

The Influence of Jiawei Wendan Decoction on the Gastric Microbiota in Depression-Anxiety Comorbidity Rat Models

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Abstract: This study leverages the high-throughput sequencing technology of 16S rRNA to investigate the effects of Jiawei Wendan Decoction on the gastric microbiota of Chronic Restraint Stress (CRS) induced depression-anxiety rat models, examining its potential as a therapy for depression-anxiety comorbidity. [Methods] SD rats were randomly divided into four groups: control, model, western medicine, and traditional Chinese medicine groups. After model establishment, treatments were administered for a continuous period of 35 days. The intestinal contents of the rats were collected, from which total DNA was extracted. Amplification of the V3-V4 region of bacterial 16S rRNA was carried out with designed primers, followed by high-throughput sequencing on the Illumina MiSeq platform. [Results] Following treatment with Jiawei Wendan Decoction, a significant increase in the *Lactobacillaceae* family and *Lactobacillus* genus was noted, becoming dominant strains. Compared to the control and model groups, the traditional Chinese medicine treatment resulted in a raised relative abundance of *Lactobacillus johnsonii*, promoting the proliferation of microbes involved in environmental and genetic information processing. [Conclusion] Jiawei Wendan Decoction exhibits anti-comorbidity effects in CRS rat models. The mechanism of action is potentially linked with the modulation of gastric microbiota, enhancing the presence of microbes involved in environmental and genetic information processing within the stomach.

1. Introduction

The co-occurrence of depression and anxiety is highly common, with 40-70% of individuals with depression also experiencing symptoms of anxiety, and around 50% of those with anxiety disorders manifesting signs of depression [1]. These individuals typically face heightened symptom severity, more complex treatments, and an increased risk of suicide. Furthermore, they often necessitate prolonged treatment durations, and generally exhibit poorer recovery rates compared to those battling a single disorder [2]. At present, the underlying causes and mechanisms of this comorbidity remain largely undefined, and the current medicinal approaches are insufficient to fully cater to the treatment requirements of these patients. Hence, the urgency to develop effective treatments is paramount.

Recent research has highlighted the significance of the gut-brain axis, a bidirectional communication system between the gastrointestinal tract and the brain, in mental health [3]. Within this axis, the composition of the gastrointestinal microbiota plays a vital role in maintaining the immune barrier of the gastrointestinal mucosa, regulating substance metabolism, and influencing emotional well-being. One key microorganism in this context is *L. johnsonii*, a probiotic bacterium naturally found in the gastrointestinal tracts of humans and other mammals [4].

L. johnsonii plays a crucial role in maintaining microbial balance in the gut, enhancing the immune system's health, and facilitating food digestion and nutrient absorption.

Wendan Decoction is based on the Jiyan Prescription of Yao Sengyuan, a renowned doctor during the Northern and Southern Dynasties. It aims to regulate the spleen and stomach, characterized by its cold, warm, gentle, and ascending and descending properties. It can regulate qi and alleviate depression, and can also reduce adversity and dispel phlegm. The Jiawei Wendan Decoction builds upon the Wendan Decoction, incorporating additional substances such as Hehuanhua and Shichangpu, which amplify its role in resolving dampness and stomach ailments, as well as calming nerves and relieving depression [5]. Clinical practice has demonstrated its regulatory effects against anxiety and depression.

Therefore, building on preliminary studies, this research utilizes the 16S rDNA high-throughput sequencing technique to observe the regulatory effects of Jiawei Wendan Decoction on the gastric microbiota composition in CRS rats. The study aims to elucidate how Jiawei Wendan Decoction exerts its antidepressant effects by modulating the structure of the gastric microbiota, thereby providing a scientific basis for the clinical application of Jiawei Wendan Decoction in treating depression-anxiety comorbidity.

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2. Materials and Methods

2.1 Grouping

SPF-grade male SD rats, weighing between 180-200g, were used in this experiment. Open-field experiments were performed to evaluate their behavioral scores prior to the initiation of the experiment. The rats with similar behavioral scores were then randomly divided into three experimental and one control group (WN-KB). The experimental groups contain a model group (WN-GM), a Chinese medicine treatment group (WN-GZ), and a Western medicine treatment group (WN-GX). The normal control group contains 8 rats.

2.2 Establishment of rats' model group

Chronic bondage stress, anxiety and depression model. The restraint stress experiment [6] lasted for 21 days. Between 8:00 and 12:00 each day, the rats were subjected to 4 hours of restraint stress by fixing the rats to the cage mesh and keeping them breathing smoothly. Food and water were prohibited during the stress, and the rats were placed back in the cage and allowed to eat and drink afterward.

