Liu-Jun-Zi decoction alleviates chemotherapy-induced anorexia by regulating gut microbiota and TLR4/MyD88/NF-κB p65 signaling pathway

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Abstract

Background: Liu-Jun-Zi decoction (LJZD), a classical nourishing formula in China, has been proven to be effective in treating chemotherapy-induced anorexia. In this study, the mechanism of LJZD in alleviating chemotherapy-induced anorexia was discussed from the aspects of regulating gut microbiota, repairing intestinal barrier injury and inhibiting inflammatory pathways. Methods: A rat model of chemotherapy-induced anorexia was established using cisplatin. The study evaluated the therapeutic effects of LJZD by observing the weight, food intake, and intestinal pathology of rats. The impact of LJZD on gut microbiota and metabolites, specifically short-chain fatty acids, was investigated through gut microbiota analysis and targeted metabolomics. The anti-inflammatory and intestinal protective effects of LJZD were assessed by examining the expression of intestinal tight junction proteins associated with the inflammatory pathway. Results: LJZD alleviated cisplatin-induced inflammation and intestinal barrier disruption, as evidenced by upregulated expression of tight junction protein 1 (TJ-1) and occludin, along with reduced serum levels of interleukin 6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and lipopolysaccharide. Additionally, LJZD alleviated microbiota imbalance and regulated the levels of short-chain fatty acids, especially increased the relative abundance of Coriobacteriales Incertae Sedis, Lactobacillus johnsonii F19785, Pseudalterella, and reduced the Tyszerella. In the hypothalamus, LJZD exerts suppressive effects on the toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/nuclear factor-κB (NF-κB) p65 signaling pathway, leading to a downregulation in the transcriptional activity of IL-6 and IL-1β, as well as Interleukin 6 receptors (IL-6R) and Interleukin-1β receptors (IL-1R1) mRNA expression levels. Conclusion: In summary, LJZD alleviate chemotherapy-induced anorexia by modulating the gut microbiota, repairing the intestinal mechanical barriers, and suppressing the TLR4/MyD88/NF-κB p65 signaling pathway.

Keywords: Liu-Jun-Zi decoction; chemotherapy-induced anorexia; cisplatin; TLR4/MyD88/IL-1/IL-6 signaling pathway; gut microbiota; rat
Liu-Jun-Zi decoction (LJZD) is a classic Chinese medicine tonic, originated in the Ming Dynasty. Clinical and animal studies have found that LJZD can effectively ameliorate chemotherapy-induced anorexia. We investigated the pathological mechanism of CIA from the aspect of intestinal microbiota and further clarified the underlying mechanism of LJZD in preventing and treating CIA from the perspective of regulating TLR4/MyD88/NF-κB p65 signaling pathway in hypothalamus and gut microbiota.

**Medical history of objective**

LJZD, a traditional Chinese nourishing prescription, was originally recorded in Yi Xue Zheng Zhan written by Tu Yuan in 1515 C.E. LJZD is commonly used to treat conditions associated with poor digestive function and excess bodily fluids/inflammation, reflecting a pattern in Chinese Medicine known as “spleen deficiency with phlegm-dampness”, as its main effects are to improve digestive function and enhance overall vitality, address fluid retention and regulate mucus production.

**Background**

Chemotherapy-induced anorexia (CIA) refers to a condition characterized by reduced appetite and decreased food intake during chemotherapy treatment [1]. The manifestation of CIA can induce inadequate intake of nutrients among patients, precipitating weight loss, fatigue, compromised immune function, and suboptimal therapeutic response [1]. Consequently, the amelioration of chemotherapy-induced anorexia assumes paramount importance in the realm of patients’ convalescence and treatment outcomes. Notably, despite the absence of specific pharmacotherapies for CIA, certain medications have been employed to alleviate symptoms associated with chemotherapy-induced anorexia, thereby fostering appetite improvement and facilitating weight restoration [2]. Noteworthy among these medications is megestrol acetate (MA), which, when administered alone or concomitantly with agents like thalidomide, ramaceton, and dexamethasone, has demonstrated its efficacy in effectively mitigating anorexia and cachexia in patients afflicted with advanced-stage cancer [3]. However, it is imperative to acknowledge that the administration of MA may engender a spectrum of severe adverse reactions, thereby potentially engendering patient noncompliance [3]. Consequently, the pursuit of efficacious interventions capable of alleviating CIA remains an urgent and formidable medical challenge, necessitating further concerted research endeavors.

The mechanism of CIA is mainly related to the central nervous system (CNS), peripheral signals, and inflammatory factors [4-6]. Research has elucidated that tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) can stimulate the degradation of orexin precursor genes within the hypothalamus, thereby precipitating CIA [7, 8]. Dai et al. have demonstrated that attenuating interleukin 6 (IL-6) levels in the brain can suppress hyperactivation of the Janus kinase/signal transducer and transmembrane activator (JAK-STAT) signaling pathway, consequently modulating the expression of anorexigenic and orexigenic peptides and ameliorating CIA [9]. Moreover, investigations have reported a close association between augmented intestinal permeability and certain neurological disorders, with bidirectional signaling across the gut-brain axis regulating inflammatory responses and immune homeostasis [10].

