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ORIGINAL RESEARCH

Jiedu Yizhi Formula Improves Cognitive Function by Regulating the Gut Dysbiosis and TLR4/NF-κB Signaling Pathway

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Objective: The objective of this study was to explore the neuroprotective mechanism of JDYZF in treating AD from the perspective of inflammation and intestinal microflora.

Methods: A total of 24 APP/PS1 mice were randomly divided into four groups: model (n = 6), JDYZF low-dose (n = 6), JDYZF high-dose (n = 6), and positive drug (n = 6), six C57 mice were used as the control group. The body weights and diets of all mice were examined daily. After 8 weeks of administration, the learning and memory of mice were evaluated by the Morris water maze test. The histopathological changes of hippocampus, liver and kidney in mice were observed by HE staining after being euthanized. The expression of p-tau in hippocampus tissue was detected by immunohistochemistry. After that, 16S rDNA sequencing was used to investigate the relationship between JDYZF and intestinal microbiota. Finally, a comparison of TLR4, p65, p-p65, iκB, p-iκB, and IL-1β protein expression in the hippocampus tissue of mice in each group was measured by Western blot.

Results: The results showed that APP/PS1 mice taking JDYZF orally were generally in good condition. Compared with the control group, JDYZF significantly improved learning and memory ability in ethology. Histology showed that JDYZF improved the hippocampal structure of mice and inhibited the deposition of p-tau. JDYZF treatment could regulate the gut microbiota of APP/PS1 mice by increasing the richness of *Lachnospiraceae, Ruminococcaceae*, and *Actinobacteria* and reducing that of *Alistipes* and *Muribaculaceae*. It also significantly inhibited the activation of the TLR4/NF-κB signaling pathway in the brain. In addition, no obvious toxic reactions were found in the liver and kidney of APP/PS1 mice after taking JDYZF for 8 weeks.

Conclusion: The findings revealed that JDYZF improved cognitive ability and alleviated the TLR4/NF-κB signaling pathway in APP/PS1 mice, and the modulating the gut microbiota presented here may help illuminate its activation mechanism.

Keywords: Jiedu Yizhi Formula, Alzheimer's disease, fecal microbial diversity, NF-κB, APP/PS1 mice

Introduction

Background

Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognitive impairment and behavioral abnormalities progressively.[1] Compared with the 2010 census figures, the AD population of China grew faster in 2022.[2] However, the country's diagnosis and treatment rate for AD remains low.[3] Therefore, improving the prevention and treatment of AD is urgent.[4] So far, Aβ protein accumulation and tau protein hyperphosphorylation are the main neuropathological of AD.[5] However, contrary to expectations, treatment options for AD are unsatisfactory.[6,7] Therefore, elucidating the molecular mechanism of AD and developing potent agents are urgent for improving patient functional status and quality of life.

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Gut microbes are the largest organ in the human body, and regulating the microbes in the gut has been sought as a promising strategy to treat AD. Studies have shown that the noncanonical nuclear factor-κB (NF-κB) signaling pathway is closely related to tau protein formation and neuroinflammation in Alzheimer's disease.[8,9] After activation, it can lead to anti-apoptotic genes and cytokines IL-1, IL-6, TNF-α, and IL-8.[10] NF-κB signaling pathway plays a key role in inflammation. Moreover, NF-κB is known to be an important modifier in the microbiome to AD, gut microbial diversity affected the TLR4/NF-κB signaling pathway in the inflammatory response. On the contrary, many studies show that TLR4/NF-κB signaling pathway can be inhibited by shifting the composition and the relative abundance of the intestinal microbiota.

Herbal remedies, which are very promising candidates for treatment, have a long history of use in a treat or preventing AD.[11] The main therapeutic principle of TCM is a holistic view of patients. In other words, TCM could treat AD progression through multiple targets and multiple channels. A gut-brain axis perspective is consistent with traditional medical thinking. Therefore, NF-κB signaling may be an important pathway to treat AD by regulating the Gut-brain axis by TCM.

Purpose

Professor Ren Jixue, a master of Chinese medicine, put forward the marrow theory. We use the standard model of marrow theory as a reference and further established that marrow deficiency and poison damage have been identified as key mechanisms of AD-induced pathogenesis. In particular, Jiedu Yizhi Formula (JDYZF) is a classical TCM prescription for AD and has been applied over 10 years in clinical. Specifically, JDYZF fills kidney essence and clears away heat and toxic material, promoting gastrointestinal motility, and effectively purging and clearing the viscera. JDYZF includes Golden thread (Latin name: Coptidis Rhizoma), Sharpleaf Glangal Fruit (Latin name: Alpiniae Oxyphyllae Fructus), Glue of tortoise shell (Latin name: Carapax et Plastrum Testudinis Colla), Rhubarb (Latin name: Rheum palmatum L), Earthworm (Latin name: Pheretima), Asiatic Cornelian Cherry Fruit (Latin name: Corni Fructus), Szechwan Lovage Rhizome (Latin name: Chuanxiong Rhizoma). Using JDYZF to treat AD has a long history of demonstrated effective clinical therapeutic effects.[12] Our previous research found that JDYZF can regulate the PI3K-Akt /Gsk3β/p53 signaling pathway, reduces the expression of inflammatory factors, and plays a protective role in the neurotoxicity of AD model cells.[13] A central signaling component of the PI3K-Akt pathway is NF-κB, which functions together with the downstream kinase, to participate in the transmission and expression of various signals.[14] However, its specific mechanism of protecting and modulating the brain-intestine axis in mice remains unclear.

In this study, we used JDYZF administration and behavioral testing in an APP/PS1 mouse model of AD, as well as standard immunohistochemical measures in the brain tissue. Next, we examined the effect of JDYZF on the gut microbacteria phenotypes and the involvement of TLR4/NF-κB signaling pathway in this process, to study the effect of JDYZF in alleviating memory deficits, and attempted to elucidate the underlying mechanisms.

Materials and Methods

Preparation of the JDYZF

JDYZF was purchased from Jilin Hongjian Pharmacy Co., Ltd. (Jilin, China), including Coptidis Rhizoma, Alpiniae Oxyphyllae Fructus, Carapax et Plastrum Testudinis Colla, Rhei Radix et Rhizoma, Pheretima, Corni Fructus and Chuanxiong Rhizoma. These herbs were mixed at a ratio of 1:2:1:1:1:1:1. The quality met the requirements of Pharmacopoeia of the People's Republic of China 2015 edition. JDYZF was boiled and then prepared into freeze-dried powder through the freeze-drying mechanism, sealed, and stored at −20°C.

