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ORIGINAL RESEARCH

Wenyang-Tianjing-Jieyu Decoction Improves Depression Rats of Kidney Yang Deficiency Pattern by Regulating T Cell Homeostasis and Inflammation Level

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Purpose: Chronic inflammation is one of the key mechanisms of depression. Wenyang-Tianjin-Jie Decoction (WTJD) is an effective antidepressant found in the course of diagnosis and treatment, but the mechanism of therapeutic effect is not clear. The study aimed to evaluate the efficacy of WTJD in the kidney yang deficiency (KYD) type of depression rats and reveal its mechanisms.

Materials and Methods: We selected forty 6-week-old male Sprague-Dawley rats for the study. We established a KYD [Phellodendron amurense Rupr (Huangbai) solution oral gavage and 4°C environments; 8 weeks] type of depression (chronic unpredictable mild stimulus; 6 weeks) rat model first. After successful modeling, we used WTJD or fluoxetine on rats for 3 weeks. Then we evaluated the depression and KYD behavior. Finally, we observed the expression of key inflammatory factors and proteins in peripheral blood and hippocampus, and further investigated the immune balance of Th17/Treg and Th1/Th2 cells and the activity of their main regulatory pathways JAK2/STAT3 and TLR4/TRAF6/NF-κB.

Results: The imbalance of Th17/Treg and Th1/Th2 cells in rats were related to KYD and depressive symptoms. Through this study, we found that WTJD can inhibit the activity of JAK2/STAT3 and TLR4/TRAF6/NF-κB pathways, balance Th17/Treg and Th1/Th2 cell homeostasis, regulate the levels of inflammatory factors in the hippocampus and peripheral blood, and reverse KYD and depression.

Conclusion: This study confirmed that WTJD had a reliable effect on depression rats with KYD, and its mechanism was to regulate the immune homeostasis of hippocampal T cells and related inflammatory factors to improve KYD and depression symptoms in rats.

Keywords: depression, immune homeostasis, inflammation, traditional Chinese medicine, hippocampus

Introduction

Depression, a common mental disorder, manifests symptoms including anhedonia, exhaustion, appetite loss, and even suicidal tendencies in severe cases.[1] The World Health Organization estimates that 5% of adults worldwide suffer from depression, and more than 75% of patients in low- and middle-income countries do not receive appropriate treatment.[2] A multi-country study found that the lifetime prevalence of depression and major depressive disorder averaged 10.6% and 30%–40%, respectively.[3] The lack of objective diagnostic indicators,[4] low cure rate and high recurrence rate are the potential causes of depression becoming a global health crisis[5,6]

The pathological mechanism of depression involves multiple systems and dimensions. There is no consensus, and the widely accepted theories are mainly the disorders of the monoaminergic neurotransmitter system, the hypothalamic-pituitary-adrenal axis, the neuroplasticity, and the inflammatory stimulation. Since Julius Wagner-Jauregg first discovered the link between inflammation and depression, many clinical and preclinical studies have supported this view.[7–9] It is difficult for a single mechanism to explain the cause of depression. Moreover, the significant difference in depression

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symptoms may be attributed to the variation in mechanisms. Therefore, by classifying depression, we can delve deeper into understanding its mechanism and ultimately achieve better treatment efficacy. Clinical investigations have found that the proportion of people with depression in people with a yang deficiency pattern is high, reaching 61.4%.[10] Furthermore, depression patients with physical symptoms as the main complaint are more likely to have a yang deficiency pattern,[11] this suggests that the yang deficiency pattern is closely related to depression.[12] Additionally, after long-term clinical observations, we found that people with kidney yang deficiency (KYD) patterns often have low energy, fatigue, lack of breath, decreased appetite, decreased libido, and other similar manifestations of depression. In animal experiments, we found that rats with KYD are prone to depression-like manifestations, including laziness, reduced food intake, and decreased fertility rate (loss of libido).[13–15] Therefore, KYD may be closely related to depression.

As a natural medicine, Traditional Chinese Medicine (TCM) plays a complementary and alternative role in treating modern depression. WenYang-TianJing-JieYu-Decoction (WTJD) is an effective prescription for depression summarized by Professor Wei hong Li based on more than 20 years of clinical experience. WTJD comprises three drugs: *Cornus officinalis Siebold & Zucc*. (Shanzhuyu), *Gynochthodes officinalis (F. C. How) Razafim. and B. Bremer* (Bajitian), and *Curcuma longa L*. (Yujin) in a ratio of 6:3:2. WTJD has been used in the clinical treatment of depression for several years with significant clinical efficacy. Meanwhile, our preliminary study suggests that WTJD can reduce depression by intervening in microbiome-gut-brain axis-mediated microbiota dysregulation and inflammation (results to be published). Based on the above theories and research results, we suspect that WTJD may improve depression by reducing inflammation of the hippocampal tissue. To test this hypothesis, we generated a chronic unpredictable mild stimulus (CUMS) model combined with a modified KYD model[14] based on our previous research to study the antidepressant inhibition of WTJD. Our findings provide reliable evidence and new insights for treating depression with WTJD.

Materials and Methods

Animals and Groupings

Considering the advantages of strong plasticity and stability and obvious behavioral performance,[16] we chose Sprague-Dawley rats for research. A total of 40 male Sprague-Dawley rats (aged 6–8 weeks old and weighed 200 ± 20 g) were purchased from Dossy Laboratory Animal Co., Ltd. (Chengdu, Sichuan, SCXK (Chuan): 2020-030). All rats were housed in temperature-controlled (22 ± 2 °C) and humidity (60%) controlled rooms with a standard light-dark cycle (12 h/12 h). Rats are free to drink water but limit food access (30 g/day/pc). After 7 days of adaptive feeding, rats were randomly assigned to the Normal, Model, Fluoxetine, WTJD high-dose (WTJD-H), and WTJD medium-dose groups (WTJD-M). All procedures were approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine (License Number: 2019-32) and followed the National Institutes of Health Laboratory Animal Care and Use Guidelines.

Plant Materials, Drugs, Reagents and Main Instruments

Phellodendron amurense Rupr (Huangbai) (2306047), Shanzhuyu (220922–72), Yujin (221201), and Bajitian (2212117) were purchased from Sichuan Neautus Chinese Medicine Pieces Co., Ltd. and certified by Professor Qin wan Huang of Chengdu University of Traditional Chinese Medicine. Fluoxetine hydrochloride capsules (H20064844) were purchased from Suzhou Sinochem Pharmaceutical Industry Co., Ltd. The enzyme-linked immunosorbent assay (ELISA) kits for Interleukin-17A (IL-17A, CV0964J88151), Tumor Necrosis Factor-α (TNF-α, CV0488644504), Interleukin-6 (IL-6, CV086F6P4712), Interleukin-10 (IL-10, CV0500449504), Interleukin-4 (IL-4, CV03R6N61988), Interleukin-1β (IL-1β, CV12288P5047), Interferon-γ (IFN-γ, CV02R8820112), and transforming growth factor-β1 (TGF-β1, CV07LH602190) were purchased from Elabscience Biotechnology Co., Ltd. Primary antibodies: Retinoic Acid Receptor-Related Orphan Receptor γ-t (RORγt: Bioss, BS-23110R); Forkhead box protein P3(FOXP3: affinity, 22228-1-AP); T-box gene expressed in T cells (T-bet: affinity, 42z9342); GATA Binding Protein 3 (GATA3: affinity, 77j1800). GAPDH (Proteintech Group, Inc, 10017731). Secondary antibody (antibody Goat anti-Rabbit lgG, Chengdu Zen-Bioscience Co., Ltd., L05DE73; antibody Goat anti-Mouse lgG, Chengdu Zen-Bioscience Co., Ltd., L06JL51). BCA protein concentration determination kit (Beijing Labgic Technology Co., Ltd., 21362908). Radio Immunoprecipitation

Assay lysate, PMS, protease inhibitor, protein loading buffer, 30% glue-making solution, Polyacrylamide gel electrophoresis gel accelerator, 4X separation gel buffer, 5% upper gel master mix, electrophoresis buffer, transfer buffer, and BSA were purchased from Beijing Solarbio Technology Co., Ltd. APS and skim milk powder were purchased from Shanghai Beyotime Biotechnology Co., Ltd. Marker was purchased from Wuhan Servicebio Biotechnology Co., Ltd. Polyvinylidene fluoride (PVDF) membrane was purchased from Sigma Aldrich (Shanghai) Trading Co., Ltd. The luminescent liquid was purchased from Baoguang Biotechnology Research (Chongqing) Co., Ltd. cDNA first-strand synthesis kit (Chengdu Rongwei Gene Biotechnology Co., Ltd., 221010-A5), 2×SYBR Green PCR Mastermix (Chengdu Rongwei Gene Biotechnology Co., Ltd., 230304-A4), RNA extraction kit (Chengdu Foregene Biotechnology Co., Ltd., R230101).