Cisplatin, a widely utilized chemotherapeutic agent in cancer treatment, often elicits adverse reactions, particularly gastrointestinal toxicity, during its administration [11]. Notably, cisplatin can severely disrupt the composition and functionality of the gut microbiota, resulting in intestinal structural and functional disturbances [11]. The gut microbiota serves a pivotal role in maintaining dynamic equilibrium within the intestines and safeguarding intestinal integrity [12, 13]. Gut microbiota metabolism-mediated production of short-chain fatty acids (SCFAs) has been identified as a regulator of intestinal immune function and reinforcement of the intestinal mechanical barrier by augmenting the expression of various tight junction proteins in intestinal epithelial cells [14]. Both foundational and clinical investigations have highlighted the potential key involvement of the gut microbiota in the development of chemotherapy-induced intestinal inflammation and injury [11]. The disruption of dynamic balance within the gut microbiota exerts deleterious effects on the body’s levels of SCFAs and lipopolysaccharide (LPS), ultimately instigating intestinal inflammation and oxidative stress [15]. These inflammatory processes within the intestines can trigger the release of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α. Left uncontrolled, these factors can yield severe systemic consequences [16]. Circulating pro-inflammatory agents such as LPS can compromise the integrity of the blood-brain barrier and gut-blood-brain barriers by disrupting tight junction epithelia, thereby inducing increased intestinal permeability or “intestinal leakage” [10]. Coupled with the breakdown of the CNS barrier, this allows intestinal-derived molecules, toxins, and pathogens to access the brain parenchyma, activate local immune cells, and serve as a conduit for triggering neuroinflammation [16]. Furthermore, SCFAs prompt intestinal endocrine cells to generate and release hormones such as ghrelin-like peptide 1 and peptidely, indirectly modulating the impact of the CNS on postprandial satiety and emotional states [17, 18].

Liu-Jun-Zi decoction (LJZD), a traditional Chinese nourishing prescription, was originally recorded in Yi Xue Zheng Zhan written by Tu Yuan in 1515 C.E. LJZD is primarily employed in the management of the syndrome characterized by spleen deficiency accompanied by phlegm-dampness, as its main effects are to improve digestive function and enhance overall vitality, address fluid retention and regulate mucus production [19]. Its ingredients include Ginseng Radix et Rhizoma, Atractylodis Macrocephalae Rhizoma, Poria, Glycyrrhizae Radix et Rhizoma, Citri Reticulatae Pericarpium, Pinelliae Rhizoma, Zingiberis Rhizoma Recens, Jujubae Fructus. Clinical and animal studies have found that LJZD can effectively treat CIA [9, 20, 21]. Our prior investigations have put forth the notion that the potential mechanism underlying the therapeutic effects of LJZD in CIA involves the inhibition of the janus kinase-signal transducer and activator of transcription signaling pathway, as well as the modulation of appetite regulatory peptides expression [9]. At present, the mechanism of action by which LJZD alleviates CIA has not been extensively investigated, and most studies have focused on the ghrelin hormone and its receptors [4, 21]. However, investigations regarding the impact of LJZD on the regulation of gut microbiota, restoration of intestinal barrier integrity, and inhibition of the toll-like receptor 4 (TLR4)-myeloid differentiation factor 88 (MyD88)-nuclear factor-κB (NF-κB) p65 signaling pathway within the brain have not been documented, and a limited number of studies have established a connection between CIA and the gut-brain axis. To bridge this knowledge gap, our study seeks to examine the potential of LJZD in ameliorating CIA by rebalancing the gut microbiota, repairing the intestinal barrier, and inactivating the TLR4-MyD88-NF-κB p65 signaling pathway responsible for generating appetite-inhibiting cytokines. By doing so, we endeavor to provide a scientific and theoretical foundation for the treatment and management of CIA.

**Material and methods**

**Chemicals and reagents**

Megestrol acetate was purchased from Guohai Biological Pharmaceutical (Qingdao, China, Lot. No. 21060506). Cisplatin was purchased from Melun Biotechnology Co., Ltd. (Dalian, China, Lot. No. J0102A). Normal saline was purchased from Guangdong Kelun Medicine & TradeGroup Co., Ltd. (Guangdong, China, Lot. No. H2007070702). Sodium pentobarbital was purchased from Deshang.
**Preparation of LJZD**

The proportion of each herb in LJZD is 9:9:6:3:4.5, and the preparation method was mentioned in our previous investigation [9]. High-performance liquid chromatography method validation and fingerprint analysis of 10 batches of LJZD have been completed previously [22]. Based on the fingerprint analysis, we observed that the similarity between each sample and the reference fingerprint (S1) was above 0.927, which suggests that the S1 batch was highly consistent, with minimal variations in the main ingredient groups, and a scientifically sound and rational preparation process [22]. We conducted quantitative analysis of the main components in batches S1–S10 of LJZD (Supplementary Table S1). Following, batch S1 sample was used for this pharmacological study.

**Animal care and experiment details**

Thirty male Sprague-Dawley rats (6–8 weeks old, admission weight: 180–210 g) with specific-pathogen-free status were sourced from Guangdong Medical Laboratory Animal Center (Guangdong, China, License No. SCXK (yue) 2018-00002). The feeding conditions of rats were as follows: temperature: 20–25 °C; humidity: 40%–70%; light/dark cycle: 12 h; feeding standard food and water. This protocol was ethically approved by the Animal Care Committee of Guangdong Pharmaceutical University (Protocol No.: gdpulacspf20170001). The animal experiment was performed in line with the Guidelines for the Management and Use of Laboratory Animals by the Chinese National Institutes of Health. Finally, criteria established for euthanizing rats were performed according to ARRIVE 2.0 guidelines rigorously.