Experimental Animals

Twenty-four male APP/PS1 mice and 6 male C57 mice (SPF level; 4 months age-old; weigh 25±5 g) were purchased from Nanjing Junke Bioengineering Co., Ltd. (Nanjing, China; certification no. SCXK-SU 2020–0009). All the mice had access to water and food in freedom and were housed in a feeding room at a temperature of 22 ± 1°C and relative humidity of 60 ± 5% on a 12-hour light/dark cycle.

Seven days after in the SPF room, the APP/PS1 mice were randomly divided into four groups. Six mice each in the low-dose group (LG), and high-dose group (HG), respectively, received 10.536 g/kg/d and 20.268 g/kg/d JDYZF by gavage. Meanwhile, six mice in the positive drug group (PG) were administered donepezil hydrochloride (0.45 g/kg) by gavage. Furthermore, six APP/PS1 mice in the model group (MG) and six C57 mice in the control group (CG) were administered normal saline (0.1 mL/10 g) by gavage. The treatment period is 8 weeks. We weighed the weights and diets of mice at a fixed time every day and observed the general state and appearance characteristics of each mouse.

Morris Water Maze Test

After 8 weeks of intervention, the Morris water maze (MWM) test was used for evaluation.[15] Briefly, the navigation experiment lasted for 6 days. After 6 days, the platform was removed and the mice were placed in the quadrant opposite the platform for the probe trial. Behavior Analysis System EthoVison XTVersion 11.0 (Noldus, the Netherlands) was used for recording and analysis.

Haematoxylin and Eosin (HE) Staining

After the behavioral experiment, the mice were euthanized. Brain tissues, liver, and kidney samples of 3 mice in each group were randomly taken quickly and fixed in 4% paraformaldehyde. The specimens were dehydrated, paraffin embedded, sectioned, stained in hematoxylin and eosin, dehydrated with gradient alcohol, transpired with xylene, and sealed with neutral gum. The basic pathological changes of brain tissue, liver, and kidney were observed under an optical microscope.[16]

Immunohistochemistry

Paraffin sections of brain tissue were dewaxed and washed, antigen repaired, serum blocked, DAB chromogenic after incubation with p-tau (1:5000, servicebio, China) antibody and secondary antibodies, restained nuclei, and microscopic examination after dehydration and sealing. The Eclipse Ci-L (NIKON, Japan) photographic microscope was used to select the target area of the tissue for 400 times imaging, to ensure that the background light of each photo was consistent. After the completion of imaging, the immunohistochemical method was processed by Image Pro Plus 6.0 analysis, ImageJ software was used to analyze the cumulative optical density (IOD) value and positive cell count, and calculate the area density = IOD value/area of the tissue.

16S rRNA Microbial Community Analysis

At the end of the treatment period, two fresh feces in the middle piece were collected from each mouse and frozen at −80°C for further analysis. The microbiome was analyzed on the HiSeq2500 PE250 sequencing platform of Novogene Bioinformatics Technology Co., Ltd. In short, the sample DNA was extracted with QIAamp Fast DNA Stool Mini Kit (Qiagen), diluted to 1 ng/μL for PCR amplification, and the V3-V4 region of 16S rDNA gene was targeted with Barcoded primers (515F and 806R) for sequencing analysis. Cluster OTU according to 97% sequence similarity, and then analyze the intra-group abundance and difference. α-Diversity was selected Chao diversity index by using Qiime v1.7.0., and evaluated the sample reliability by plotting the rarefaction curve with R v2.15.3. These calculations were analyzed by the Tukey's test.[17] β-Diversity was assessed using PCoA. Then, MetaStat was used to analyze differential microbiota by using R v2.15.3.

Western Blotting

Homogenize the fresh mouse hippocampal and put it into the lysis buffer. Extract the total protein from the brain tissue, after quantitative determination of protein, SDS-PAGE electrophoresis, membrane transferred, blocked, added primary antibody (including anti-iκB-α (1:500, cell signaling technology, USA), anti-NF-κB p65 (1:1000, cell signaling technology, UK), anti-p-NF-κB p65 (1:1000, Santa Cruz Biotechnology, USA), anti-IL-1β (1:1000, Santa Cruz Biotechnology, USA), anti-TLR4 (1:1000, Santa Cruz Biotechnology, USA) and anti-p-iκB-α (1:200, Santa Cruz Biotechnology, USA)) and then used the appropriate secondary antibody to detect the protein.[18] After imaging, the protein of TLR4 and IL-1β expression was expressed by the ratio of the gray value of the target protein to the gray value of GAPDH. The expression

of other proteins was expressed by the ratio of the gray value of phosphorylated protein to the gray value of total protein. The Western blot results were analyzed by image lab and Image J, and the experimental data were processed by GraphPad prism 8.

Statistical Analysis

GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA) was used to analyze data. All experiments were repeated at least three times and the obtained data were presented as the mean ± standard deviation (x ± s). One-way ANOVA was applied to analyze statistical significance. P-value less than 0.05 indicated a statistically significant difference.

Results

Effects of JDYZF for Weight and General Status in APP/PS1 Mice

Some studies have shown that under the intervention of lipopolysaccharide, the neuroinflammation of AD mice is negatively correlated with weight.[19] As shown in Figure 1, the weight of mice in the CG increased steadily within 8 weeks, which was in line with the normal growth law of mice. Compared with the CG, APP/PS1 mice have a high initial weight generally. However, with the standard feeding and treatment, the food intakes and body weights of mice in each group have changed significantly. LG was closer to CG, while PG intakes were more than MG, but the weight change was not obvious. HG intakes were similar to MG, and body weight fluctuate greatly. Further, the mice in the MG had obvious domain occupation consciousness and had fights and biting behaviors. These basic informations showed that LG is developing in a more favorable direction for AD mice.

