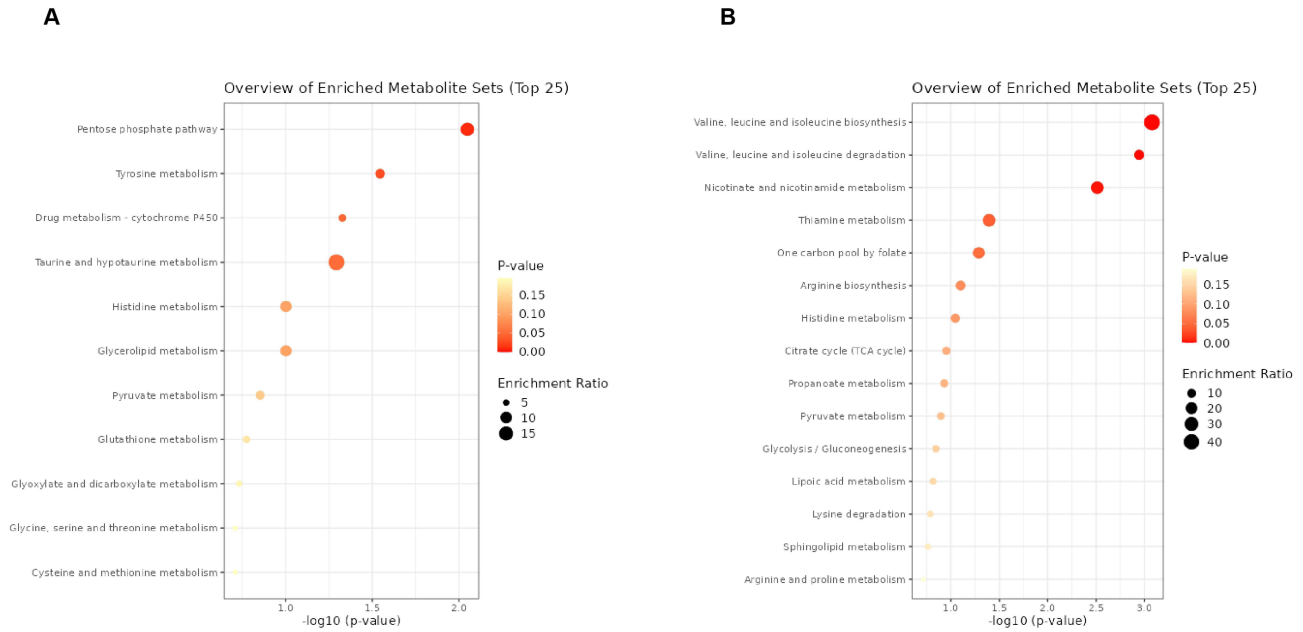

Figure 6 KEGG enrichment analysis of differential fecal metabolites. (**A**) Control vs CUMS comparison showing enrichment of pathways involved in amino acid and neurotransmitter metabolism, including tyrosine, tryptophan, taurine/hypotaurine, and glutathione metabolism.(**B**) CUMS vs MMWA comparison showing enrichment of pathways associated with amino acid biosynthesis (arginine, valine, leucine, isoleucine), taurine/hypotaurine, and thiamine metabolism, suggesting partial recovery of neuromodulatory and energy-related functions. Bubble size represents enrichment ratio, and bubble color indicates statistical significance (p-value).

Warm Acupuncture Restores Gut Barrier Integrity and Inhibits Histidine Metabolism

To clarify whether MMWA improves gut-brain homeostasis through intestinal protection, we examined colonic tight junction proteins and histidine metabolism-related enzymes. RT-qPCR revealed that Occludin, Tjp1 (ZO-1), and Claudin4 expression was significantly reduced in CUMS rats, indicating impaired barrier function, whereas MMWA markedly restored these genes (Figure 9A–C). Western blot analysis confirmed parallel changes at the protein level (Figure 9D). In contrast, the pro-inflammatory enzymes HAL and HDC were upregulated in the CUMS group but suppressed by MMWA intervention (Figure 9E). These findings suggest that warm acupuncture strengthens intestinal barrier integrity and reduces histidine-driven inflammatory signaling, thereby contributing to its antidepressant effects.