A pre-experimental acclimation period of six days was allotted to allow the rats to acclimate to both their environment and the designated diet. After acclimation, the rats were stratified by weight and then subsequently allocated into five groups using a randomized assignment approach (n = 6) including (1) Control group (i.g. distilled water b.i.d, i.p. saline, Control group); (2) LJZD normal control group (i.g. 4.8 g/kg LJZD b.i.d, i.p. saline, LJZD group); (3) Cisplatin group (i.g. distilled water b.i.d, i.p. 6 mg/kg cisplatin, Cis group); (4) MA treatment group (i.g. 25 mg/kg MA b.i.d, i.p. 6 mg/kg cisplatin, MA + Cis group) and (5) LJZD treatment group (i.g. 4.8 g/kg LJZD b.i.d, i.p. 6 mg/kg cisplatin, LJZD + Cis group). In our previous multi-dose study, we examined the dose-effect relationship of LJZD and determined that the dose of 9.6 g/kg per day was the optimal dosage for mitigating CIA [9]. Based on this finding, the present study employed the most effective dose of LJZD (4.8 g/kg LJZD b.i.d) instead of multi-dose study. Cisplatin was diluted to 0.6 mg/mL with saline, and a single intraperitoneal injection at a dose of 10 mL/kg was established for CIA model. The rats received intragastric administration twice daily for 0–3 days (8 a.m. and 8 p.m.). The food intake and body weight of rats were meticulously documented at predetermined time points of ~48, ~24, 0, 24, 48, and 72 h, respectively. An hour after the final gavage, fresh feces (about 2–3 pellets) of rats were collected in frozen tubes and quickly stored in liquid nitrogen for intestinal microbiota analysis. Sodium pentobarbital (45 mg/kg, i.p.) was used to anesthetize all groups. After anesthetizing the rats (confirmed by the absence of tail pinch reflex), blood samples were collected via the abdominal aorta. Segments of 1 cm were taken from the jejunum (15 cm below the pylorus), ileum (1 cm above the ileocecal valve), and colon (1 cm below the cecum), fixed in a 4% paraformaldehyde solution for a duration of 24 h and subsequently transferred to 70% ethanol for histopathological and immunofluorescence analysis. The hypothalamus was also collected and rapidly frozen in liquid nitrogen for subsequent analysis of relevant indicators (Figure 1A illustrates the experimental timeline for the animal study).

**16S rDNA analysis of gut microbial community**

Total microbial DNA was extracted from the samples using the HiPure fecal DNA kit (Magen Co., Ltd., Guangzhou, China). The concentration and purity of the samples were determined through agarose gel electrophoresis. Subsequently, the V3–V4 region of the 16S rDNA gene was amplified via PCR using specific primers, resulting in the preparation of a 16S rDNA gene library. The Illumina platform was employed for end-to-end sequencing (PE250) following standard protocols. All PCR reagents were sourced from New England Biolabs (Ipswich, MA, USA). Based on the assigned labels, we conducted abundance statistics of operational taxonomic units, diversity analysis,
species composition analysis, functional research, and environmental relationship research. The sequencing and bioinformatics analysis were performed using the Omicsmart online platform (http://www.omicsmart.com). Furthermore, we express our gratitude to Genedeno Bio technology Co., Ltd. (Guangzhou, China) for their assistance in 16S rDNA gene sequencing. The raw data obtained from the experiment has been deposited in the NCBI Sequence Read Archive database under the accession number PRJNA97993.

LC-MS detection of SCFAs in rat feces
The levels of SCFAs in rat fecal samples were determined using LC-MS analysis. The samples were diluted with 50% acetonitrile (Sigma-Aldrich Co., Ltd., St Louis, MO, USA) and centrifuged at 12,000 rpm and 4 °C for 15 min. The resulting supernatant was combined with 3-NPH (Aladdin Biochemical Technology Co., Ltd., Shanghai, China) and EDC (6% pyridine, Aladdin Biochemical Technology Co., Ltd., Shanghai, China) in a volume ratio of 2:1:1. The mixture was then centrifuged again at 12,000 rpm and 4 °C for 15 min. The supernatant was further diluted 100 times with 50% acetonitrile and water for UPLC-MS analysis. The UPLC system used was the Waters Acquity Series (Waters Technologies), equipped with an Acquity UPLC HSS T3 column (2.2 mm × 100 mm, 1.8 μm, Waters Technologies Inc.), coupled with a mass spectrometry system (AB SCIEX 5500 Qtrap-MS, AB SCIEX Inc., Milwaukee, WI, USA).

The standard curve was constructed by graphing the peak area of each component on the y-axis against the corresponding mass concentration in (ng/mL) on the x-axis. The regression equation and linear range of the standard curve can be found in Supplementary Table S2. To assess the stability of all SCFAs, QC samples were incorporated randomly during sample injection. The RSD of peak area values obtained from the QC samples was calculated as an indicator of stability. A comprehensive summary of the results can be found in Supplementary Table S3.

ELISA assay
Each intestinal tissue was supplemented with an appropriate volume of PBS for protein extraction, followed by homogenization. The concentrations of LPS, IL-6, IL-1β, and TNF-α in the serum were quantified utilizing commercially available ELISA kits.