JDYZF Can Improve Cognitive Impairment in APP/PS1 Mice

We used the Morris water maze test to evaluate and compare the learning ability and long-term and short-term memory ability of mice in each group. The experiment was divided into two parts: the escape latency and the probe trial. The results showed that in the escape latency, the CG had a good learning ability. The learning and memory of mice in the MG decreased significantly. Compared with the CG, the escape latency was prolonged on the 4th and 5th days ($p < 0.05$, $p < 0.01$). Compared with the MG. LG, HG, and PG showed various degrees of cognitive improvement on the 4th day ($p < 0.05$) (shown in Figure 2A). During the probe trial, the CG looked for the quadrant where the platform was located, which was targeted and tended to be a linear strategy. The search strategy of the LG and the PG were close to the CG, which could cross the platform many times, showing a trend strategy. The search strategy of the HG randomly crossed the quadrant with the platform, and the memory was improved. The mice in the MG always showed marginal exploration, learning, and memory has not improved (shown in Figure 2D). In addition, compared with the MG, the time of spending in the platform quadrant in PG, LG and HG were prolonged ($p < 0.05$), and the

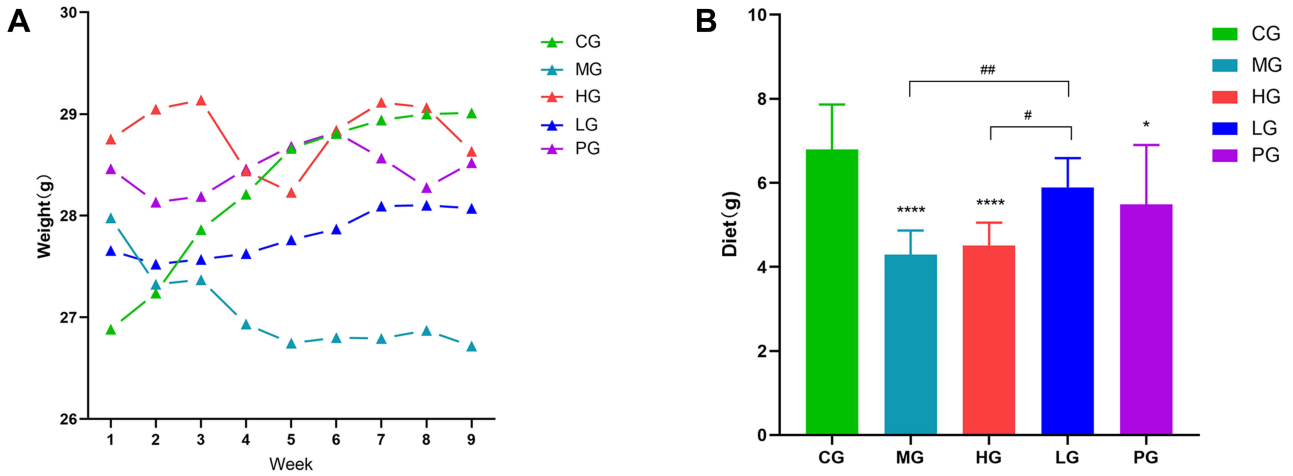

Figure 1 Effects of JDYZF on the levels of weight and diet. (**A**) Weight; (**B**) Diet. *$p < 0.05$, ****$p < 0.01$ compared with the CG; #$p < 0.05$, ##$p < 0.01$ compared with the LG, (**B**) $F = 12.12$, N=6.

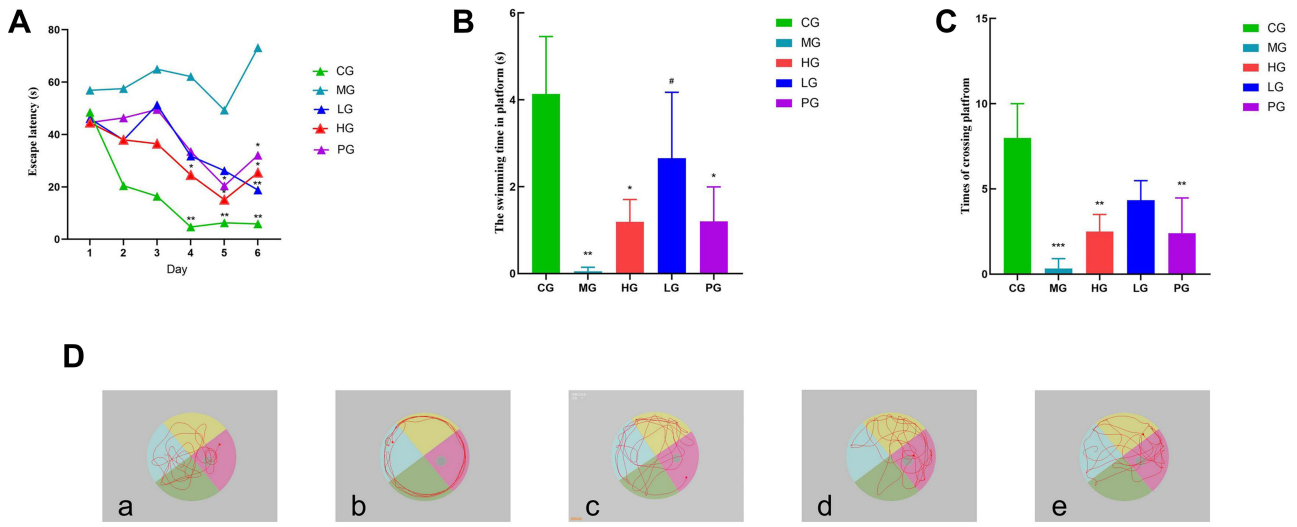

Figure 2 Oral administration of JDYZF rescues cognitive deficits in APP/PS1 mice. (**A**) Escape latency; (**B**) Probe trial time in the quadrant; (**C**) Probe trial number of times crossing platforms; (**D**) Representative swimming trajectories of different groups of mice after removing the platform. (**a**) CG; (**b**) MG; (**c**) HG; (**d**) LG; (**e**) PG. The results were shown as mean ± SEM or representative pictures. Data in panels were analyzed by one-way ANOVA. *$p < 0.05$, **p or ***$p < 0.01$ compared with the CG; $^{\#}p < 0.05$ compared with the MG, (**B**) $F = 8.447$, (**C**) $F = 10.56$. N = 6.

number of crossing the platform were increased ($p < 0.01$) (shown in Figure 2B and C). This showed that APP/PS1 mice have neurological damage at the age of 6 months. A drug intervention can reduce this damage to varying degrees, and the effect of LG seemed to be more significant.