Preparation of WTJD

Before preparation, we used high-performance liquid chromatography to identify the main chemical components of WTJD. The main chemical structure and identification results of WTJD are shown in Supplementary Material 1. WTJD is prepared according to our established methodology. We ground 300 g of Shanzhuyu, 150 g of Yujin, and 100 g of Bajitian together, soaked them in ten times the volume of purified water for 30 min, and heated them to keep slightly boiling for 1 h. After filtering with gauze, decoct the herbal residues again at 100 °C for 1 h with eight times the volume of purified water. Subsequently, the two extracted solutions were combined, concentrated under reduced pressure, and then lyophilized to produce 87.285 g of extract powder (extraction ratio 15.87% [w/w]). The lyophilized powder of Huangbai was also prepared according to the above method, and the extraction ratio was 13.25–15.21% [w/w], and the dosage was 23.85 mg/kg for subsequent KYD modeling. The lyophilized powder was stored in a –80 °C freezer.

Experimental Design

KYD Model

Our team has been engaged in the study of KYD patterns and constitutions for a long time. The KYD rat modeling method used in this study was invented on the basis of following the etiology of traditional Chinese medicine. The previous research verified the model from a more comprehensive animal macroscopic characterization and microscopic molecular biology level,[14,15,17] indicating that the rat model of kidney yang deficiency constructed by this method is an animal model that conforms to the pathogenesis and symptoms of traditional Chinese medicine. The specific implementation methods are as follows: Lyophilized powder of Cortex Phellodendri (Huangbai) is prepared according to the preparation method 2.3. Besides the Normal group, rats in other groups received Huangbai solution gavage (0.36 g/kg) and 4 °C low-temperature environment for 1 h (programmable constant temperature and humidity box, Xiamen Fubes Testing Equipment Co., Ltd., FBS-225L-20) for eight weeks daily.

CUMS Model

Chronic unpredictable mild stimulation is one of the most recognized depression modeling methods in recent years.[18] This modeling method simulates the induced characteristics of depression (long-term, low-intensity, random). After modeling, it shows a highly similar phenomenon and pathophysiological characteristics to patients with depression.[19,20] Starting from the third week, a random stimulus intervention was introduced daily. These interventions included fasting for 24 h, water fasting for 24 h with an empty bottle as a form of stimulation, exposure to noise at 85 dB for 1 h, tilting the rat cage at a 45° angle for 12 h, keeping the litter wet for 24 h, clamping the rat's tail for 1 min at a point 2 cm away from the tip, and binding the rat for 4 h using a smooth cylindrical plastic bottle with a diameter of 7 cm and a length of 20 cm, which was perforated to ensure air circulation.

Behavioral evaluation of KYD and depression were performed on each group of rats before and after treatment, and the appropriate drug was administered for gavage of rats in each group (Normal and Model: normal saline 10 mL·kg; Fluoxetine: compound fluoxetine capsules 3.6 mg/kg; WTJD-H: WTJD 9.9 g/kg, WTJD lyophilized powder 1.57 g/kg; WTJD-M: WTJD 4.95 g/kg, lyophilized powder 0.79 g/kg), once daily for three weeks. KYD and CUMS modeling are given simultaneously with the treatment. The experimental process and CUMS operation diagram can be seen in Figure 1.

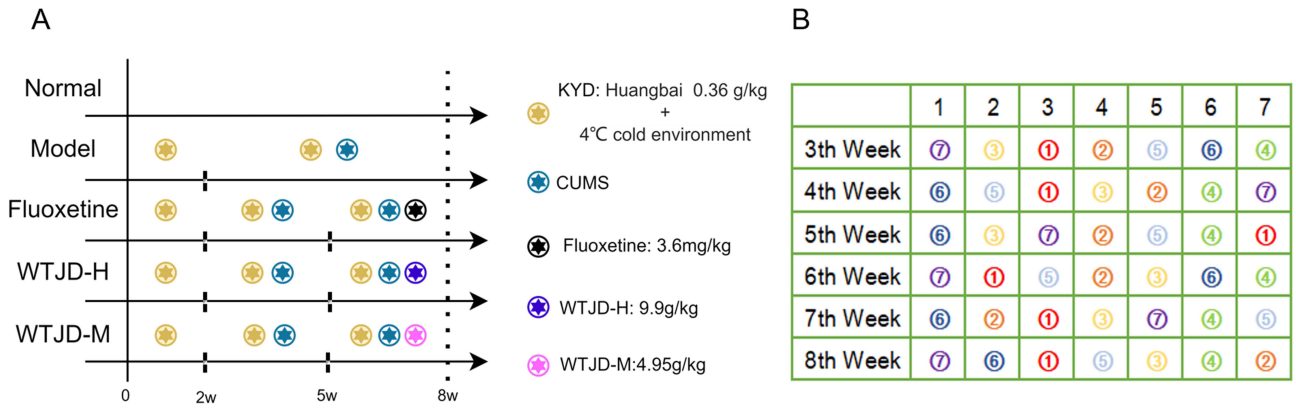

Figure 1 (**A**) Outline of the experimental process. (**B**) Operation diagram of CUMS.

Behavioral Evaluation

Evaluation of Physical Characteristics of KYD

The assessment was performed using the evaluation scale of KYD physical characteristics developed by the author's team (Supplementary Material 2), including the body temperature, weight, behavior, tongue image, and body hair of rats, and the total score > 12 was evidence of KYD.

Sugar Water Preference Experiment

The rats in each group were trained for two days of adaptation period before testing, and two bottles of 1% sucrose water were placed in each cage at 20:00 on the first day, and one of the bottles was replaced with normal drinking water simultaneously on the second day. Rats fasted for 24 h on the third day, and quantitative 1% sucrose water and normal drinking water were randomly placed on all rats at 20:00 on the fourth day, and water bottles were collected after 2 h and weighed separately. Rat's sugar water preference rate = sucrose water consumption / (sucrose water consumption + normal drinking water consumption) × 100%.

Open Field Experiments

The rat was brought to the testing chamber 3 h before the trial to acclimate to the spatial environment fully. The tests of each group of rats were completed sequentially in the afternoon (18:00–22:00), and each rat was tested once. The experiment was performed in a quiet environment, and the rats were placed in the bottom center of a black open box of 40 cm × 100 cm × 100 cm. After their free movement for 2 min, the total activity distance and rest time of the rats were recorded within 5 min. Before the next experiment, the feces were removed, and the bottom of the box was wiped with 75% ethanol.

Forced Swimming Experiments

The rats were put into a transparent test barrel with a diameter of 25 cm and a height of 60 cm, a water temperature of 25 ± 2 °C, and after acclimatization for 2 min, the swimming time, climbing time, and immobility time of the rats within 5 min were recorded. Each rat needs to change the water one time after the test.

Acquisition Materials and Inspection Methods

After completion of drug administration and behavioral tests, the rats in each group were fasted without water for 12 h, anesthetized with 1% pentobarbital sodium solution (50 mg/kg), blood was collected from the abdominal aorta, static, centrifuged, serum collected and stored in a –80 °C refrigerator. The rats were then sacrificed by cervical dislocation, and the head was immediately severed on ice to remove the brain and then stored at –80 °C or placed in 4% paraformaldehyde for more than 24 h for subsequent use.

Histological Observation

The brain specimens were fixed with a 4% paraformaldehyde solution and then incubated for 24 hours. Afterward, we used a traditional method of dehydrating ethanol through a gradient process. Once transparency was achieved using xylene, the samples were embedded in paraffin and then incubated at 70 °C for 90 min. The samples were sectioned and then subjected to HE staining. They were observed under a light microscope to examine the pathological morphology, and images were captured.

Bioassays

The hippocampus were rinsed with pre-chilled PBS to remove residual blood. Subsequently, the specimens were weighed and dissected into smaller sections, which were then transferred into a centrifuge tube. Precooled PBS (tissue: PBS in a 1:9 ratio) was added, homogenized, and placed in an ice bath for 30 min, during which four times of sonication. Finally, the homogenate 5000 Xg was centrifuged for 5–10 min, and the supernatant was collected. The study used the ELISA kit to determine the levels of IL-1β/4/6/10/17A, IFN-γ, and TGF-β1 following the protocols.

Western Blotting

RORγt, FOXP3, T-bet, and GATA3 protein expression was detected. RIPA lysate was used to extract total tissue proteins from hippocampal tissues, while BCA kits were used to measure total protein concentrations in the supernatant. Samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis transferred to the PVDF membrane and then blocked with 5% skim milk in TBS-T. The membrane was incubated with primary antibodies and GAPDH (1:10,000 dilution) overnight at 4 °C. The PVDF membrane was rinsed with TBST three times for 10 min in a room temperature shaker, then the corresponding secondary antibody (1:1000 dilution) was added and incubated in a room temperature shaker for 60 min. After the secondary antibody incubation, the PVDF membrane was rinsed with TBST in a room-temperature shaker three times for 10 min. The PVDF film was tilted to the exposure plate. The chromogenic solution was prepared according to the 1:1 volume ratio of A and B liquids added to the front of the PVDF film. The exposure time, area, and background were adjusted according to the results in the gel imaging system to obtain the exposure result map. A gray value analysis of the bands using Image J software is expressed as the ratio of target protein intensity to GAPDH band intensity.

qRT-PCR

Hippocampal tissue was homogenized using a tissue homogenizer, and the total RNA of cells was extracted following the RNA extraction kit's procedure. The cDNA First Strand Synthesis Kit was used to transcribe total RNA to cDNA reversely, and the cDNA synthesis conditions were as follows: incubation at 25 °C for 10 min, 55 °C for 15 min, 85 °C for 5 min, and then placed on ice. The RT-PCR reaction system totals 20 μL. The PCR reaction conditions were 50 °C for 2 min, 94 °C for 10 min, 95 °C for 3 min, 95 °C for 5 s, 60 °C for 30s, and 40 cycles in steps 4 and 5. The relative expression is calculated using the 2-ΔΔCt method. The primer sequences are shown in Table 1.