Discussion

This study provides compelling evidence that MMWA significantly ameliorates depression-like behaviors and cognitive deficits in rats subjected to CUMS. Key findings include: (1) MMWA reversed behavioral abnormalities, including anhedonia and impaired spatial memory; (2) it restored gut microbial diversity and suppressed the overgrowth of pathogenic taxa such as Escherichia-Shigella; (3) it normalized fecal metabolic profiles, particularly amino acid and SCFA-related metabolites; and (4) it repaired intestinal barrier integrity and suppressed histidine metabolic inflammation. These data collectively suggest that MMWA confers antidepressant-like effects via modulation of the gut–microbiota–metabolite–barrier axis.

Accumulating studies underscore the central role of the gut-brain axis in depression pathophysiology. Chronic stress disrupts intestinal microbiota and metabolic outputs, which in turn modulate neuroinflammation and neurotransmitter biosynthesis.[22] In line with our findings, recent reports demonstrated that CUMS models exhibit reduced microbial richness, elevated *Proteobacteria*, and suppressed SCFA-producing genera, all of which were reversed by microbiota-targeting interventions.[18,19] Moreover, we identified a downregulation of neuroprotective fecal metabolites, including taurine, nicotinamide, and arginine, in CUMS rats—metabolites shown to be crucial for redox homeostasis, blood–brain barrier function, and synaptic plasticity.[16] MMWA effectively normalized their levels, suggesting a strong neurometabolic link. Furthermore, intestinal barrier dysfunction is increasingly recognized as a contributing factor in depression via endotoxemia and peripheral immune activation.[23] In our study, the decline in tight junction proteins (Occludin, Tjp1,