Hematoxylin-eosin (HE) staining
Tissue samples of the jejunum, ileum, and colon were collected from each experimental group, dissected into small 0.5 cm fragments. The specimens were fixed overnight at 4 °C in 4% paraformaldehyde. Subsequently, these samples were embedded in paraffin and sectioned into slices of 4 μm thickness. HE staining was performed on these sections for histological examination. The height of intestinal villi, the thickness of intestinal wall and the thickness of intestinal mucus were measured by a trinocular compound microscope (CKX41, Olympus Corporation, Tokyo, Japan) with digital camera. The severity of intestinal injury was evaluated semi-quantitatively according to the histological scoring criteria [23].

Immunofluorescence staining
The slides were subjected to citric acid antigen retrieval solution in a microwave oven for repair, followed by permeabilization and blocking with 5% BSA containing 0.5% Triton X-100 at room temperature for 2 h. Subsequently, the slides were incubated with rabbit anti-mouse TJ-1 antibody and rabbit anti-mouse occludin antibody overnight at 4 °C. Following the washing step, it was subsequently incubated with a combination of Alexa Fluor 594-labeled donkey anti-mouse antibody (1:200, Beyotime Bio Technology, Shanghai, China, Lot. No. A04553), at room temperature for 1 h. After cleaning again, added an appropriate amount of anti-fluorescence quenching mounting agent and observed by CKX41 inverted phase contrast microscope.

Real time quantitative PCR assay
Total RNA was extracted from the hypothalamus using Trizol reagent, followed by reverse transcription into cDNA. The primer sequence (refer to Supplementary Table S4) used for analysis was custom-designed and synthesized by Sangon Bioengineering Co., Ltd. (Shanghai, China, https://www.sangon.com/). The reaction mixture was prepared using the TB Green® Premix Ex Taq™ II kit, while GAPDH served as the internal reference for normalization. The relative expression levels of individual mRNA transcripts were determined using the 2^ΔΔCT method.

Western blot (WB) assay
Hypothalamic tissues were collected and lysed in pre-chilled RIPA protein extraction reagents supplemented with protease inhibitors. The total protein content was determined using a BCA kit (Tianyu Biotechnology Co., Ltd., Shanghai, China). Equal amounts of protein were loaded into wells and denatured in sodium dodecyl sulfate sample buffer at 95 °C for 10 min. The gel was prepared following the guidelines provided by the SDS-PAGE gel kit (Beyotime Bio Technology, Shanghai, China) and subjected to electrophoresis. Subsequently, the protein samples were transferred onto a polyvinylidene fluoride membrane (Millipore, Boston, MA, USA) using electroblotting techniques. Following Tris-buffered saline containing 1% Tween-20 washing, the membrane was blocked with 3% bovine serum albumin or 5% skimmed milk powder at room temperature for 1 h. Next, the membrane was incubated overnight at 4 °C with the primary antibody. After incubation with HRP-labeled secondary antibodies, chemiluminescent signals were detected using highly sensitive ECL chemiluminescence reagents (Beyotime Bio Technology, Shanghai, China). Band intensities were quantified utilizing ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis
The experimental results were presented as mean ± standard error of the mean. Statistical analysis was conducted using GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA). Two-way analysis of variance (ANOVA) was applied to assess food intake and body weight, while one-way analysis of variance followed by Tukey’s multiple comparison test was employed for other calculations. P-value less than 0.05 was deemed to indicate a statistically significant difference.

Results
LJZD alleviated cisplatin-induced anorexia and the decrease in food intake
In order to study the impact of LJZD and MA on Cia, we recorded food intake and body weight. The findings revealed that the food intake and body weight of rats in all cisplatin-modeled groups significantly decreased when compared to the Control group after 24, 48, and 72 h (P < 0.001, Figure 1B, 1C). At 72 h post-modeling, the food intake of rats in the MA + cis group and LJZD + cis group exhibited a significant increase compared to the Cis group (P < 0.01, P < 0.001, Figure 1B), but there was no significant recovery of body weight in two treatment groups (Figure 1C). Interestingly, the food intake of the LJZD group was higher than that of the Control group at 24 and 72 h, but there was no significant difference. The results suggest that LJZD administration has the potential to attenuate cisplatin-induced anorexia and reestablish food intake in rats.

LJZD eliminated cisplatin-induced systemic inflammation and intestinal mechanical barrier destruction
ELISA results indicated a significant increase in peripheral IL-6, IL-1β, TNF-α, and LPS levels following cisplatin administration (Figure 2A). LJZD can significantly reduce the effect of cisplatin on the above cytokines, which is consistent with the changes of the above inflammatory factors and their related receptor mRNA in the hypothalamus (Figure 2B). The histological morphology of the jejunum, ileum, and colon was evaluated using HE staining. (Jejunum and ileum’s HE staining results were shown in Supplementary Figure S1). Taking the colon as an example, the results demonstrated that the intestinal tissue structure of the Control group was complete, the
intestinal villi were arranged neatly and densely, the mucosal muscle layer was tight, and no cell inflammatory infiltration was observed. Combined with histological semi-quantitative analysis, compared with the Control group, the intestinal structure of rats in Cis group was incomplete, the muscular layer of mucosa was damaged, inflammatory cell infiltration appeared, and the thickness of intestinal wall (blue column, Figure 2C) was significantly thinner ($P < 0.001$, Figure 2D); intestinal villus atrophy, disordered arrangement, increased crypt depth, and decreased villus height ($P < 0.01$, Figure 2E, Supplementary Figure S1c); the thickness of mucous (green short column, Figure 2C) was significantly thinner ($P < 0.01$, Figure 2F). The two treatment groups significantly ameliorated the intestinal barrier damage caused by cisplatin, which was consistent with the immunofluorescence results (Figure 3). TJ-1 and occludin as transmembrane proteins showed blue nuclei and green protein expression on cell membranes under immunofluorescence microscopy (Figure 3A, 3B, jejunum and ileum’s immunofluorescence results were shown in Supplementary Figure S2). The analysis of integral optical density value obtained from immunofluorescence staining indicated a substantial reduction in the expression of TJ-1 and occludin proteins in the rat intestine treated with cisplatin, when compared to the Control group ($P < 0.001$). In contrast, there was a notable elevation in the expression of TJ-1 and occludin proteins in the intestinal tract of the MA + Cis and LJZD + Cis groups, as compared to the Cis group (TJ-1 in MA + Cis group: $P < 0.01$; occludin: $P < 0.001$; TJ-1 in LJZD + Cis group: $P < 0.05$; occludin: $P < 0.01$, Figure 3C, 3D).