Table 1 Primer Sequences

Gene Name	5'→3'	Gene ID	Product Length
JAK2—F	GCTCCTCTCCTTGACGACTT	24514	288
JAK2—R	AATGAACCTGCGGAATCTGT		
STAT3—F	CGGATCGCTGAGGTACAATCC	25125	313
STAT3—R	TGACTCTTTGCTGGCTGCAT		
TLR4—F	CCGCTCTGGCATCATCTTCA	29260	108
TLR4—R	TCCCACTCGAGGTAGGTGTT		
TRAF6—F	TGGAGTTTGACCCACCTTTG	11245	242
TRAF6—R	GCCTTTATTTGGACACTTTACCG		
NF-κB—F	ATCGTAAGAATGGACAGAACAGC	81736	303
NF-κB—R	TTCGGGGTAGTAGAGAAAGGG		
GAPDH-F	GAAGGTCGGTGTGAACGGAT	24383	250
GAPDH-R	CCCATTTGATGTTAGCGGGAT		

Statistical Analysis

All data for this study are expressed as mean ± standard deviation (mean ± SD). Brown-Forsythe test was used for normality and homogeneity testing ($p > 0.05$). Then, one-way analysis of variance and LSD test of GraphPad Prism 9 software (Graph Pad Software, San Diego, California) were used to compare the differences between groups. $P < 0.05$ was statistically significant. Statistical analysis of the difference before and after treatment within the group was performed using the paired-sample *T*-test of SPSS 21.0 software (IBM, Armonk, USA), and the difference was statistically significant at $P<0.05$.

Results

WTJD Improved the KYD Phenotype in Rats with KYD Type of Depression

Figure 2 shows that from the second week to the end of the experiment, rats outside the Normal group showed a similar degree of slowdown in weight gain, and none of the treatments significantly reversed this trend. Furthermore, from the second week, the body temperature of rats outside the Normal group was quite low, and the body temperature of rats treated with WTJD-H gradually increased. Conversely, the rats in the WTJD-M and Fluoxetine groups did not change.

Before treatment, the rats treated with KYD + CUMS showed a significant KYD phenotype ($P < 0.01$). Furthermore, apart from the previously indicated physiological characteristics such as reduced body temperature and body weight, the rats also exhibited significant changes in their nails, tongue, and pinna color and thinning of body hair. Additionally, they displayed behaviors such as an arched back and a preference for huddling, further indicating the effectiveness of the KYD modeling approach. After three weeks of treatment, the KYD trait score was significantly reduced in the WTJD groups ($p < 0.01$), while there was no significant change in the model group.

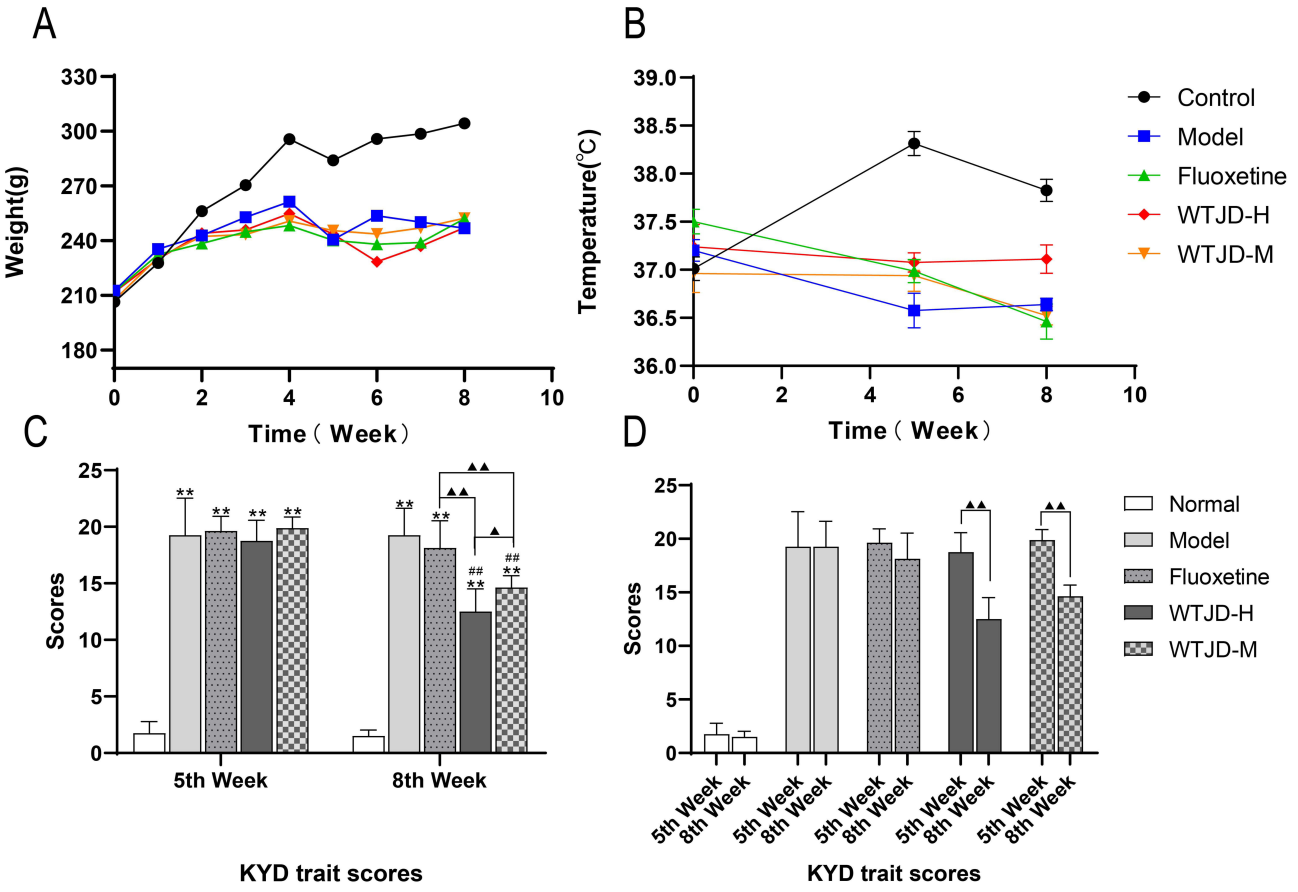

Figure 2 WTJD improved the KYD trait in rats with KYD type of depression. (**A**) Trends in body weight. (**B**) Trends in body temperature. (**C**) Differences in KYD traits between the five groups. (**D**) Differences in KYD traits before and after treatment. The data is represented by mean ± SD, n = 8. Compared to the Normal group, **p < 0.01. Compared to the Model group, ##p < 0.01. Comparison between groups in Fluoxetine group, WTJD-H group and WTJD-M group, ▲▲p < 0.01, ▲p < 0.05.

WTJD Improved Depressive Behavior in Rats with KYD Type of Depression

We evaluated the depressive behavior of each group of rats before and after treatment (Figure 3). In the absence of therapy, CUMS + KYD significantly reduced the swimming time of rats ($P < 0.01$). Additionally, there was a significant increase in resting time ($P < 0.01$) and varying degrees of decreased climbing time ($P < 0.05$ or $P < 0.01$). Simultaneously, the sugar water consumption of rats after CUMS + KYD intervention was significantly lower than the Normal group ($P < 0.01$). After CUMS+KYD intervention, the total distance of open field in the Model group, Fluoxetine group, and WTJD-M group was shortened ($P < 0.01$), and the resting time was prolonged ($P < 0.01$). The swimming test, sugar water preference test, and open field test results indicated that depression modeling was established.