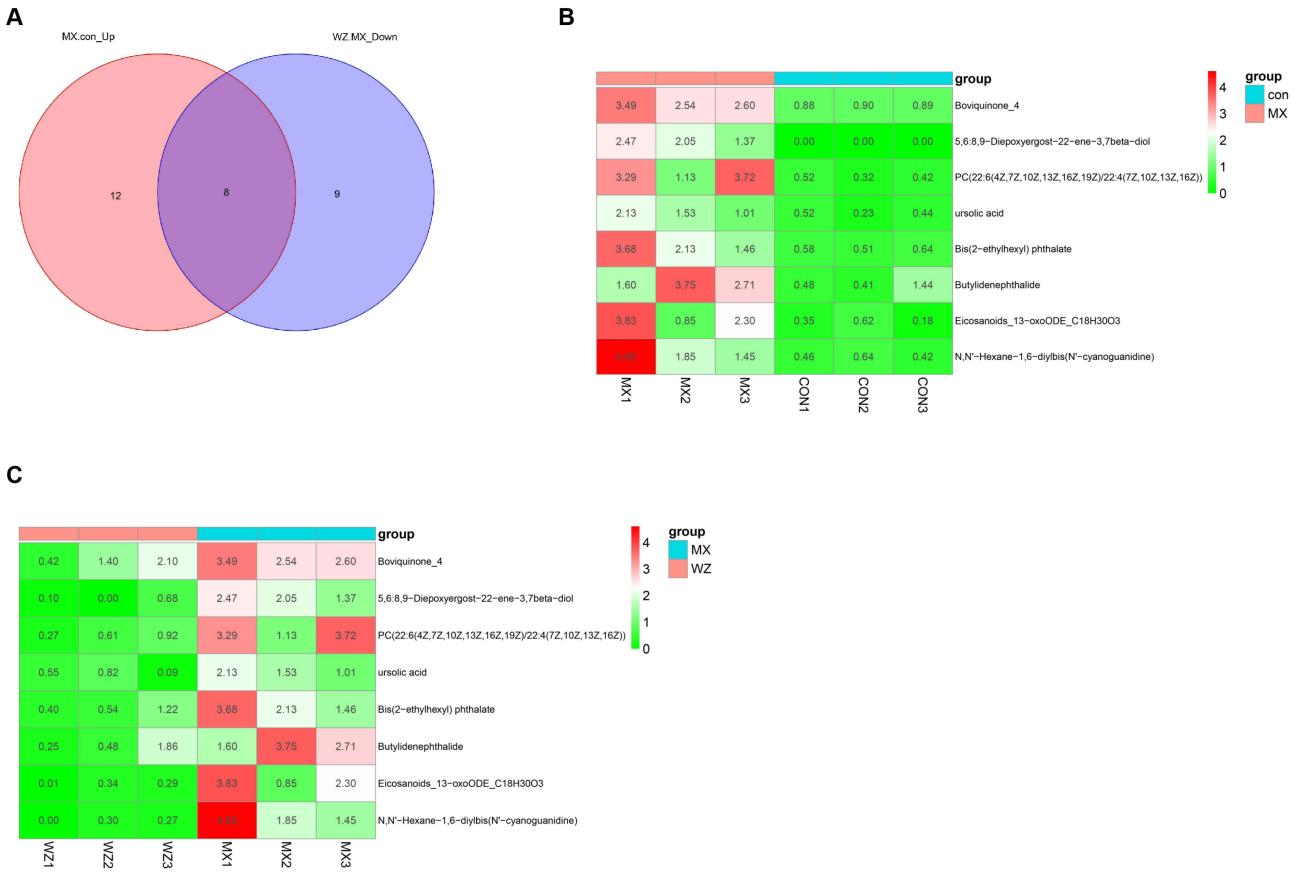

Figure 7 Identification of key metabolites showing an "abnormal–recovery" trend across groups. (**A**) Venn diagram illustrating eight overlapping differential metabolites between the Control vs CUMS and CUMS vs MMWA comparisons. (**B**) Heatmap of relative expression levels of key metabolites between the Control and CUMS groups, showing significant downregulation in CUMS rats. (**C**) Heatmap showing partial or complete recovery of these metabolites following MMWA treatment. Color intensity reflects Z-score standardized abundance.

Claudin4) and upregulation of pro-inflammatory histidine metabolism enzymes (HAL, HDC) in CUMS rats were both significantly reversed by MMWA, corroborating previous findings on the anti-inflammatory role of acupuncture on gut epithelial integrity.

Importantly, this study extends the literature by employing integrated 16S rRNA gene sequencing, untargeted metabolomics, and transcriptomic profiling in a traditional medicine context. Recent multi-omics studies have identified similar gut-metabolite signatures in both rodent and human depression cohorts, highlighting the translational relevance of our findings.[24]

This study demonstrates that MMWA alleviates depression by regulating the gut microbiota-metabolite-barrier axis in a validated CUMS model, highlighting the recovery of key microbial genera (Lactobacillus, Prevotella) and neuroactive metabolites such as taurine and arginine. By integrating 16S rRNA sequencing with untargeted metabolomics, the work reveals a functional link between microbial remodeling and host metabolic regulation, suggesting potential targets for microbiota-based interventions in depression. Furthermore, it provides scientific support for traditional Mongolian medicine by aligning the concept of "Heti regulation" with contemporary gut-brain axis paradigms, thereby bridging ethnomedicine and evidence-based neuroscience.

Several limitations should be acknowledged. Male rats were used in this study to minimize the confounding effects of hormonal fluctuations, which significantly influence gut microbiota composition, intestinal barrier function, immune activation, and stress susceptibility.[5–12] Female rodents exhibit estrous cycle–dependent changes in circulating sex hormones, producing substantial variability in microbiota profiles, cytokine secretion, and stress-related behavioral outcomes.[5–7] Such intra-group variability can reduce statistical power and obscure microbiota–metabolite associations