JDYZF Attenuates Hippocampal Neuron Damage in APP/PS1 Mice

JDYZF can improve the learning and memory of APP/PS1 mice and alleviate hippocampal neuronal damage. Hyperphosphorylation of p-tau protein is the early pathological basis of AD, resulting in a large number of neuroinflammation and degeneration. HE staining showed that the nuclei of the hippocampal CA1 area in the CG were large and round, with distinct layers, clear structure, complete membrane and nuclear membrane, normal staining of nuclei, complete morphology of organelles, and neat arrangement of nerve fibers. Additionally, the hippocampal tissues of MG showed pyknotic nuclei, some neurons were damaged, the nerve cells in the CA1 area were disordered, the normal nerve cells decreased, and the neuronal gap increased. JDYZF could recover the number and morphology of nerve cells in the hippocampal area and showed good neurons and nucleoli (shown in Figure 3A). This finding indicated that JDYZF has a protective effect on neurons and can delay the pathological progress of AD in the early stage.

The immunohistochemical method showed that the cumulative optical density of p-tau protein in mice brain tissues from high to low was the MG, HG, PG, LG, and CG ($p < 0.01$) (shown in Figure 3B and C). APP/PS1 mice have more p-tau protein deposition in the hippocampus, which was consistent with the pathological changes of AD mice. JDYZF can clear the excessive p-tau protein deposition in the hippocampus of APP/PS1 mice.

JDYZF Improved the Fecal Microbiota of APP/PS1 Mice

Next, we examined the effect of APP/PS1 mice on the gut microbacteria phenotypes and the involvement of JDYZF in this process. OTUs with 97% similarity were obtained using cluster tags and plotted Petal diagram (shown in Figure 4). Four hundred and sixty-two OTUs are in common use by all groups. As shown in Figure 5A and B, the alpha diversity analysis in the Chao1 index indicated that the whole microbial assortments significantly decreased in the MG ($p < 0.05$). However, JDYZFL treatment significantly increased in response to APP/PS1 mice ($p < 0.05$). Furthermore, PCoA analysis showed that the gut microbial clusters in the MG were significantly separated from those in the CG, whereas JDYZFL treatment inhibited the segregation and demonstrated a marked shift close to those in the CG (shown in Figure 5C).

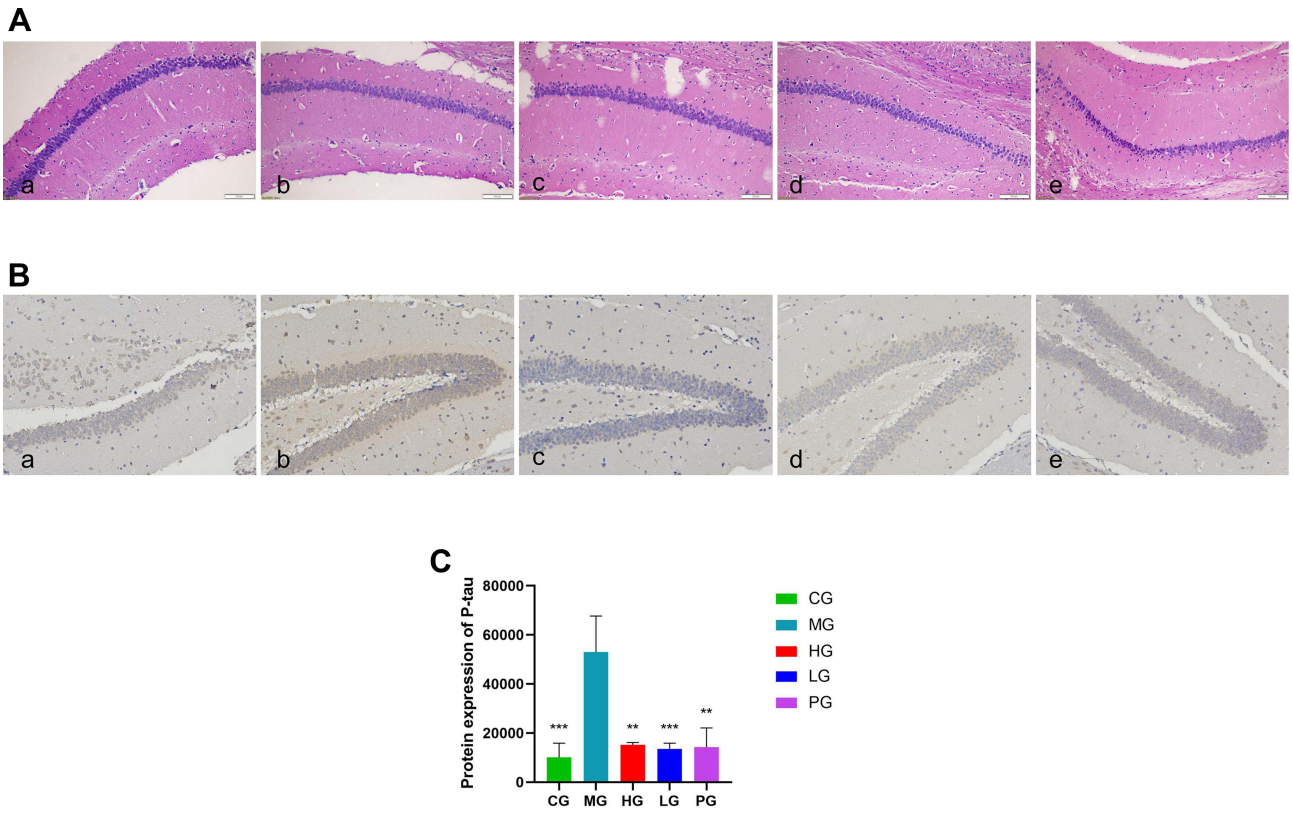

Figure 3 Representative histopathological and immunohistochemical photos of hippocampal CA1 area tissue sections from each group. (**A**) Histological examinations were performed in the hippocampal CA1 area tissue. The scale bar was 100 μm; (**B**) Levels of p-tau were measured by immunohistochemical method. The scale bar was 100 μm; (**C**) Quantitative analysis of p-tau. (**a**) CG; (**b**) MG; (**c**) HG; (**d**) LG; (**e**) PG. $^{**}p < 0.01$, $^{***}p < 0.01$ compared with the MG, $F = 15.09$, N = 3.