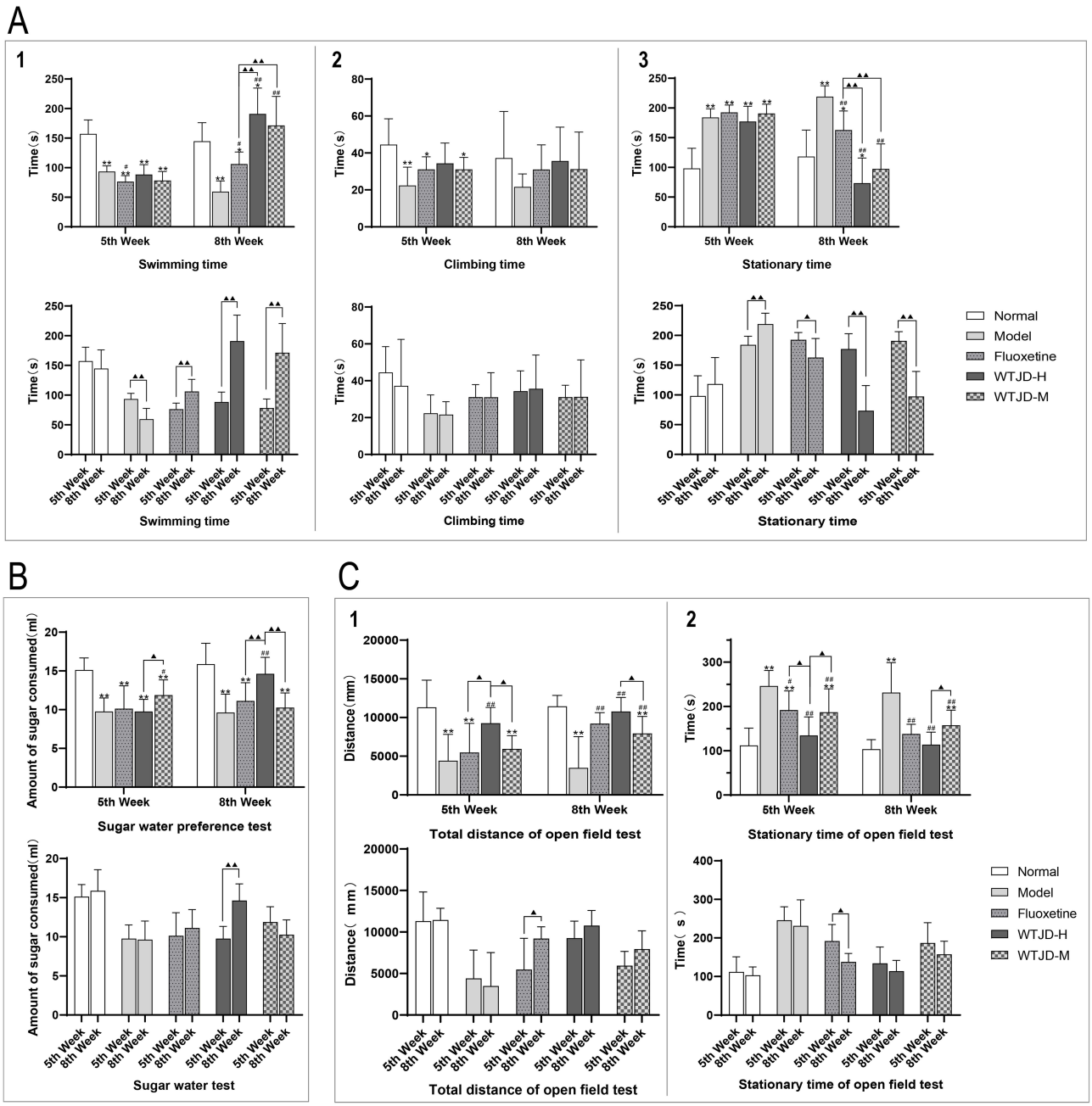

Figure 3 WTJD improves depressive behavior in the KYD type of depression rats. (**A**) Forced swimming experiments results: (1) Swimming time; (2) Climbing time; (3) Stationary time. (**B**) Sugar water preference test. (**C**) Open field test: (1) Total distance of open field; (2) Static time in the open field. The data is represented by mean ± SD, n = 8. Compared with the Normal group, $^{**}p < 0.01$, $^{*}p < 0.05$. Compared to the Model group, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$. Comparison between groups in Fluoxetine group, WTJD-H group and WTJD-M group, $^{\blacktriangle\blacktriangle}p < 0.01$, $^{\blacktriangle}p < 0.05$.

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After three weeks of treatment, rats were evaluated for depressive behavior again (Figure 3). In the swimming experiment, the swimming time of the Model group was significantly shorter than that before (P<0.01), while the swimming time of rats in three treatment groups was significantly higher than that before (P<0.01), the effect of increasing swimming time in both groups of WTJD was better than that of the Fluoxetine group (P<0.01). None of the treatments had a significant effect on climbing time in the forced swimming experiments. The resting time in the Model group was considerably higher than that of the previous measurement (P<0.01), and the resting time in all three treatment groups was shorter than before (P<0.05 or P<0.01). When comparing the groups after treatment, the resting time of the WTJD-H and the WTJD-M was significantly shorter than that of Fluoxetine group. The consumption of sugar water after treatment in the WTJD-H group was significantly higher than that before (P<0.01), which was significantly better than that in the WTJD-M and Fluoxetine groups (P<0.01), and the changes in the remaining groups were not obvious. In the open field test, the resting time of rats in the three treatment groups after treatment was significantly lower than that of the Model group (P<0.01), and the resting time of the WTJD-H group was lower than that of the WTJD-M group (P<0.05). The largest difference before and after treatment was the Fluoxetine group, which showed an increase in total distance and a decrease in resting time (P<0.05).

WTJD Improved Hippocampal Histopathological Morphology in Rats with KYD Type of Depression

The effect of WTJD on the hippocampus in rats with KYD type of depression was explored at the histological level (Figure 4). H&E staining showed that many pyramidal cells can be observed in the hippocampus area of the Model group, which is triangular or irregularly shaped. The nucleoli were unobvious, the fine matter was scarce, and the boundary between the nucleus and cytoplasm was blurred. WTJD can reduce the occurrence of cytonuclear abnormalities in pyramidal cells. However, the hippocampus of rats in the Fluoxetine group showed pathological manifestations of irregular arrangement of pyramidal cells and telangiectasia and congestion.

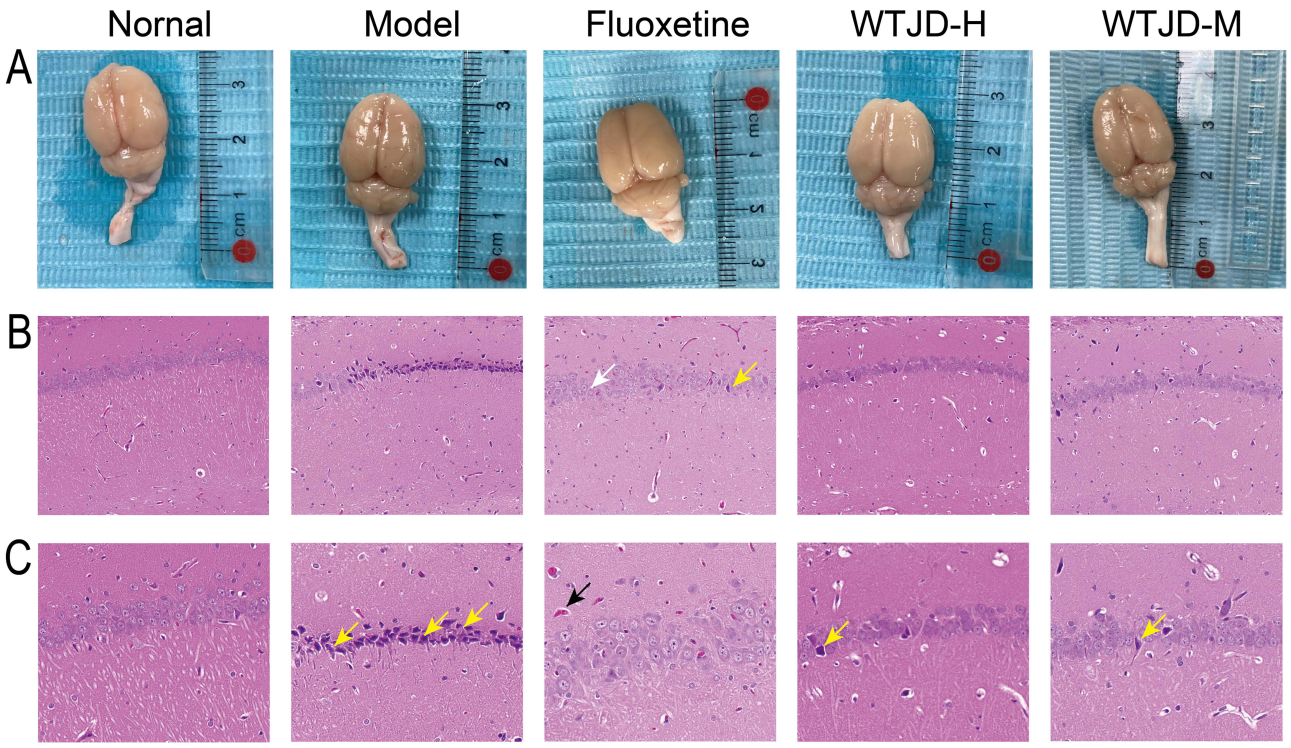

Figure 4 WTJD can alleviate histologic abnormalities of the hippocampus in rats with KYD type of depression. (**A**) Complete Brain Anatomy of Rat. (**B**) Representative micrographs of rat hippocampal H&E in each group under 200x microscopy. (**C**) Representative micrographs of HE staining of rat hippocampal tissues in each group under 400x microscopy. The white arrow indicates that the pyramidal cells are irregularly arranged, the black arrow indicates telangiectasia congestion, and the yellow arrow indicates that the pyramidal cell nucleus is deeply stained, solidified, triangular or irregular, the nucleoli are not obvious, and the boundary between the nucleus and the cytosol is blurred.