![Figure 2 Effect of LJZD on cisplatin-induced systemic inflammation and intestinal mechanical barrier destruction.](https://www.tmrjournals.com/tmr)

(A) Plasma IL-6, IL-β, TNF-α and LPS levels were detected by ELISA kits. (B) n = 6. Relative mRNA levels of IL-6, IL-6R, IL-1β and IL-1RI were detected by real time quantitative polymerase chain reaction. (C) n = 6. H&E staining of colon, scale bar = 100 μm, the magnification was 200× (i). (D–F) The thickness of intestinal wall, the thickness of mucous and the height of villus measurement results of colon. Data were expressed as mean ± SEM (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the Cis group; **$P < 0.01$, ***$P < 0.001$, compared with the Control group. LJZD, Liu-Jun-Zi decoction; Cis, cisplatin; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin 6; LPS, lipopolysaccharide; MA, megestrol acetate.
The administration of cisplatin can lead to alterations in the diversity of various intestinal microbial communities

After cisplatin administration, the species richness and evenness of the Cis, MA + Cis, and LJZD + Cis groups were determined to be significantly lower compared to both the Control and LJZD groups, as indicated by the α-diversity indices, Shannon, Simpson, Chao 1 and ACE (Figure 4A, 4B). The data of ACE and Chao1 can be seen in Supplementary Figure S3). It can be seen from the Simpson analysis that compared with the Cis group, the MA + Cis and LJZD + Cis groups can significantly improve α diversity ($P < 0.01$, $P < 0.05$, Figure 4B). However, compared with the Cis group, the LJZD + Cis groups showed an improvement trend in Shannon, Chao 1 and ACE analysis, but there was no significant difference, which may be caused by different analysis models. For β-diversity, we used the weighted Bray matrix for nonmetric multidimensional scaling analysis and the accuracy of the model was assessed by measuring stress values. Our findings demonstrated significant differences in gut microbiota among the five groups, and the reliability of the data model was confirmed by a stress value of 0.03. It was obvious that the intestinal microbiota was separated from the Control group after cisplatin modeling (Figure 4C). Similar microbial distinctions between the Control group and the LJZD group were also evident in our observations. A notable disparity was observed in the gut microbiota of the two treatment groups when compared to the Cis group. The bacterial communities of the two groups clustered together, but did not completely return to the level observed in the Control group.

The administration of cisplatin has been linked to distinct changes in the composition of intestinal microbial communities

As Bacteroidetes, Firmicutes, Verrucomicrobia, and Proteobacteria account for more than 90% of the total gut microbiota at the phylum level, our analysis primarily focused on changes in these four phyla (Figure 4D). Of particular note is the fact that compared with the Cis group, the Firmicutes/Bacteroidetes ratio (F/B) of the two treatment groups increased (Figure 4E). The two treatment groups could positively regulate the above microbiota, but there was no significant
difference. At the genus level, most sequences belong to Akkermansia, Bacteroides, Lachnospiraceae NK4A136 group, Romboutsia, Roseburia, Alloprevotella, Prevotellaceae NK3B31 group, Lactobacillus, Ruminoccus 1, Ruminiclostridium 9 (Figure 4F). There was a pronounced rise in the relative abundance of Akkermansia within the cisplatin modeling groups, in comparison to both the Control and LJZD groups (Figure 4G). On the other hand, the relative abundances of Lachnospiraceae NK4A136 group, Roseburia, Lactobacillus, and Alloprevotella were found to be higher in the two treatment groups compared to the Cis group. Nevertheless, it is noteworthy that these differences did not reach statistical significance (Figure 4H–4K).

In conjunction with linear discriminant analysis effect size analysis (Supplementary Figure S4), the key microbiota such as Clostridium, Lachnospiraceae NK4A136 group and Ruminococcus 1 in the Cis group were significantly reduced, while the relative abundance of Verrucomicrobia, Akkermansia, Streptococcus and Bacteroides increased significantly. It was unexpected to observe an increase in the relative abundance of Muribaculaceae in the LJZD group. Compared with Cis group, the relative abundance of Clostridia, Lachnospira, Alloprevotella, Lachnospiraceae NK4A136 group and Adlercreutzia in MA + Cis group increased significantly. The relative abundance of Coriobacteriales Incertae Sedis, Lactabacillus johnsonii F19785, Clostridiales vadinBB60 group and Parasutterella increased significantly in the LJZD + Cis group, and it is noteworthy that a decrease was observed in the relative abundance of Tyzzerella. (Supplementary Figure S4b).