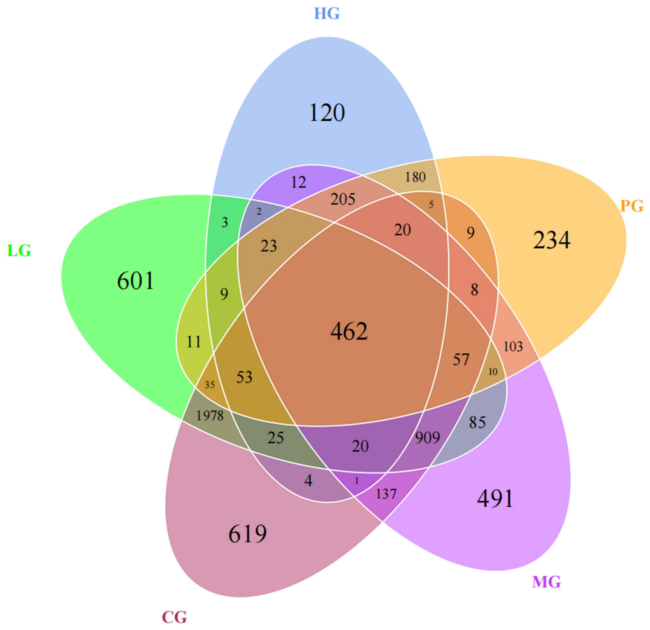

Figure 4 OTUs-based Venn diagram.

Then, one-way ANOVA was used to identify the microbial multivariate. The results showed that JDYZF influenced species abundance of Alzheimer's disease. We performed the MetaStat analysis to screen different microbiota in each group (shown in Figure 6). *Lachnospiraceae, Ruminococcaceae, Actinobacteria*, and *Muribaculaceae*, were the most representative.

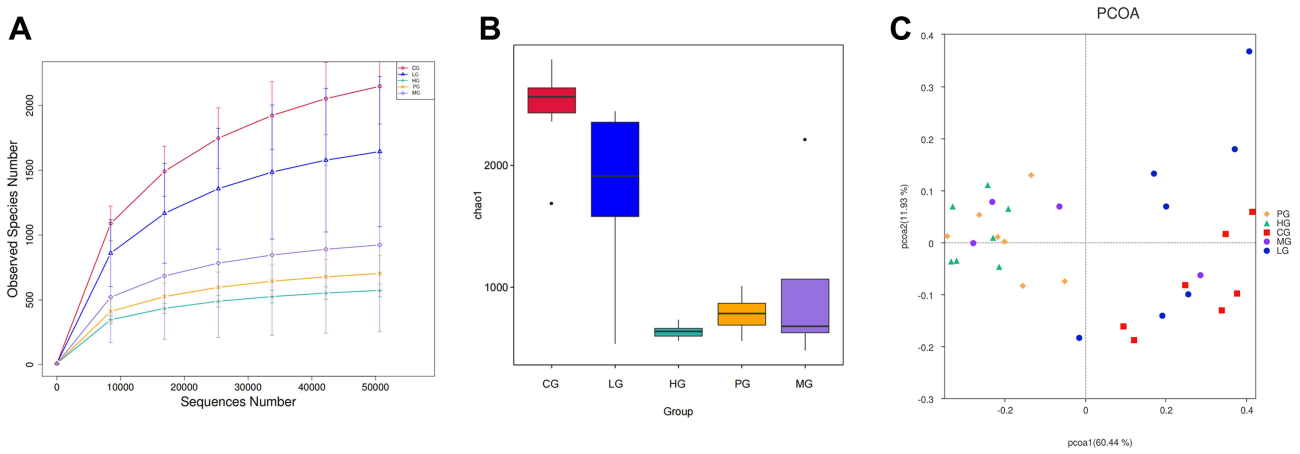

Figure 5 Alpha and beta diversity. (**A**) Observed_species; (**B**) chaos analysis (Wilcoxon test); (**C**) PCoA (weighted).

JDYZF Inhibits the Expression of Proteins Related to the Proinflammatory Pathway

Finally, the interaction between JDYZF and TLR4/NF-κB signaling pathway was investigated due to the important role of this pathway in inflammation and oxidative stress of hippocampus. Quantitative analysis revealed that the expression of TLR4, p-p65/p65, and p-iκB/iκB in the hippocampus were enhanced ($p < 0.01$), and the expression of these proteins was down-regulated in the hippocampus of PG ($p < 0.01$). The LG seemed to be more effective. Notably, IL-1β as a star protein downstream in the NF-κB signaling pathway is positively correlated with LPS and APOE4. LPS and APOE4 are the most closely related inflammatory indicators with AD.[20–22] Therefore, we evaluated the expression of IL-1β. Surprisingly, the results were consistent with the upstream protein level (shown in Figure 7). These data showed that JDYZF can down-regulation the protein expression related to the NF-κB pathway, reduced neuroinflammatory response, and improve cognitive function.

JDYZF Has No Hepatotoxicity and Nephrotoxicity

AD is a chronic disease of the nervous system, which needs to be treated with long-term oral Chinese medicine in the clinic. Therefore, we analyzed the HE staining of the liver and kidney for mice. We found that the hepatocytes were scattered outward with the interlobular artery as the center through liver slices, and the hepatocytes were arranged regularly. The morphology of the hepatic lobules was normal, and no obvious abnormalities were found (shown in Figure 8A). In the kidney tissue, the kidney medulla were oval, the tube wall was thin, and no obvious abnormalities were found in the glomerulus (shown in Figure 8B). It showed that oral administration of JDYZF for 8 weeks will not cause significant changes in the structure of the liver and kidney in mice. This finding indicated a preliminary basis for the safety of oral JDYZF.

Discussion

Currently, most drugs developed to treat AD remain in Phase III clinical trials, and there are no innovative drugs approved for the specific treatment of AD. The drugs already on the market include Acetylcholinesterase and N-methyl-Daspartate, etc., which only moderately improve symptomatic in Alzheimer's patients but do not slow disease progression.[23] Therefore, there is an urgent unmet need for the development of new therapeutics for this unfortunate disease. Herbal medicine has been widely used in Asian medicine, notably used in the treatment of AD.[24] At the same time, TCM also advocates the principle of preventing disease, which is also necessary for the treatment of AD. Prior studies indicate that patients have prodromal symptoms such as constipation and psychiatric symptoms for approximately ten years ago.[25–27] An early-stage and timely management of the patients may be a promising therapeutic strategy in the management of AD. A previous study reported that JDYZF effectively improved memory deficits and reduced AD-like pathological changes in Aβ-injected AD rats, attenuated the expression of NLRP3/caspase-1/GSDMD, and reduce the