2.3 Open-field experiment

The Open Field Maze, a prevalent tool for assessing mouse behavior, was utilized in this study [5]. A wooden box (80cm x 80cm x 40cm) was segmented into 25 equal squares where a mouse was observed for 3 minutes per session. The horizontal crossings indicated activity levels, while rearing behavior showcased curiosity towards the new environment. Each mouse was tested once, with both types of movements recorded. The box was cleaned with water and 75% alcohol between sessions.

2.4 Chinese medicine recipe

The composition of the Jiawei Wendan Decoction includes Pinellia ternata (6g), Citrus peel (9g), Fructus Aurantii (2 pieces), Concha Bambusae (6g), Ginger (12g), Licorice (3g), Albizia julibrissin flower (12g), and Acorus calamus (9g). High-quality herbs were selected and verified in the phytochemistry lab. Standard operating procedures were established to decoct and concentrate the medicine into a paste. The paste was then stored at 4°C for future use.

2.5 Medicine administration

The Chinese medicine and Western medicine groups began medication at the time of model establishment, starting daily at 8:00 am with a duration of 35 days. 0.5% sodium carboxymethyl cellulose (CMC) was used to dissolve Jiawei Wendan Decoction at the concentration of 12g/kg in the Chinese medicine group. Probiotics were utilized in depression and anxiety western medicine groups. Each group was given the same volume of drug at 0.1ml/10g, once per day. The model group and control group were fed with an equal volume of normal saline. All animals had free access to food and water.

2.6 16S rRNA Gene Sequences

Rat stomach microbiome samples were collected and genomic DNA extracted. The 16S rRNA gene region was amplified via PCR and sequenced. PCR products were purified to eliminate residual components and used to create libraries. These were sequenced using Illumina MiSeq, and diversity metrics analyzed in QIIME, with microorganisms categorized and displayed as a heatmap.

3. Results

3.1 Alpha-diversity analysis of intestinal microbiota

Alpha-diversity is often used to measure species richness and evenness in community ecology. The experimental results show that, a marked diminution in the *chao1*, *PD_whole_tree*, and Shannon indices in the stomach was also observed after TCM treatment (Fig. 1A-C). The pronounced decline in these metrics indicates a contraction in the microbial diversity of the stomach, coupled with a potential loss of equilibrium among resident species. Additionally, Simpson index was used to assess species diversity. The higher the Simpson index value, the lower the bacterial diversity. A notable decrease in the Simpson index was recorded, further echoing the findings of a simplified microbial environment (Fig. 1D).

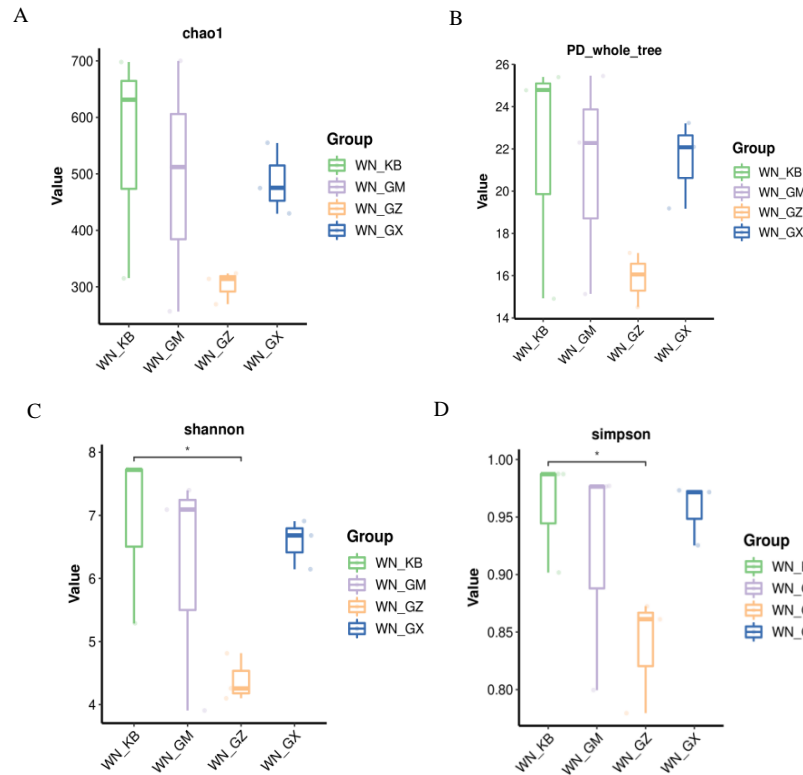


Figure 1. Alpha-diversity in the stomach in medicine treatment depression-anxiety rat.