https://doi.org/10.2147/NDT.S566386

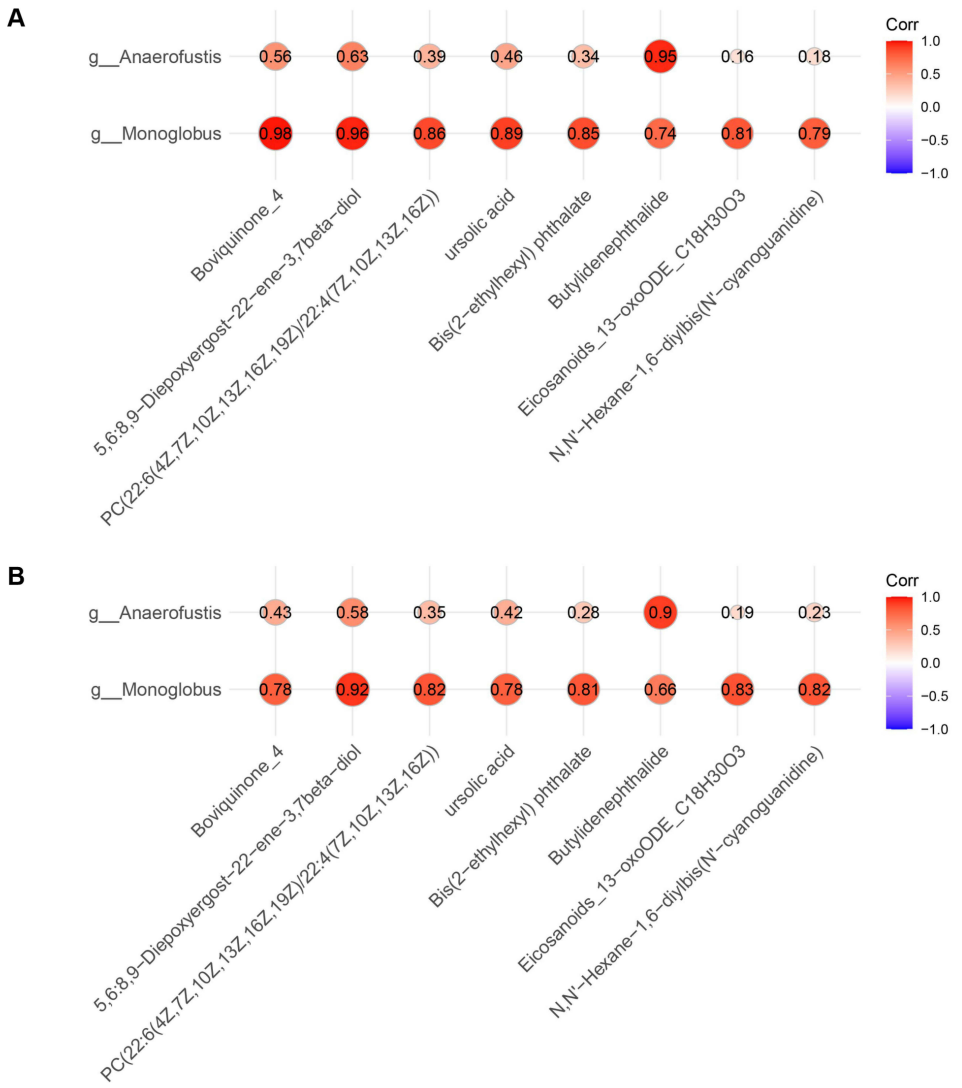

Figure 8 Correlation between key fecal metabolites and gut microbial genera. (**A**) Heatmap showing Spearman correlation coefficients between selected differential genera and key metabolites in the Control and CUMS groups. (**B**) Correlation heatmap between microbial genera and metabolites in the CUMS and MMWA groups. Red and blue indicate positive and negative correlations, respectively. Only significant correlations are shown ($p < 0.05$). All correlations were calculated based on n = 9 biological samples per group, and Spearman rho coefficients are represented by the heatmap color scale.