WTJD Regulated the Immune Function Axis of Hippocampal Th17/Treg and Th1/Th2 Cells in Rats with KYD Type of Depression

We observed the protein expression levels of key transcription factors of Th1/2/17 and Treg cells to determine the effect of WTJD on the immune function axis of Th17/Treg and Th1/Th2 cells (Figure 5). Compared with the Normal group, the protein levels of RORγt and T-bet were significantly increased in the Model group ($p < 0.01$), and the protein expression levels of FOXP3 and GATA3 were significantly reduced ($p < 0.01$). Both treatments can affect Th17/Treg cells and Th1/Th2 cells, manifested by reducing RORγt and T-bet expression while increasing FOXP3 and GATA3 expression, of

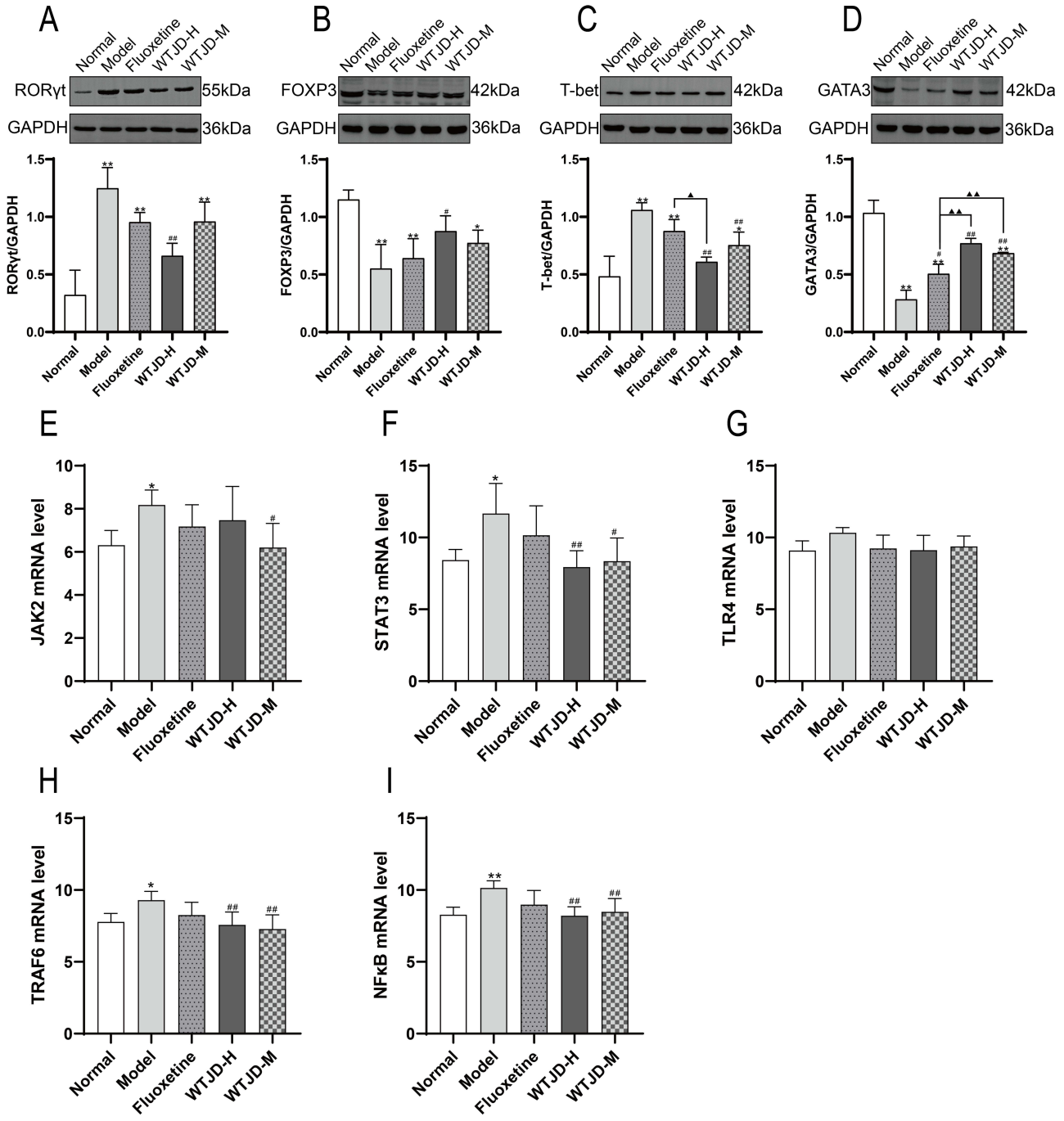

Figure 5 WTJD regulated hippocampal RORγt/FOXP3 and T-bet/GATA3 protein levels and JAK2/STAT3 and TLR4/TRAF6/NF-κB pathway activities in rats with KYD type of depression. (**A**–**D**) expression levels and semi-quantitative analysis of RORγt, FOXP3, T-bet, GATA3, n = 4. (**E**–**I**) The mRNA expression levels of JAK2, STAT3, TLR4, TRAF6, and NF-κB in the hippocampus were detected by qRT-PCR. The data is represented by mean ± SD, n = 6. Compared to the Normal group, $^{**}p < 0.01$, $^{*}p < 0.05$. Compared with the Model group, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$. Comparison between groups in Fluoxetine group, WTJD-H group and WTJD-M group, $^{▲▲}p < 0.01$, $^{▲}p < 0.05$.

which the change in T-bet/GATA3 expression is more obvious. In RORγt/FOXP3 expression, no significant difference was observed among the three treatment groups. The two groups of WTJD were superior to the Fluoxetine group in inhibiting the expression of T-bet and increasing the expression of GATA-3, which was closest to the expression level of the normal group. Instruction of Prestained Protein Marker II, raw data and image for Western blot are all visible in Supplementary Materials 3–5. JAK2/STAT3 and TLR4/TRAF6/NF-κB are key regulatory pathways of Th17/Treg cells and Th1/Th2 cells immune homeostasis. We detected the expression of mRNA of key proteins of the above two pathways and found that the Model rats had increased activity of JAK2/STAT3 and TLR4/TRAF6/NF-κB pathways, WTJD could inhibit the overexpression of the above pathway proteins. There were insignificant differences between the three treatment groups, but the down-regulation of JAK2, STAT3, TLR4 and NF-κB mRNAs by WTJD seemed to be more significant.

WTJD Regulated the Level of Inflammatory Factors in Hippocampal Tissue in Rats with KYD Type of Depression

We investigated the levels of important inflammatory factors in hippocampal tissue to access the inflammatory effects of CUMS and KYD on the hippocampus (Figure 6). The hippocampus of CUMS + KYD rats showed different degrees of high pro-inflammatory factors (IL-1β/6/17A, TNF-α, and IFN-γ), WTJD and fluoxetine can reduce the expression of pro-inflammatory factors in hippocampal tissues, and WTJD is slightly more potent than fluoxetine. IL-4/10 and TGF-β1 in the Model group were lower than those in the Normal group, and IL-4/10 and TGF-β1 also showed different degrees of improvement after treatment, but all differences were insignificant.

WTJD Regulated the Level of Peripheral Blood Inflammatory Factors in Rats with KYD Type of Depression

We measured inflammatory factor levels in the peripheral blood of rats in each group (Figure 7). IL-1β/6, TNF-α and INF-γ in the Model group were significantly higher than in the Normal group. WTJD and fluoxetine could downregulate these pro-inflammatory factors to a certain extent, among which WTJD-H seem to had the most significant effect. IL-17A showed a similar trend, but no statistical difference existed between the groups. The Model group showed decreased levels of anti-inflammatory factors (IL-4/10 and TGF-β1). Fluoxetine and WTJD could increase anti-inflammatory factors in the peripheral blood, among which WTJD-M was slightly better than fluoxetine and WTJD-H.

Discussion

Inflammation is an important pathway to depression onset. Genetic predisposition interacts with the pro-inflammatory external environment and is a breeding ground for inflammation-related depression.[21] Innate immune cells are activated by various pathways to synthesize and release inflammatory mediators and stimulate inflammatory responses. Inflammatory cytokines are crucial in this process. Inflammatory factors can cause depression in the following ways: (1) excessive activation of microglia and astrocytes, causing emotional symptoms;[22] (2) activation of the hypothalamic-pituitary-adrenal axis and inhibition of the negative feedback loop, resulting in hyperglucocorticoidemia, a vicious cycle of glucocorticoid resistance and uninhibited release of inflammatory factors, and eventually causing emotional symptoms;[23] (3) by affecting the synthesis, metabolism, reuptake, receptor expression and other pathways of various neurotransmitters including serotonin (5-hydroxyptamine, dopamine and glutamate), resulting in depressive symptoms;[24,25] (4) damage neuronal plasticity and affect neurotrophic factor levels.[26]

The hippocampus is the core area of the brain responsible for memory and cognitive function, especially good at managing emotion-related memory.[27] In the study of the relationship between brain structure and depression, the relationship between the hippocampus and depression is the most comprehensive. The reduction of hippocampal volume is closely related to recurrent depression.[28,29] At the same time, the anatomical position of the hippocampus is closely connected with the hypothalamus. The hippocampus is rich in glucocorticoid receptors and has a regulatory effect on the hypothalamic-pituitary-adrenal axis.[30] In addition, the plasticity of neurons in the hippocampus is high, which is closely related to memory, emotion, and learning function.[31,32] Studies have shown that stress can affect the activation of