Correlation analysis of SCFAs, key gut microbial taxa and intestinal mechanical barrier indexes

Fecal SCFAs content was quantified using LC-MS (Table 1). There was a significant decrease in the total SCFAs content in the Cis group compared to the Control group (P < 0.001). In the MA + Cis group, the levels of propionic acid, acetic acid, valeric acid, and total SCFAs

![Figure 4](https://example.com/figure4.png)

**Figure 4** LJZD attenuated cisplatin-induced microbial dysbiosis and regulated the key microbiota. (A–C) The α- and β-diversity of the gut microbiota were analyzed using the Shannon index, the Simpson index, and nonmetric multidimensional scaling analysis. (D, F) The distribution of intestinal microbiota at the phylum and genus levels was assessed. (E) The relative abundance of Firmicutes/Bacteroidetes at the phylum level. (G–K) Relative abundance changes of specific microbiota at genus level. Data were expressed as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001, compared with the Cis group; ****P < 0.0001, compared with the Control group. LJZD, Liu-Jun-Zi decoction; Cis, cisplatin; MA, megestrol acetate.
in feces were significantly elevated compared to the Cis group. After LJZD treatment, most of the SCFAs levels changed, especially the propionic acid level was significantly increased compared with the Cis group. Surprisingly, the majority of SCFAs in the LJZD group showed an increasing trend in their levels.

Correlation analysis was carried out to establish a link between intestinal microbiota/metabolites and parameters pertaining to the integrity of the intestinal mechanical barrier (Figure 5A, 5B). Most of the key microbiota were found to exhibit a negative correlation with serum LPS levels and a positive correlation with SCFAs, as revealed by the results. In conjunction with the indicator species analysis (Supplementary Figure S4a-S4d), the Lachnospiraceae Nk4A136 group in the Control, MA + Cis, and LJZD groups exhibited a positive correlation with propionic acid, butyric acid, acetic acid, and total SCFAs levels, and showed significant differences (P < 0.05, P < 0.05, P < 0.01, Supplementary Figure S3c, Figure 5A); it can be observed that the relative abundance of Akkermansia and Bacteroides in the Cis group exhibited a negative correlation with the levels of all SCFAs and a positive correlation with the levels of LPS in serum (Supplementary Figure S3b, Figure 5A). In the LJZD + Cis group, the specific microbiota Lactobacillus and Roseburia displayed a positive correlation with propionic acid and isobutyric acid levels, while showing a negative correlation with Isovaleric acid, Valeric acid, and serum LPS levels (Supplementary Figure S3d-S3i, Figure 5A). The correlation analysis results indicated a positive correlation between the levels of SCFAs, particularly butyric acid, isobutyric acid, and acetic acid, and markers of intestinal barrier integrity, such as villus height, intestinal wall thickness, mucus thickness, TJ-1, and occludin. On the other hand, the indicators of intestinal barrier integrity exhibited a negative correlation with LPS levels.

LJZD alleviates brain inflammation by inhibiting the TLR4/MyD88/NF-κB p65 signaling pathway

The recognition of LPS by TLR4 triggers cellular signal transduction cascades, including the activation of NF-κB and other signaling pathways. To evaluate the expression levels of TLR4 and its associated proteins, WB analysis was employed (Figure 6A). Based on the obtained results, the expression levels of TLR4, MyD88, and NF-κB p65 in the hypothalamus of the Cis group demonstrated a significant upregulation compared to the Control group (P < 0.001, P < 0.001, P < 0.001, Figure 6B-6D). As expected, the expression levels of the aforementioned proteins in the hypothalamus showed a significant decrease in both treatment groups. Remarkably, the LJZD + Cis group displayed a more pronounced therapeutic effect (P < 0.001, P < 0.001, P < 0.001, Figure 6B-6D). The findings indicate that LJZD exerts a reduction in brain inflammation and alleviates anorexia, potentially through the inhibition of the TLR4/MyD88/NF-κB p65 signaling pathway.

Discussion

In this particular investigation, we observed the ameliorating effect of LJZD on CIA in rats. We found that its potential mechanisms involve beneficial (or harmful) intestinal microbiota regulation, the repair of intestinal barrier, the inhibition of inflammatory pathways in the hypothalamus.