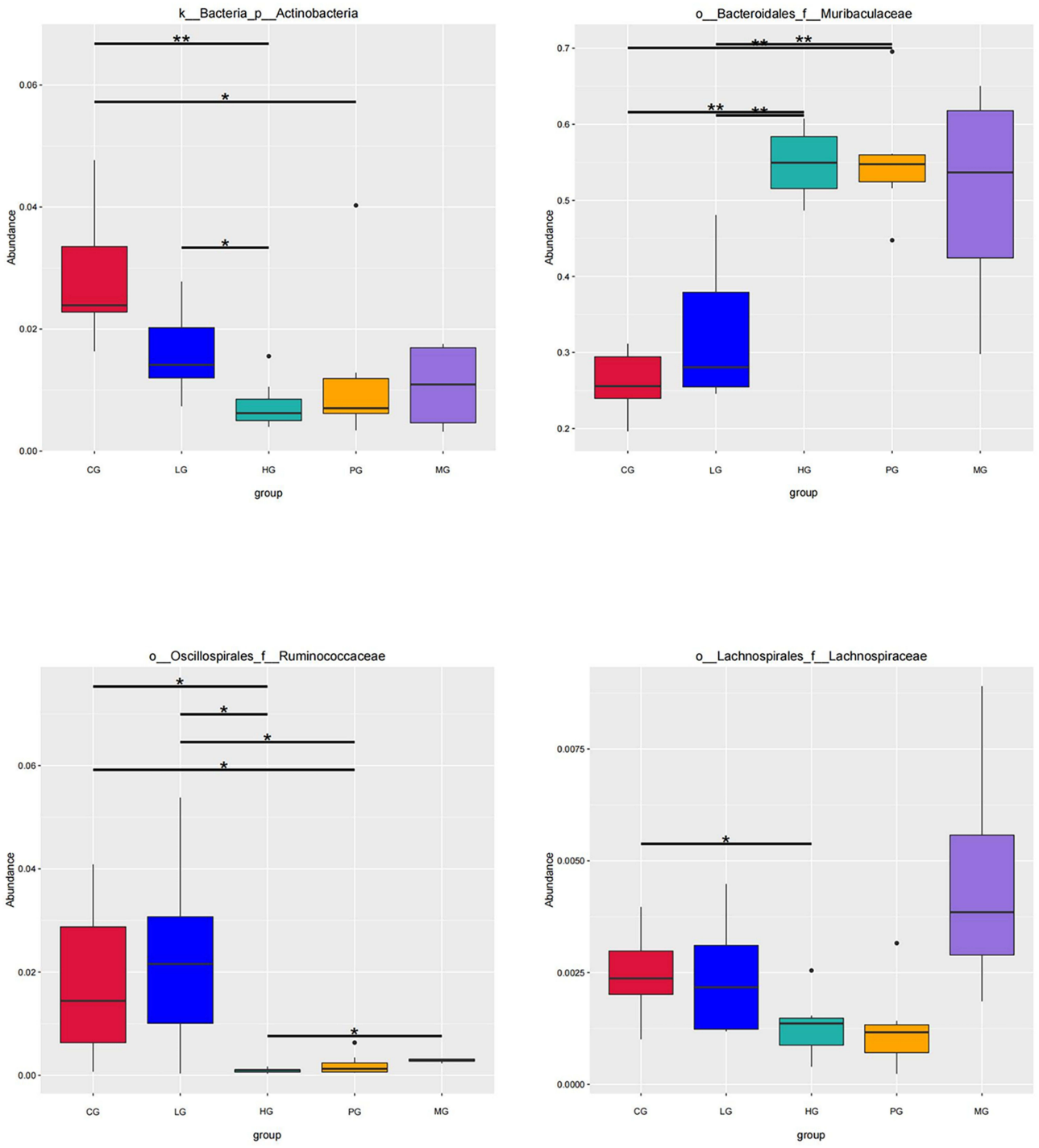

Figure 6 Boxplot of main differential bacteria relative abundance. Horizontal lines represent the difference between the groups. *Indicates significant difference between the two groups (p value <0.05). **Indicates the difference between the two groups was significant (p value <0.01).

content of IL-1β and IL-18 in hippocampus and cortex,[28] improved chronic cognitive impairment. These findings indicated that JDYZF may represent a promising candidate that could be used for neurodegeneration treatment and reduction of the risk of AD. However, it is unknown whether JDYZF beneficially affects p-tau protein and attenuates inflammatory reactions in APP/PS1 mice.

APP/PS1 mice are a forceful model for evaluating AD, which can evaluate the learning and memory function, and detect the pathological level of p-tau protein. Multiple neurological disorders reveal the importance of individualized differences,