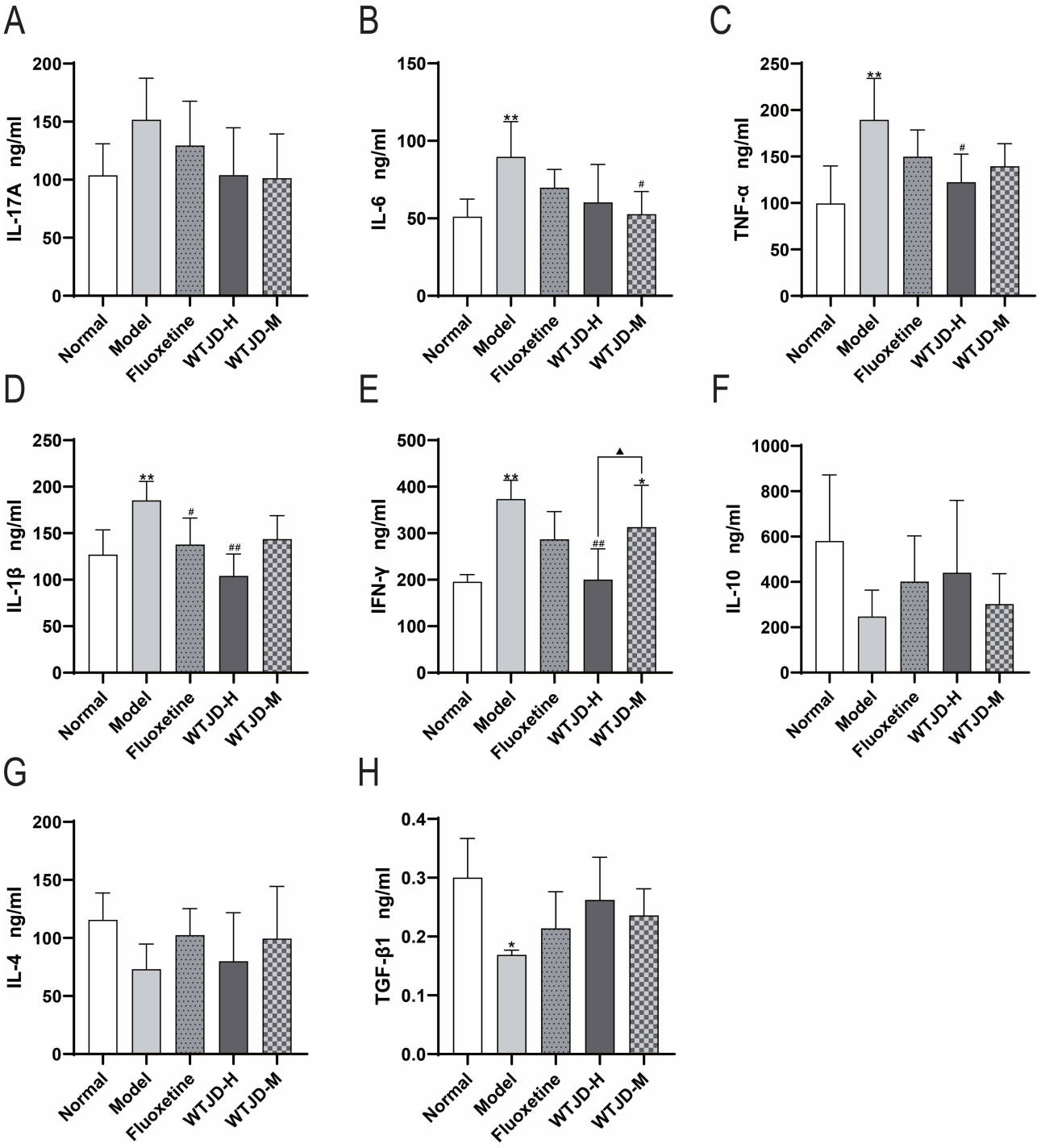

Figure 6 WTJD regulated inflammatory factor levels in the hippocampus in KYD-type depressed rats. (**A**–**H**) IL-17A, IL-6, TNF-α, IL-1β, IFN-γ, IL-10, IL-4, TGF-β1 levels in the rat hippocampus. The data is represented by mean ± SD, n = 6. Compared with the Normal group, $^{**}p < 0.01$, $^{*}p < 0.05$. Compared with the Model group, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$. Comparison between groups in Fluoxetine group, WTJD-H group and WTJD-M group, $^{\blacktriangle}p < 0.05$.

microglia in the hippocampus, which in turn increases the expression of TNF-α in the tissue[33] At the same time, hippocampal CD38 is closely related to depression-like behavior in the inflammatory model.[34] In short, the hippocampus is one of the most studied brain regions in animal models of depression, and it is also considered to be the brain region that is most closely related to depression.[35]

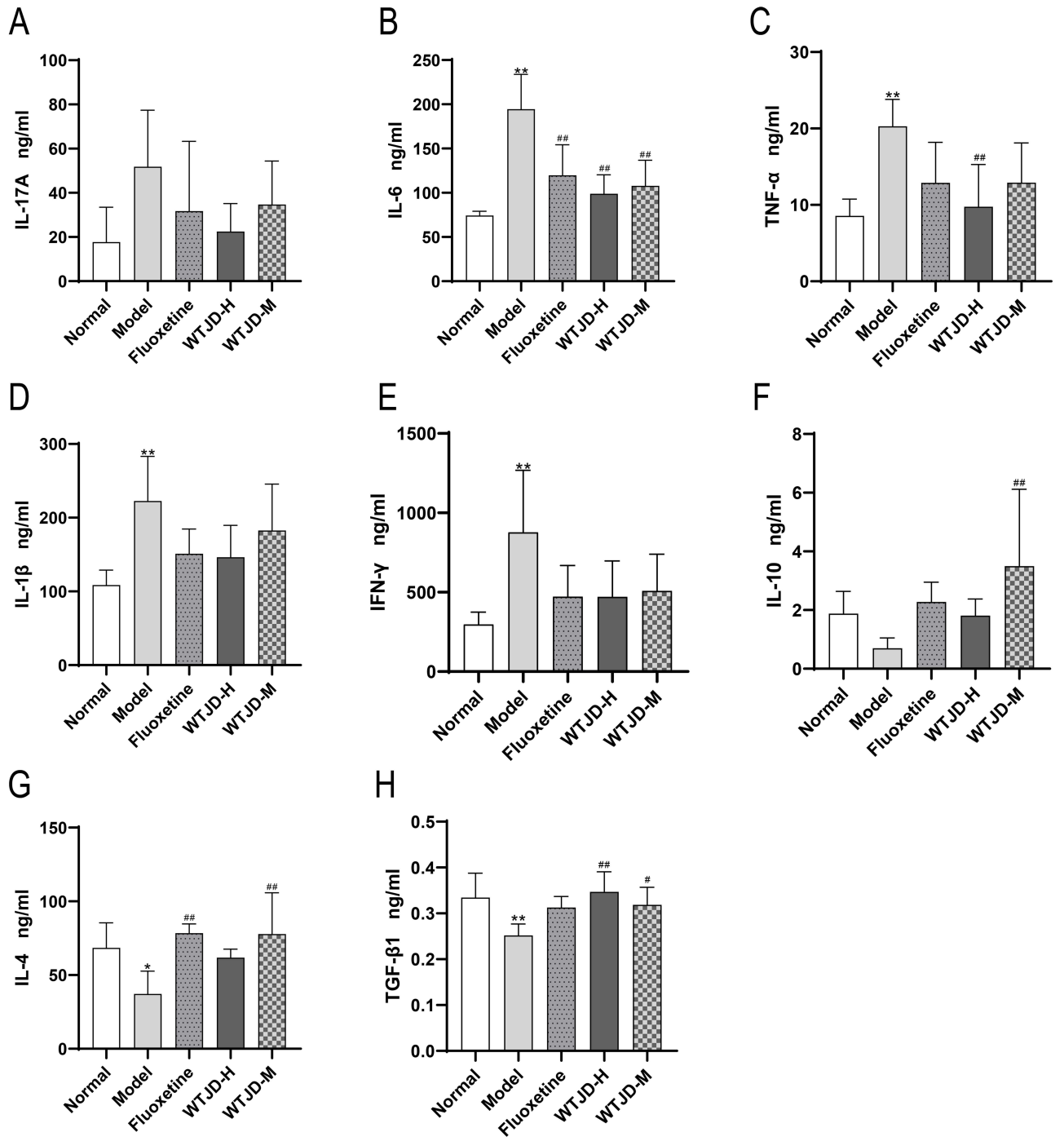

Figure 7 WTJD regulates inflammatory factor levels in peripheral blood in KYD-type depressed rats. (**A**–**H**) Levels of IL-17A, IL-6, TNF-α, IL-1β, IFN-γ, IL-10, IL-4, TGF-β1 in rat peripheral blood. The data is represented by mean ± SD, n = 6. Compared to the Normal group, $^{**}p < 0.01$, $^{*}p < 0.05$. Compared with the Model group, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$.