3.2 Microbial community structure analysis of intestinal microbiota

Following the administration of TCM, evaluations employing Beta-diversity metrics, Venn diagrams, and Lefse (Linear Discriminant Analysis Effect Size) analyses revealed notable shifts in the microbial assemblages. These shifts manifested as increased dissimilarity in microbial compositions across the assessed sites (Fig. 2). Post-treatment, there was a decrease in the overall number of bacterial species. Interestingly, 162 distinct bacterial species emerged subsequent to the TCM intervention (Fig. 2B). Among the newly predominant taxa, *Lactobacillaceae*, *Lactobacillus* were particularly notable (Fig. 2C).

3.3 Analysis of relative abundance of species

Delving deeper, a pronounced enhancement of *L. johnsonii* populations in the stomach post TCM treatment was observed. This augmentation contrasted with the relatively stable levels observed with Western medication (Fig 3).

3.4 Analysis of cell function of species

Moreover, TCM fostered the proliferation of microbes involved in environmental information processing and genetic information processing in the stomach (Fig. 4).

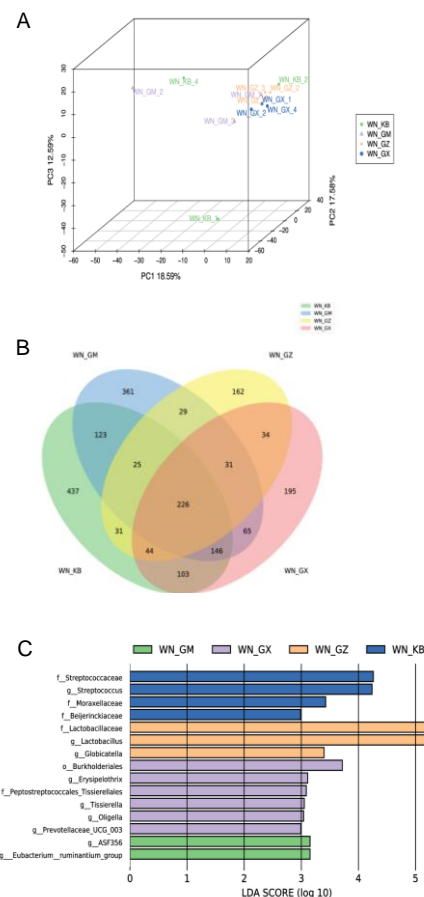


Figure 2. Change of microbiota after medicine treatment. (A. Beta-diversity, B. Venn diagram, C. LEFSE analyse)