Studies of the gut microbiota communities

The findings of this study suggest that targeting the gut microbiota with LJZD may offer a promising therapeutic approach for enhancing CIA, as demonstrated by the reduction in intestinal LPS level and the modulation of SCFAs levels. Studies have shown that an increase in the F/B ratio can effectively lead to weight gain and stimulate appetite [24]. Consistent with prior research, our findings demonstrate that LJZD treatment can increase the F/B ratio in response to cisplatin-induced modeling, which can enhance appetite. Of particular note, LJZD treatment was observed to significantly increase the abundance of specific microbiota, including Coriobacteriales Incertae Sedis, Lactobacillales, and Parasutterella [25–27], which are known to reduce intestinal mucosal inflammation, promote metabolism, and decrease LPS levels. It is noteworthy that a reduction in the relative abundance of Tyzzerella was detected in the LJZD + Cis group, which is of particular interest. It is noteworthy that a prior study examining inflammation and behavioral side effects induced by chemotherapy in female mice uncovered a positive correlation between IL-1β levels and the relative abundance of Tyzzerella [28]. Our findings are in agreement with the idea that anorexia could be relieved through the reduction of IL-1β levels and the relative abundance of Tyzzerella. Overall, the implications of our results indicate that MA and LJZD may exert therapeutic effects by modulating various intestinal colonies that contribute to the maintenance of intestinal mechanical barriers, reduction of inflammatory responses, and increase in total SCFAs levels, thereby restoring acute intestinal injury induced by cisplatin. A notable observation in the Cis group was the considerable decrease in the relative abundance of the Lachnospiraceae NK4A136 group, known for its anti-inflammatory properties, and Ruminococcus 1, which has been associated with anti-intestinal oxidative stress effects [29, 30]. In contrast, we detected an elevation in the relative abundance of potentially pathogenic Streptococcus and Bacteroides in this group. An intriguing finding of our study was the unexpectedly elevated relative abundance of Akkermansia in the Cis group when compared to the Control group. Prior studies have revealed that Akkermansia can degrade mucins, thin the thickness of mucus, and promote the transfer of pathogens from the intestinal cavity to the host tissue, thereby exacerbating inflammation caused by Salmonella typhimurium infection [31]. Emerging research has demonstrated the potential of Akkermansia to exert lipid-consuming effects and suppress appetite, making it a promising area of investigation for the treatment of obesity and bulimia [32]. Our findings are consistent with this, as we observed a sharp increase in the relative abundance of Akkermansia after cisplatin modeling, along with a decrease in both body weight and appetite. Remarkably, Akkermansia has also been demonstrated the ability to mitigate inflammation and serve as a therapeutic agent in conditions such as inflammatory bowel Disease, obesity, and diabetes [33]. We speculated that the abnormal rise in Akkermansia

Table 1 The fecal contents of SCFAs in the five groups of rats (ng/mg, mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acetic acid</th>
<th>Propionic acid</th>
<th>Isobutyric acid</th>
<th>Butyric acid</th>
<th>Isovaleric acid</th>
<th>Valeric acid</th>
<th>Total SCFAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>257.45 ± 25.31</td>
<td>51.21 ± 5.09</td>
<td>63.05 ± 8.15</td>
<td>84.22 ± 11.10</td>
<td>4.39 ± 0.81</td>
<td>3.89 ± 0.74</td>
<td>467.54 ± 41.07</td>
</tr>
<tr>
<td>Cis group</td>
<td>132.05 ± 23.79**</td>
<td>21.22 ± 3.15**</td>
<td>6.98 ± 5.94</td>
<td>10.62 ± 9.30</td>
<td>0.81 ± 0.39**</td>
<td>0.78 ± 0.45**</td>
<td>172.46 ± 21.36***</td>
</tr>
<tr>
<td>MA + Cis</td>
<td>214.60 ± 27.71</td>
<td>49.80 ± 3.69</td>
<td>15.35 ± 1.32</td>
<td>22.28 ± 2.58</td>
<td>2.87 ± 0.38</td>
<td>3.16 ± 0.41</td>
<td>308.07 ± 29.74'</td>
</tr>
<tr>
<td>LJZD + Cis</td>
<td>169.87 ± 19.30</td>
<td>40.56 ± 8.57</td>
<td>8.52 ± 0.38</td>
<td>12.54 ± 0.88</td>
<td>2.60 ± 1.79</td>
<td>0.54 ± 0.16</td>
<td>234.42 ± 26.52''</td>
</tr>
<tr>
<td>LJZD group</td>
<td>257.72 ± 43.10**</td>
<td>55.51 ± 11.32**</td>
<td>88.78 ± 56.61</td>
<td>136.63 ± 88.22</td>
<td>3.95 ± 22.00</td>
<td>4.50 ± 1.18***</td>
<td>547.10 ± 89.04''</td>
</tr>
</tbody>
</table>

One-way analysis of variance (ANOVA) was employed to assess significant differences. Data were expressed as mean ± SEM (n = 3). * P < 0.05, ** P < 0.01, *** P < 0.001, compared with the Cis group; " P < 0.05, "" P < 0.01, """ P < 0.001, compared with the Control group.
levels following cisplatin treatment could be a mechanism of self-protection in the body. Following cisplatin-induced acute intestinal injury, the relative abundance of *Akkermansia* with anti-inflammatory and protective intestinal barrier was increased. It is encouraging to note that we also observed a rise in the relative abundance of Muribaculaceae in the LJZD group, which is a symbiotic intestinal bacterium known to reduce the colonization of pathogenic microbiota [34]. It is worth noting that most of the above-mentioned specific microbiota (except *Akkermansia*) have a changing trend but no statistical difference. We infer that it is because the experimental sample size is not large enough and more sensitive sample detectors are needed. This is one of the main limitations of this study, even if LJZD shows good therapeutic effect.

![Figure 5](image5.png)

Figure 5 Correlation analysis of SCFAs, key gut microbial taxa and intestinal mechanical barrier indexes. (A) Pearson correlation analyses were conducted to investigate the relationship between key gut microbial taxa and metabolites, including SCFAs and LPS. (B) Pearson correlation analyses were performed to assess the relationship between SCFAs and markers of the intestinal mechanical barrier. Statistically significant correlations were indicated by * or X. The strength of the correlation was represented using a color scale, ranging from 0.75 (or 1.0) for strong positive correlation to –0.75 (or –1.0) for strong negative correlation.