Th1/2/17 and Treg cells are recognized as significant subsets of CD4+ T cells within the human immune system, preserving immunological homeostasis.[36] Under normal circumstances, Th1/2/17 and Treg cells are closely related in differentiation, mutually restricting each other in function, and are in dynamic equilibrium. Numerous studies have confirmed the decrease in Treg cells, Th1/Th2 cell ratios, and increased Th17 cells in patients with depression.[37,38] Among them, Th17 cell changes have the strongest association with depression.[39] The balance of Th17/Treg cells and Th1/Th2 cells is regulated by various factors, including TGF-β and IL-1β/6, which can induce Th17 cells,[36,40] while IL-

4/10 can promote Treg cell expansion.[41] Th17 cells are highly plastic, and under different cytokine microenvironments and inflammatory conditions, Th17 cells can change their phenotype and function to obtain Th1-, Th2-, and Treg-like phenotypic cells.[42,43] IL-17, a key cytokine of Th17, can promote the production of various inflammatory mediators, leading to chronic neurological inflammation and depression.[44] Unlike Th17 cells, Treg cells can express anti-inflammatory cytokines IL-10 and TGF-β, inhibit various immune cell activities and responses, and maintain immune tolerance.[45,46] IFN-γ and IL-4/10 are the main cytokines secreted by Th1/Th2 cells, respectively, and play an antagonistic role in jointly maintaining internal environmental homeostasis.[47] JAK2/ STAT3 is a key pathway that regulates Th17/ Treg cell immune homeostasis. Activated JAK families can mediate STAT3 phosphorylation, causing Th17/Treg cell imbalance and eventually causing inflammation and depression.[48,49] TLR4/TRAF6/NF-κB signaling pathway is one of the important pathways for regulating specific immune inflammation,[50] inhibiting this pathway activation reduces inflammatory cytokine levels, weakens microglial overactivation, reduces neuroinflammation, and improves Th1/Th2 cell immune imbalance, alleviating chronic stress-induced depression-like behavior.[51–53] Therefore, lowering pro-inflammatory factor levels, increasing anti-inflammatory factor levels, and maintaining Th17/Treg and Th1/Th2 cell homeostasis are feasible mechanisms for alleviating depression.

Herein, the KYD scores of rats in all groups decreased significantly after WTJD treatment, suggesting that WTJD can significantly improve KYD traits. In the forced swimming test, rats in each group that underwent CUMS and KYD modeling showed a significant extension of resting time and a significant reduction of swimming time, accompanied by different degrees of reduction in climbing time. After treatment, the swimming time of rats increased significantly, and the resting time decreased significantly. This showed that CUMS + KYD rats were less resistant to non-ideal environments and did not have enough energy to challenge. Meanwhile, fluoxetine and WTJD could improve the despair and fatigue of rats. The sugar water consumption of CUMS+KYD rats increased significantly after WTJD-H treatment, suggesting that WTJD-H can alleviate anhedonia caused by CUMS and KYD. In the open field trial, CUMS + KYD rats showed more "laziness" (reduced total distance and increased quiescent time), which was improved by WTJD and fluoxetine, but fluoxetine was superior.

Our study found that CUMS + KYD can activate JAK2 / STAT3 and TLR4 / TRAF6 / NF-κB signaling pathways, leading to the immune imbalance of Th17 / Treg and Th1 / Th2 cells in the hippocampus of rats, the increase of pro-inflammatory factors (such as IL-6/17A), the decrease of anti-inflammatory factors (IL-4/10) and the infiltration of inflammatory cells or the abnormal morphology of pyramidal cells, which eventually lead to depression-like behavior. The inflammatory state of hippocampal tissue was improved somewhat after fluoxetine and WTJD treatment, but WTJD was more prominent. Specifically, WTJD can regulate the pathological changes of hippocampal tissues, inhibit JAK2/ STAT3 and TLR4/TRAF6/NF-κB signaling, correct the immune imbalance of Th17/Treg and Th1/Th2 cells, exert anti-inflammatory and neuroprotective effects, and finally achieve the effect of alleviating KYD pattern and depression.

Currently, the first-line treatment of depression is selective serotonin reuptake inhibitors, which are widely used because they perform best in treating depression. However, after administration, they are prone to side effects on the digestive tract and central nervous system, and the dosage needs to be strictly controlled.[54] Since depression is a systemic disease with mixed mechanisms, designing a promising drug that passes through a single target is quite challenging. Multi-target therapy or combination therapy has gradually become the development trend in depression treatment. Encouragingly, TCM with multi-component, multi-target, and multi-pathway characteristics can treat depression.[55,56] While improving depressed mood, anxiety, sleep disorders, and somatic symptoms, TCM has the advantages of safety, non-toxic side effects, and non-addiction.[57] According to the Chinese Pharmacopoeia (China, 2020), we recognize that the key ingredients of WTJD include morroniside, loganin, curcumin and nystose. Morroniside can inhibit pro-inflammatory factor levels, increase PGC-1α expression, and improve rat neurological function.[58,59] Loganin can inhibit microglial activation and reduce inflammatory factor release by inhibiting the NF-κB signaling pathway.[60] Curcumin can reduce oxidative stress and inflammatory response, regulate TLR-4/NF-κB pathway protein expression, and correct Th1/ Th2 cell homeostatic imbalance.[61,62] Nystose may be effective in improving ulcerative colitis, a high-risk disorder for depression, by maintaining the epithelial barrier and reducing the secretion of inflammation-associated cytokines.[63,64] Simultaneously, the anti-inflammatory and antidepressant effects of morroniside and loganin, which are the main components of WTJD formulations, have been confirmed in animal and clinical trials.[65–67] Curcumin can regulate

changes in serum corticosterone levels in rats caused by chronic stress, stimulate neurocentral neurotransmitter expression, and exert good antidepressant effects in depressed animals and patients.[68,69] The extract of Bajitian, inulin oligosaccharide, has antidepressant effects and has been recommended by the Neurology Professional Committee of the Chinese Association of Integrative Medicine as a treatment option for patients with depression and KYD.[70] These findings suggest that WTJD is a promising multi-target, multi-pathway treatment option for KYD type of depression. The results of this study preliminarily clarified the efficacy and mechanism of WTJD on KYD type of depression rats, and provided a data basis for the future research and development of WTJD and its entry into clinical trials. WTJD may offer a safe and cheap treatment option for patients with KYD type of depression or depression. However, convincing evidence requires long-term and in-depth research.

TCM theory believes that the brain is the sea of marrow; the kidney stores essence, governs the bones, and generates marrow. Only when the kidney essence is abundant and constantly replenishes the marrow sea in the brain can the spirit be nourished so that people are refreshed and their psychological functions are normal. The kidney yang is the main driving force for maintaining human mental activity and life movement, which is born from the kidney essence. Hence the manifestation of the kidney essence deficiency pattern often accompanies the KYD pattern. Warming and tonifying the kidney yang while replenishing the essence can achieve twice the effect with half the effort. Based on long-term clinical experience, our team concluded that the incidence of depression is not only related to one zang-fu or one Pathogenesis but a process of multi-zang-fu dysfunction with "essence deficiency-yang deficiency- qi stagnation" as the core. In clinical diagnosis and treatment of depression, it is necessary to grasp its deep-seated pathological mechanism, and the three methods of tonify essence, warming yang, and regulating qi should run through the whole process so as to achieve a good curative effect. Shanzhuyu in WTJD nourishes kidney essence; it can be converted into kidney yang, and the body and spirit are in harmony without depression. Bajitian tonifies kidney yang, which can promote the free flow of qi and mood and the transformation of kidney essence into kidney yang. When the kidney essence is full, the patient's originally negative psychosomatic activity will be stimulated; Yujin can circulate blood and resolve stagnation while regulating qi, which makes the Shanzhuyu and Bajitian play a tonic effect without stagnation. Because the Bajitian and Shanzhuyu are interior-warming medicines, Yujin, as a counteracting assistant medicine, can warm the whole decoction but not dry it. WTJD regulates the physiology and psychology of patients with KYD type of depression and has the effects of tonifying yang and essence, calming the mind, relieving depression and anti-fatigue, and achieving the purpose of treating both the tip and root. The whole decoction has remarkable efficacy, and the medicinal is simple and cheaply refined.

There are still some things that could be improved in our research. (1) The molecular mechanism of WTJD in improving KYD-type depression was partially revealed. However, the ingredients of WTJD have yet to be detected by appropriate methods, and the interaction mechanism between ingredients and the therapeutic mechanism of each ingredient needs to be further studied. (2) Some inflammatory factors have both pro-inflammatory and anti-inflammatory effects, such as IL-6 and TGF-β1. Our experiment cannot determine which effect WTJD treatment specifically causes them to produce. Therefore, more multi-angle and in-depth study of the mechanism of WTJD on KYD type of depression is needed. (3) In this study, rats were selected as the research object, and whether the results can be extended to humans requires broader and deeper verification. (4) This study mainly focuses on the animal model of depression with kidney-yang deficiency, and other types of depression may not apply to the conclusions and findings of this study. (5) Although there were no obvious adverse reactions and deaths in rats within the dose range of this study, we failed to conduct a safety study of WTJD. (6) This study focused on the effect of WTJD on the immune and inflammatory levels of the hippocampus but did not observe the effect of WTJD on important participants in brain immunity, such as the blood-brain barrier and other immune cells, we will continue to study in this direction in the future.

Conclusion

This study found that there was T cell immune homeostasis imbalance and chronic inflammation in the hippocampus of KYD-type depression rats. WTJD can restore hippocampal immune balance, alleviate chronic inflammation, and improve KYD and depressive symptoms in rats to some extent.