in multi-omics analyses. Therefore, the use of male rats helped ensure biological consistency when examining the effects of chronic stress and warm acupuncture. Nevertheless, sex differences in microbiota–brain interactions are biologically relevant, and future studies incorporating both sexes will be essential to determine the broader generalisability of our findings. Although the sample size of nine animals per group is modest, it is consistent with established practice in CUMS-based behavioral and microbiota research. A recent meta-analysis confirmed that the CUMS model typically produces medium-to-large effect sizes, allowing reliable detection of group differences with comparable sample sizes.[4] Moreover, key alterations in gut microbiota and metabolite profiles associated with depression have been shown to exhibit robust effect sizes in both clinical and preclinical studies.[5] Nonetheless, we acknowledge that detecting subtle molecular differences in high-dimensional omics datasets may require larger cohorts, and future studies with expanded sample sizes will be needed to validate small-effect changes. Additionally, causal relationships between microbiota and behavior remain correlative; future studies employing fecal microbiota transplantation or germ-free models are warranted. While multi-omics data were comprehensive, central nervous system markers such as hippocampal BDNF and neuroinflammatory cytokines were not assessed, which constrains mechanistic insight.

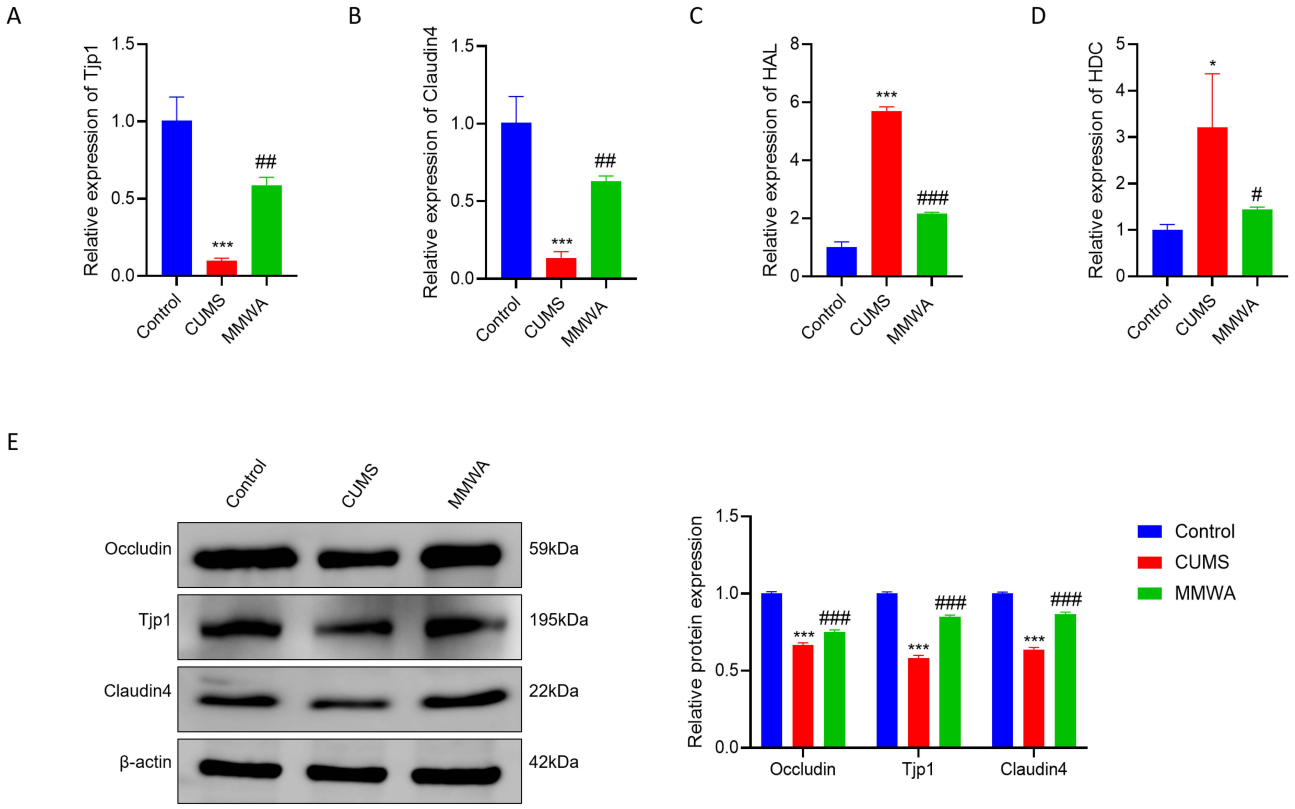

Figure 9 Warm acupuncture enhances intestinal barrier integrity and suppresses histidine metabolism in CUMS rats. (**A**–**C**) mRNA expression levels of tight junction–related genes Occludin, Tjp1 (ZO-1), and Claudin4 in colonic tissues, determined by RT-qPCR. (**D**) Representative Western blot and quantitative analysis of Occludin, Tjp1, and Claudin4 protein expression in the colon. (**E**) mRNA expression of histidine metabolism–related enzymes HAL and HDC, showing upregulation in CUMS rats and suppression by MMWA intervention. Data are presented as mean ± SD (n = 3–6/group). $^{*}p < 0.05$, $^{***}p < 0.001$ vs Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs CUMS.