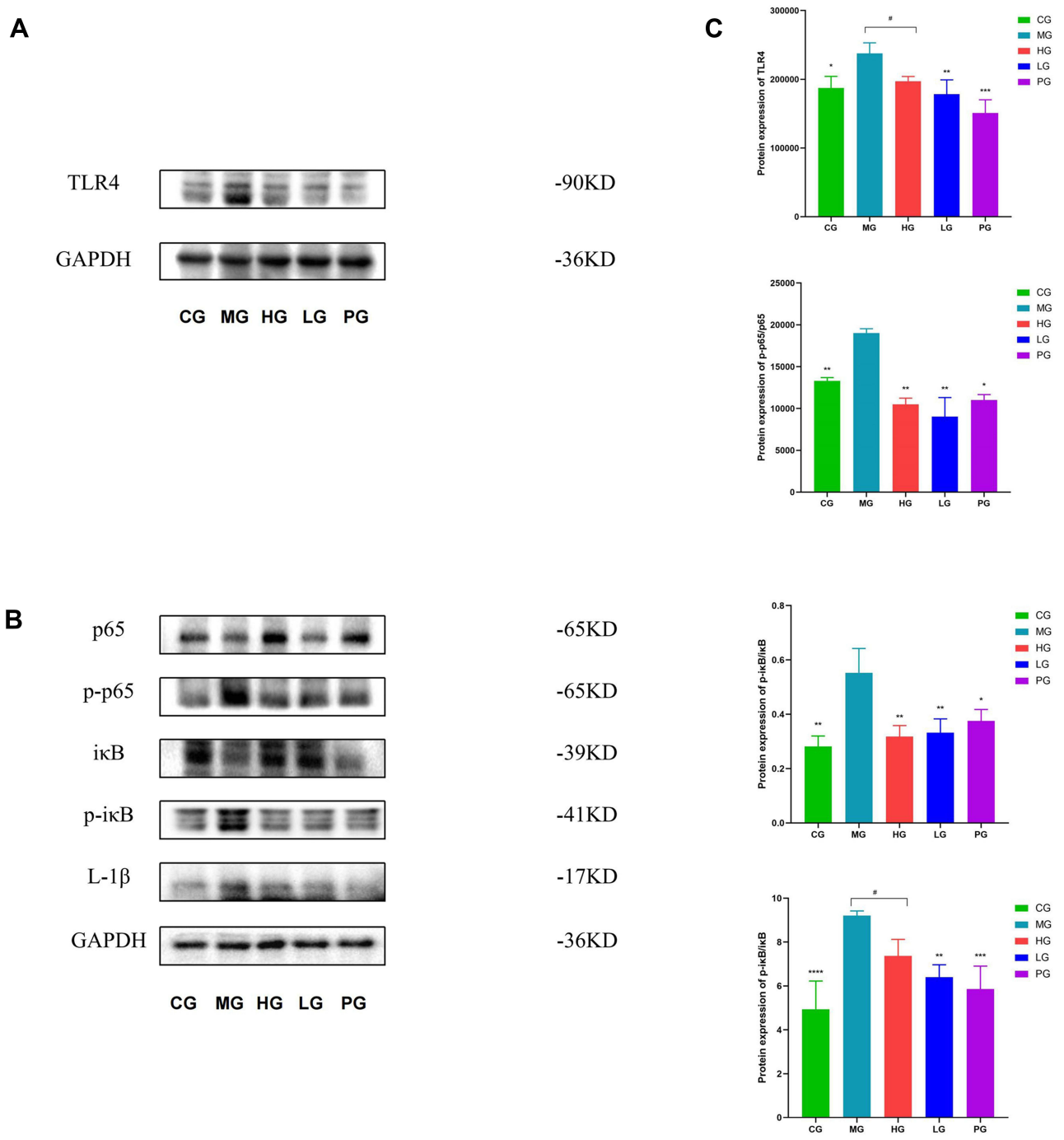

Figure 7 (**A**) The Western blot assay images for the levels of TLR4 in APP/PS1 mice. (**B**) The Western blot assay images for the levels of p-iκBiκB, p-p65, p65, and IL-1β in APP/PS1 mice. (**C**) The protein level of TLR4, p-iκB/iκB, p-p65/p65, and IL-1β in JDYZF-treated APP/PS1 mice were measured by Western blot assay. **p* < 0.05, ***p* or ****p* or *****p* < 0.01 compared with the MG, #*P* < 0.05 compared with HG. *F* = 10.91, *F* = 10.93, *F* = 23.53, *F* = 14.46. N = 3 in each group.

including gender, and age.[29] Existing studies in humans and other animals of AD reported the importance of gender.[30,31] Therefore, we chose male mice that were used as recipients. We paid attention to the weight changes and diet of mice. Prior studies that have noted the change in body weight are related to AD.[32] Being overweight is still one of the predisposing factors of AD.[33] However, AD patients are negatively correlated with body weight.[34] In this experiment, APP/PS1 mice lost weight and diets significantly. Although the specific reasons are not clear, we can speculate that the reasons include enriching pro-inflammatory bacteria and suppressing probiotics in APP/PS1 mice gut,[35,36] low dietary intake,[37] nutrient deficiency,[38] high metabolic consumption, and regular irregular physical exercise.[39,40] Among them, dietary restriction is

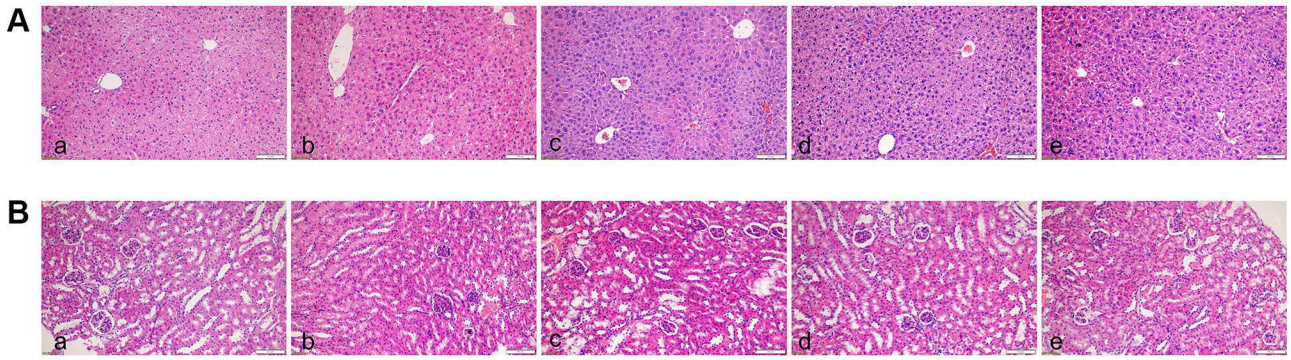

Figure 8 Histopathological changes in each group. (**A**) Pathological tissue of APP/PS1 mice liver; (**B**) pathological tissue of APP/PS1 mice kidney. (a) CG; (b) MG; (c) HG; (d) LG; (e) PG. (HE staining, ×200).

not only related to decreased vigilance and memory deterioration, but also related to Aβ protein and p-tau protein deposited and atrophy in the medial temporal cortex and other locations.[41,42] Higher metabolic consumption may be related to neurotoxicity, which consumes nutrients in the body, thus oxidizing and damaging brain tissue.[43] Therefore, we believe that appropriate weight gain has a protective effect on AD patients. Interestingly, JDYZF can achieve this result.[44]

Tau protein is the core factor of Alzheimer's disease and can predict disease,[45,46] which may affect cognition in any regulation.[47] Dr. Arne Ittner said, "the toxic effect of tau protein for brain cells is necessary, which leads to the impairment of memory".[48] Therefore, p-tau is the main target of AD diagnosis and treatment,[49] down-regulation of endogenous p-tau protein helps to slow down the development of AD and its inflammatory response.[50] In this study, JDYZF could inhibit p-tau protein, alleviate axonal neuron damage caused by p-tau protein. Compared with JDYZF groups, the hippocampal formation in MG include a trisynaptic loop from the entorhinal cortex to the dentate gyrus, from the dentate gyrus to CA3 showed pyknotic nuclei, some neurons were damaged, the nerve cells in the CA1 area were disordered, the normal nerve cells decreased, and the neuronal gap increased.