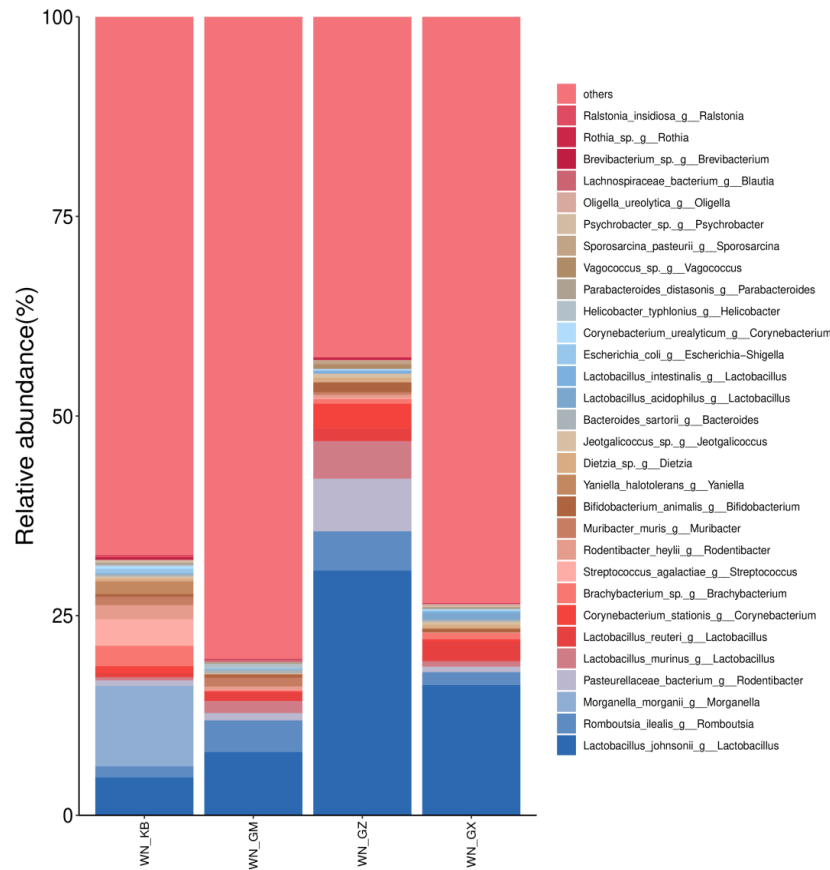


Figure 3. Relative abundance of species in stomach of depression-anxiety rat.

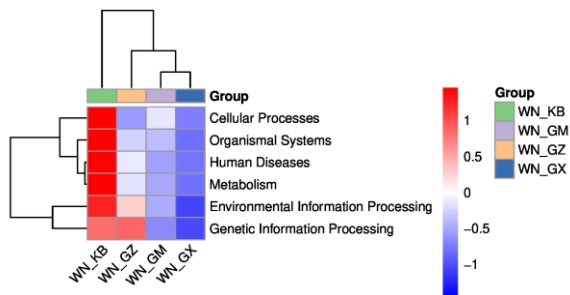


Figure 4. Heat-map of microbiome-related cell function changes in stomach of depression-anxiety rat.

4. Conclusions

This study extensively investigates the potential antidepressant and anti-anxiety effects of Jiawei Wendan Decoction on CRS depression-anxiety rat models, primarily focusing on its influence on gastric microbiota. Utilizing the high-throughput sequencing technique of 16S rRNA, we discerned a beneficial role of this traditional Chinese medicine in augmenting the abundance of salutary gut microbiota, predominantly amplifying the population of the *Lactobacillaceae* family, including *L. johnsonii*. Furthermore, a notable increase was observed in the proliferation of microbes implicated in environmental and genetic information processing post-treatment.

Our findings align with several preceding studies demonstrating the substantial association between the

Lactobacillaceae family and the mitigation of depression and anxiety symptoms. Han [7] delineated a critical link between the decreased abundance of the *Lactobacillaceae* family and heightened symptoms of depression and anxiety. Additionally, studies by Cheng [8] and Janssen [9] emphasized the potential of various *Lactobacillus* strains in reducing depressive symptoms, potentially by modulating mechanisms such as tryptophan metabolism and the hypothalamic-pituitary-adrenal axis.

Concentrating on *L. johnsonii*, various researches have accentuated its considerable role in curtailing anxiety-like behaviors and modulating neural signals in rodent models [10]. This bacterium is known to influence brain serotonin levels, aiding in emotional regulation as evidenced by Desbonnet [11] and Sharma [12]. Supporting this, Jang [13] and Marcial [14] showcased its potential in offering sustained anxiety relief, particularly concerning gastrointestinal inflammation.

In the context of the current research, Jiawei Wendan Decoction surfaces as a promising therapeutic agent, likely enhancing the abundance of *L. johnsonii*, consequently alleviating co-morbid depression and anxiety symptoms in CRS rats. This study substantiates the theory associating the growth of the *Lactobacillus* genus, particularly *L. johnsonii*, with symptom alleviation in depression and anxiety disorders.

Looking ahead, it becomes paramount to further explore *L. johnsonii*'s role and mechanisms in depression, paving the way for its prospective clinical applications in treating depressive and anxiety disorders. This endeavor could revolutionize treatment strategies, fostering personalized

and potentially side-effect-free interventions. The incorporation of this approach with existing therapies might foster a more comprehensive treatment method, thereby enhancing the battle against depression and anxiety. Future research should prioritize clinical trials to substantiate these findings in humans, potentially establishing the foundation for novel clinical guidelines. Moreover, this venture embarks on the development of diagnostic tools based on gut microbiome profiles, steering healthcare towards a more individualized approach. Ultimately, while this research suggests Jiawei Wendan Decoction's potential role as a formidable ally in addressing depression and anxiety through gut microbiota modulation, further studies remain essential to unravel its mechanisms of action and ascertain its clinical efficacy.

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