![Figure 6](image6.png)

Figure 6 The impact of LJZD on the expression of proteins associated with TLR4/MyD88/NF-κB p65 was evaluated. The study presented representative WB results (A) and corresponding quantitative analyses (B–D) of the hypothalamus. Data were expressed as mean ± SEM (n = 3). *P* < 0.05, **P** < 0.001, compared with the Cis group; ***P** < 0.001, compared with the Control group. LJZD, Liu-Jun-Zi decoction; Cis, cisplatin; MA, megestrol acetate; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; TLR4, toll-like receptor 4.
It is widely believed that LPS can disrupt and penetrate the intestinal barrier, leading to chronic inflammation [35]. Intestinal barrier dysfunction is commonly associated with a range of intestinal and systemic diseases, underscoring the importance of this phenomenon. Based on our observations, we postulated that LJZD treatment can effectively decrease the abundance of detrimental microbiota responsible for LPS production, while concurrently repairing damage to the intestinal barrier and restricting the translocation of LPS into the bloodstream. Furthermore, SCFAs are a critical group of microbial metabolites that are centrally involved in regulating host physiological functions and maintaining intestinal homeostasis, with particular emphasis, the research focuses on fortifying the integrity of the intestinal barrier [36]. The results of our study are consistent with those of the correlation analysis, which indicate that LJZD may have an additional mode of action in managing CIA by promoting the production of SCFAs-generating microbiota.

The impact of LJZD on the TLR4-MyD88-NF-κB p65 signaling pathway

Our study on the CIA rat model found that LJZD notably enhanced food intake and mitigated cisplatin-induced injury to the jejunum, ileum, and colon, which suggests the therapeutic potential of LJZD in CIA. LJZD mitigated the elevation of IL-6, IL-1β, and TNF-α levels induced by cisplatin, which is in line with prior research. Simultaneously, we assessed the activation of the TLR4-MyD88-NF-κB p65 signaling pathway by LPS in the hypothalamus. Based on our results, it is apparent that LJZD administration can effectively alleviate the symptoms of CIA by promoting the restoration of the intestinal mechanical barrier, which in turn inhibits the activation of the TLR4-MyD88-NF-κB p65 signaling pathway, a pathway that is typically triggered by LPS. Additionally, it effectively decreases the deleterious effects of multiple appetite-suppressing inflammatory factors induced by cisplatin.

Future research directions for LJZD on CIA

In this study, the observed impact of the LJZD + Cis group on the overall gut microbiota did not align as closely with the Control group as initially anticipated. However, in view of the role of LJZD group in increasing food intake and reducing some inflammatory factors in this study, we believe that there is still a certain research prospect to improve CIA from the perspective of restoring intestinal microbiota homeostasis. Our inference suggests that the dosage of LJZD may require further adjustments, warranting the establishment of multiple gradient concentrations of LJZD in future investigations. Simultaneously, it is imperative to conduct an in-depth examination of fecal bacteria transplantation to elucidate the intricate involvement of the gut microbiota in CIA treatment, thereby corroborating our prior findings pertaining to the gut microbiota.

Due to the multi-component and multi-target nature of traditional Chinese formulas, it is difficult to elucidate their therapeutic mechanisms. The therapeutic effects of the principal chemical constituents of LJZD, selected according to the fingerprint spectrum, on CIA were not investigated as part of this study. Literature suggests that hesperidin, one of the major components of LJZD, has antioxidant, antibacterial, and anti-inflammatory effects [37]. In a rat model of acute intestinal injury induced by intraperitoneal injection of LPS, hesperidin can improve the morphology of rat ileum by regulating the gut microbiota and reducing inflammatory responses, thereby enhancing intestinal health [37]. In a mice experimental study utilizing LPS to induce disease behavior and anorexia, it was observed that hesperidin exhibited a pronounced ameliorative effect attributed to its antioxidative properties [38]. Ginsenosides are the principal constituents of ginseng. Several studies have consistently demonstrated the notable effectiveness of ginsenosides Rg1, Rb1, and Rg3 in alleviating anorexia induced by neuroinflammation [39–41]. Notably, our study identified ginsenoside Rg1 as one of the potential active ingredients in LJZD for the treatment of CIA. Future research can focus on the active ingredients of LJZD to investigate the mechanisms of action of individual compounds on CIA.

Conclusion

To summarize, LJZD exerts its therapeutic effect on CIA by inactivating TLR4-MyD88-NF-κB p65 signaling pathway and regulating gut microbiota to repair of intestinal barrier. Nevertheless, our study is limited by the fact that we did not employ fecal microbiota transplantation and metabolomics technology to directly investigate the therapeutic effect of key indicator intestinal microbiota and metabolic pathways on CIA. This limitation may have weakened the overall strength of our findings. Therefore, additional investigation is necessary to fill these knowledge gaps and attain a comprehensive understanding of the mechanisms responsible for the therapeutic benefits of LJZD in CIA. Furthermore, this study exclusively focused on investigating the microbiota-to-intestine-to-brain connection in relation to chemotherapeutic anorexia. In subsequent investigations, we envision conducting a bidirectional regulatory examination encompassing the brain-to-intestine-to-microbiota pathway, with the aim of elucidating the potential correlation between chemotherapeutic anorexia and the intricate gut-brain axis mediated by microbial entities.

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