The use of beneficial microorganisms has been proven to have one of the most beneficial effects on AD. As the intestinal flora is the largest receiving organ in the body, we hypothesized that the intestinal environment may be a valuable strategy for developing novel AD therapeutics. Preclinical studies provide direct evidence for a link between disorders in flora and AD.[51] Most studies found that probiotic administration conferred neuroprotective benefits and could attenuate cognitive deficits. Meanwhile, gut microbiota imbalance may regulate the TLR4/NF-κB signaling pathway in the brain immune system.[52,53] In this study, we identified that JDYZF treatment could regulate gut microbiota by increasing the richness of *Ruminococcaceae* and *Actinobacteria* and reducing that of *Alistipes, Lachnospiraceae, Deferribacteres*, and *Muribaculaceae*. The *Ruminococcaceae* abundance is associated with short-chain fatty acids production and APOE genotypes. This suggests that *Ruminococcaceae* is worth further investigation as a potential target to mitigate cognitive decline.[54,55] Similarly, *Actinobacteria* as potential therapeutic options for AD in the same way.[56] Research has shown that the content of *Deferribacteres* is a negative correlation with cognitive parameters.[57] *Deferriactors*, as harmful Gram-negative bacteria, are closely related to the inflammatory response and obesity.[58] Several studies have shown that *Deferribacteres* abundance is positively correlated with LPS, TNF-α, and NF-κB-p65 levels.[59] It can regulate TLR4/MyD88/NF-κB signal path in inflammatory response by inhibiting *Deferribacteres*.[60] *Lachnospiraceae* is less abundant in AD,[61] and it is positively correlated with TLR4/NF-κB.[62,63] Preliminary studies have shown that the administration of berberine fumarate significantly ameliorated metabolic disorders, and reduced the populations of *Lachnospiraceae*. In addition, it reduced inflammation, inhibiting the overexpression of TLR4.[64] Furthermore, Ginkgolide B extracts against cognitive impairment with neuroprotective activity and can reverse the increased abundance of *Muribaculaceae*.[65] *Muribaculaceae* was associated with cognitive impairment and depressive mood and participates in the TNF-α/NF-κB signaling pathway.[66]

NF-κB and other members of its family are important regulators in the inflammatory process and participate in the inflammatory and stress process of the organisms.[67,68] TLR4, an upstream member of the NF-κB pathway, widely exists

on the surface of microglia. TLR4 activates the NF-κB pathway and its downstream events after p-tau protein deposition and makes p65 and p50 in its downstream change from the inactive state to the phosphorylated active state, and binding to specific DNA common sequences, thereby enhancing the transcription of inflammation-related proteins. In addition, TLR4 is a specific receptor for LPS and has an important role in gram-negative infections.[69] It connects with intestinal flora, neuroinflammation, and nutrients.[70]

As a traditional Chinese medicine compound, JDYZF has complex components, involving alkaloids, nucleosides, flavonoids, and other compounds. Modern pharmacological research showed that the main component of Coptis Chinensis is berberine. Berberine has a good neuroprotective effect.[71] It down-regulates NF-κB and activates iκB, IL-1β and TNF-α by blocking PI3K-Akt and MAPK signaling pathways,[72] and regulating apoptosis and oxidative stress.[73] Therefore, berberine can alleviate cognitive impairment, and this potential therapeutic mechanism is related to inhibiting p-tau hyperphosphorylation and NF-κB signal.[74] Yan found that bitter cardamom has an anti-inflammatory effect and can reduce the damage of lipopolysaccharide for nerve cells through PI3K-Akt/NF-κB pathway,[75] and improve the learning and memory of model mice. In order to clarify the potential mechanism of this traditional prescription, we further determined whether JDYZF affected the NF-κB signal in APP/PS1 mice through Western blot. We found that TLR4 was activated in APP/PS1 mice and contributed to the NF-κB signal downstream, which is characterized by iκBα and p65 expression. In terms of dose, the HG did not bring more benefits to the mice's cognition. On the contrary, the LG significantly improved the gut microbiota diversity and composition, inhibited the up-regulated expression of TLR4, suppression iκBα phosphorylation and degradation, and inhibit subsequent nuclear translocation of NF-κB p65. Suppress p-iκBα and p-p65 can regulate neuroinflammation and oxidative stress, reduce the activation of neuroglia cells and reverse p-tau protein, thereby improving cognition. Based on these findings, we believe that the effect of JDYZF on the activation of inflammatory corpuscles in APP/PS1 mice is through the down-regulation of TLR4/NF-κB signal mediated.

In summary, this thesis has provided that JDYZF can save cognition, inhibit microglia activation, and reduce neuroinflammation and neuron loss. The anti-inflammatory mechanism of JDYZF may involve regulating intestinal microecology and blocking TLR4/NF-κB pathway. Moreover, the pathological changes in the liver and kidney were not observed after 8 weeks of treatment, which provided a basis for the safety of the clinical medication. These findings further reveal the understanding of a part of the mechanism for JDYZF and provide new evidence for the beneficial role of traditional medicine in the prevention and treatment of AD.

Conclusion

In summary, this study verified the mechanism of JDYZF on AD across the TLR4/NF-κB signal pathway. JDYZF can regulate gut microbiota imbalance, negatively regulate TLR4/NF-κB signaling pathway in the brain immune system, clear the phosphorylation of p-tau protein, inhibit the vicious cycle of cognitive damage caused by an inflammatory reaction, and thus treat Alzheimer's disease. Based on the current research, the inflammatory reaction and apoptosis may be the tip of the iceberg in the treatment of AD,[76] and other pathways may also be involved in the mechanism of JDYZF. Developing new drugs based solely on the pathological formation of AD is not satisfactory.[77] The human body is a complex system. It is better to look at AD from the perspective of a holistic view than as an independent individual. Traditional Chinese medicine has the characteristics of multi-channel and multi-target. Based on the existing research, clarifying the mechanism of JDYZF ulteriorly will provide a basis and new ideas for the treatment of Alzheimer's disease.

Abbreviations

AD, Alzheimer's disease; PG, the positive drug group; p-tau, Phosphorylated Tau, MG, the model group; JDYZF, Jiedu Yizhi Formula, CG, the control group; NF-κB, Nuclear factor-κB, HE staining, Haematoxylin and Eosin staining; LG, the low-dose JDYZF group, MWM, Morris water maze; HG, the high-dose JDYZF group.

Ethics Approval

The whole process of animal experiments passed the ethical review of the Ethics Committee of Changchun University of Traditional Chinese Medicine (No.2021195 Changchun, China).

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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.

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Disclosure

The authors report no conflicts of interest in this work.

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