Conclusion

This study demonstrates that MMWA alleviates depression-like behaviors in CUMS rats by restoring gut microbiota composition, correcting metabolic disturbances, and enhancing intestinal barrier function. These findings suggest potential mechanisms through which MMWA may influence depressive-like behavior in animal models. Possible pathways—including TLR4/NF-κB signaling, neurotransmitter biosynthesis, and SCFA-mediated gut–brain communication—warrant further investigation. Given the use of only male rats and the absence of germ-free or FMT validation, the translational relevance should be interpreted cautiously. Overall, MMWA appears to modulate the microbiota–metabolite–barrier axis and may serve as a complementary approach for improving depressive-like behaviors in preclinical settings.

Data Sharing Statement

The data used to support the findings of this study are available from the Dr. Aruna and Dr. Silengge upon request.

Ethic Statement

This study was approved by the Medical Ethics Committee of the Inner Mongolia Medical University (YKD202301169) and conducted in compliance with the institutional guidelines (Directive 2010/63/EU in Europe) for the care and use of animals.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by National Natural Science Foundation of China (No. 82460981); Mongolian pharmacy "First-Class Discipline" of Inner Mongolia Medical University (No. MYX2023-S01); First-class scientific research projects of Inner Mongolia Medical University (YLXKZX-NYD-001; YLXKZX-NYD-002); Chinese Medicine (Mongolian Medicine) "first-class discipline" construction of scientific and technological innovation team (2024MYYLXK004); Inner Mongolia Cultivating Center of Cooperative Innovation for Mongolian Medicine (No. MYYXTPY202207); Science and Technology Development Initiative for Advanced Clinical Specialties in Public Hospitals of Inner Mongolia Autonomous Region (No. 2023SGGZ131) and Natural Science Foundation of Inner Mongolia Autonomous Region (No. 2024MS08014).

Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Marwaha S, Palmer E, Suppes T, Cons E, Young AH, Upthegrove R. Novel and emerging treatments for major depression. *Lancet*. 2023;401 (10371):141–153. doi:10.1016/S0140-6736(22)02080-3
2. Patel V, Saxena S, Lund C, et al. Transforming mental health systems globally: principles and policy recommendations. *Lancet*. 2023;402 (10402):656–666. doi:10.1016/S0140-6736(23)00918-2
3. Liu G, Miao L, Niu H, et al. Human Umbilical Cord Mesenchymal Stem Cells Ameliorated Chronic Unpredictable Mild Stress-Induced Depression and Anxiety by Alleviating Neuroinflammation. *J Neuroimmune Pharmacol*. 2025;20(1):45. doi:10.1007/s11481-025-10198-2
4. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. *Neurosci Biobehav Rev*. 2019;99:101–116. doi:10.1016/j.neubiorev.2018.12.002
5. Liu L, Wang H, Chen X, Zhang Y, Zhang H, Xie P. Gut microbiota and its metabolites in depression: from pathogenesis to treatment. *EBioMedicine*. 2023;90:104527. doi:10.1016/j.ebiom.2023.104527
6. Li N, Wang Q, Wang Y, et al. Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice via the gut microbiota-inflammation-brain axis. *Stress*. 2019;22(5):592–602. doi:10.1080/10253890.2019.1617267
7. Rutsch A, Kantsjo JB, Ronchi F. The Gut-Brain Axis: how Microbiota and Host Inflammasome Influence Brain Physiology and Pathology. *Front Immunol*. 2020;11:604179. doi:10.3389/fimmu.2020.604179
8. Mhanna A, Martini N, Hmaydoosh G, et al. The correlation between gut microbiota and both neurotransmitters and mental disorders: a narrative review. *Medicine*. 2024;103(5):e37114. doi:10.1097/MD.0000000000037114
9. 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(8):461–478. doi:10.1038/s41575-019-0157-3
10. Hays KE, Pfaffinger JM, Ryznar R. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. *Gut Microbes*. 2024;16(1):2393270. doi:10.1080/19490976.2024.2393270
11. 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. doi:10.1016/j.cpr.2020.101943
12. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*. 2015;48:186–194. doi:10.1016/j.bbi.2015.03.016
13. Shui L, Yi RN, Wu YJ, et al. Effects of Mongolian Warm Acupuncture on iNOS/NO and Inflammatory Cytokines in the Hippocampus of Chronic Fatigue Rats. *Front Integr Neurosci*. 2019;13:78. doi:10.3389/fnint.2019.00078
14. G A, Li X, Su B, et al. Effect of Mongolian warm acupuncture on the gene expression profile of rats with insomnia. *Acupunct Med*. 2019;37 (5):301–311. doi:10.1136/acupmed-2016-011243
15. Xu Y, Li X, Man D, Su X, G A. iTRAQ-based proteomics analysis on insomnia rats treated with Mongolian medical warm acupuncture. *Biosci Rep*. 2020;40(5). doi:10.1042/BSR20191517
16. Wang L, Wang Z, Lan Y, Tuo Y, Ma S, Liu X. Inulin Attenuates Blood-Brain Barrier Permeability and Alleviates Behavioral Disorders by Modulating the TLR4/MyD88/NF-kappaB Pathway in Mice with Chronic Stress. *J Agric Food Chem*. 2023;71(36):13325–13337. doi:10.1021/acs.jafc.3c03568
17. Xiao B, Wang J, Yang H, et al. Study on preparation and properties of silver alloy for Mongolian medicine acupuncture. *Heliyon*. 2024;10(8): e29145. doi:10.1016/j.heliyon.2024.e29145
18. Wang J, Zhu H, Song X, et al. Electroacupuncture regulates gut microbiota to reduce depressive-like behavior in rats. *Front Microbiol*. 2024;15:1327630. doi:10.3389/fmicb.2024.1327630
19. Wang M, Sun P, Li Z, et al. Eucommiae cortex polysaccharides attenuate gut microbiota dysbiosis and neuroinflammation in mice exposed to chronic unpredictable mild stress: beneficial in ameliorating depressive-like behaviors. *J Affect Disord*. 2023;334:278–292. doi:10.1016/j.jad.2023.04.117
20. Bo A, Si L, Wang Y, et al. Clinical Trial Research on Mongolian Medical Warm Acupuncture in Treating Insomnia. *Evid Based Complement Alternat Med*. 2016;2016(1):6190285. doi:10.1155/2016/6190285
21. Cao L, Shen M, Yu H, Lv H, G A. Clinical effect of the warm acupuncture of Mongolian medicine on insomnia in the elderly and its brain-gut peptide mechanism: a randomized controlled trial. *Zhongguo Zhen Jiu*. 2024;44(7):787–791. doi:10.13703/j.0255-2930.20230816-k0001
22. Góralczyk-Bińkowska A, Szmajda-Krygier D, Kozłowska E. The Microbiota-Gut-Brain Axis in Psychiatric Disorders. *Int J Mol Sci*. 2022;23 (19):11245. doi:10.3390/ijms231911245

23. Geurts L, Neyrinck AM, Delzenne NM, Knauf C, Cani PD. Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics. *Benef Microbes*. 2014;5(1):3–18. doi:10.3920/BM2012.0065
24. Qian X, Tian P, Guo M, et al. Determining the emotional regulation function of Bifidobacterium breve: the role of gut metabolite regulation over colonization capability. *Food Funct*. 2024;15(3):1598–1611. doi:10.1039/D3FO02739B