Abbreviation

WTJD, Wenyang-tianjing-jieyu decoction; KYD, Kidney yang deficiency; CUMS, Chronic unpredictable mild stimulus; JAK2, Janus kinase 2; STAT3, Signal transducer and activator of transcription 3; TLR4, Toll-like receptor 4; TRAF6,

Tumor necrosis factor receptor-associated factor 6; NF-κB, Nuclear transcription factor-κB; TCM, Traditional Chinese Medicine; RORγt, Retinoic acid receptor-related orphan receptor γ-t; FOXP3, Forkhead box protein P3; T-bet, T-box 21 transcription factor; GATA3, GATA binding protein 3; IL-, Interleukin-; TNF-α, Tumor necrosis factor-α; IFN-γ, Interferon-γ; TGF-β1, Transforming growth factor-β1.

Data Sharing Statement

The data that support the findings of this study are available upon request from the corresponding author, upon reasonable request.

Credit Authorship Contribution Statement

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.

References

1. Malhi GS, Mann JJ. Depression. *Lancet*. 2018;392(10161):2299–2312. doi:10.1016/S0140-6736(18)31948-2
2. Depressive disorder (depression). Secondary depressive disorder (depression); 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/depression. Accessed March 14, 2024.
3. Cambridge University Press. *Mental Disorders Around the World: Facts and Figures from the WHO World Mental Health Surveys*. Cambridge: Cambridge University Press; 2018.
4. Wei ZX, Xie GJ, Mao X, et al. Exosomes from patients with major depression cause depressive-like behaviors in mice with involvement of miR-139-5p-regulated neurogenesis. *Neuropsychopharmacology*. 2020;45(6):1050–1058. doi:10.1038/s41386-020-0622-2
5. Cuijpers P, Karyotaki E, de Wit L, Ebert DD. The effects of fifteen evidence-supported therapies for adult depression: a meta-analytic review. *Psychother Res*. 2020;30(3):279–293. doi:10.1080/10503307.2019.1649732
6. Herrman H, Patel V, Kieling C, et al. Time for united action on depression: a Lancet-World Psychiatric Association Commission. *Lancet*. 2022;399(10328):957–1022. doi:10.1016/S0140-6736(21)02141-3
7. Li H, Song L, Cen M, et al. Oxidative balance scores and depressive symptoms: mediating effects of oxidative stress and inflammatory factors. *J Affect Disord*. 2023;334:205–212. doi:10.1016/j.jad.2023.04.134
8. Moriarity DP, Grehl MM, Walsh RFL, Roos LG, Slavich GM, Alloy LB. A systematic review of associations between emotion regulation characteristics and inflammation. *Neurosci Biobehav Rev*. 2023;150:105162. doi:10.1016/j.neubiorev.2023.105162
9. Du Y, Wang YL, Chen L, Li QE, Cheng Y. Anti-depressant-like effects of rannasangpei and its active ingredient crocin-1 on chronic unpredictable mild stress mice. *Front Pharmacol*. 2023;14:1143286. doi:10.3389/fphar.2023.1143286
10. Min J, Jinfang D, Qingkun S, Xin W. Investigation on TCM constitution of Beijing high-speed rail crew and its correlation with depression and anxiety. *J Beijing Univ Tradit Chin Med*. 2018;41(03):248–252.
11. Ganlu L. *TCM Constitution Determination and Syndrome Observation of Patients with Somatic Symptoms as the Main Complaint of Anxiety or Depression Before and After Treatment* [Master]. Beijing University of Traditional Chinese Medicine; 2019.
12. Study on personality psychological characteristics of TCM constitution types based on 505 college students. The 8th Chinese Medicine Constitution Symposium of China Association of Chinese Medicine and Chinese Medicine Health Status Cognition and Constitution Identification Research Forum; 2010; Qingdao, Shandong, China.
13. Shilin Y, Feng G, Bin W, et al. Study on semi-quantitative operation standard of kidney yang deficiency syndrome. *Mod J Integr Traditional Chinese and Western Med*. 2004;06:701–702.
14. Han L, Huang X, Cai H, et al. Mitochondrial dysfunction in a rat model and the related risk of metabolic disorders. *J Tradit Chin Med*. 2023;43(1):95–104. doi:10.19852/j.cnki.jtcm.20221017.001

15. Huang X, Cai H, Li H, et al. Cinnamon as dietary supplement caused hyperlipidemia in healthy rats. *Evid Based Complement Alternat Med*. 2021;2021:9892088. doi:10.1155/2021/9892088
16. Overstreet DH, Friedman E, Mathé AA, Yadid G. The flinders sensitive line rat: a selectively bred putative animal model of depression. *Neurosci Biobehav Rev*. 2005;29(4–5):739–759. doi:10.1016/j.neubiorev.2005.03.015
17. Shuang O, Han L, Haiyang C, Jing W, Zhaohui T, Weihong L. Sequencing and analysis of whole genome DNA methylation of liver cells in rats with kidney-yang deficiency constitution. *J Traditional Chin Med*. 2022;63(09):875–881.
18. Wang Y, Wang X, Wang K, et al. Chronic stress accelerates glioblastoma progression via DRD2/ERK/β-catenin axis and dopamine/ERK/TH positive feedback loop. *J Exp Clin Cancer Res*. 2023;42(1):161. doi:10.1186/s13046-023-02728-8
19. Dang H, Chen Y, Liu X, et al. Antidepressant effects of ginseng total saponins in the forced swimming test and chronic mild stress models of depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009;33(8):1417–1424. doi:10.1016/j.pnpbp.2009.07.020
20. Ning J, Yiwen Z, Rainbow Y, red Y, Xinmin L. Overview of experimental methods of depressive behavior in rats and mice. *Acta Lab Animalis Sci Sin*. 2021;29(06):830–838.
21. Goldsmith DR, Bekhbat M, Mehta ND, Felger JC. Inflammation-related functional and structural dysconnectivity as a pathway to psychopathology. *Biol Psychiatry*. 2023;93(5):405–418. doi:10.1016/j.biopsych.2022.11.003
22. Jia X, Gao Z, Hu H. Microglia in depression: current perspectives. *Sci China Life Sci*. 2021;64(6):911–925. doi:10.1007/s11427-020-1815-6
23. Wiebenga JXM, Heering HD, Eikelenboom M, van Hemert AM, van Oppen P, Penninx B. Associations of three major physiological stress systems with suicidal ideation and suicide attempts in patients with a depressive and/or anxiety disorder. *Brain Behav Immun*. 2022;102:195–205. doi:10.1016/j.bbi.2022.02.021
24. Li B, Yang W, Ge T, Wang Y, Cui R. Stress induced microglial activation contributes to depression. *Pharmacol Res*. 2022;179:106145. doi:10.1016/j.phrs.2022.106145
25. Zipp F, Bittner S, Schafer DP. Cytokines as emerging regulators of central nervous system synapses. *Immunity*. 2023;56(5):914–925. doi:10.1016/j.immuni.2023.04.011
26. Qiu J, Fang Q, Xu T, et al. Mechanistic role of reactive oxygen species and therapeutic potential of antioxidants in denervation- or fasting-induced skeletal muscle atrophy. *Front Physiol*. 2018;9:215. doi:10.3389/fphys.2018.00215
27. Oliet SH, Malenka RC, Nicoll RA. Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. *Neuron*. 1997;18(6):969–982. doi:10.1016/S0896-6273(00)80336-0
28. Roddy DW, Farrell C, Doolin K, et al. The Hippocampus in depression: more than the sum of its parts? Advanced hippocampal substructure segmentation in depression. *Biol Psychiatry*. 2019;85(6):487–497. doi:10.1016/j.biopsych.2018.08.021
29. Treadway MT, Waskom ML, Dillon DG, et al. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. *Biol Psychiatry*. 2015;77(3):285–294. doi:10.1016/j.biopsych.2014.06.018
30. Ciufolini S, Dazzan P, Kempton MJ, Pariante C, Mondelli V. HPA axis response to social stress is attenuated in schizophrenia but normal in depression: evidence from a meta-analysis of existing studies. *Neurosci Biobehav Rev*. 2014;47:359–368. doi:10.1016/j.neubiorev.2014.09.004
31. Borsini A, Giacobbe J, Mandal G, Boldrini M. Acute and long-term effects of adolescence stress exposure on rodent adult hippocampal neurogenesis, cognition, and behaviour. *Mol Psychiatry*. 2023;28(10):4124–4137. doi:10.1038/s41380-023-02229-2
32. Chen X, Cui QQ, Hu XH, et al. CD200 in dentate gyrus improves depressive-like behaviors of mice through enhancing hippocampal neurogenesis via alleviation of microglia hyperactivation. *J Neuroinflammation*. 2023;20(1):157. doi:10.1186/s12974-023-02836-4
33. Brás JP, Guillot de Suduiraut I, Zanoletti O, et al. Stress-induced depressive-like behavior in male rats is associated with microglial activation and inflammation dysregulation in the hippocampus in adulthood. *Brain Behav Immun*. 2022;99:397–408. doi:10.1016/j.bbi.2021.10.018
34. Zhang X, He T, Wu Z, et al. The role of CD38 in inflammation-induced depression-like behavior and the antidepressant effect of (R)-ketamine. *Brain Behav Immun*. 2024;115:64–79. doi:10.1016/j.bbi.2023.09.026
35. Song Q, Fan C, Wang P, Li Y, Yang M, Yu SY. Hippocampal CA1 βCaMKII mediates neuroinflammatory responses via COX-2/PGE2 signaling pathways in depression. *J Neuroinflammation*. 2018;15(1):338. doi:10.1186/s12974-018-1377-0
36. Lee GR. The balance of Th17 versus treg cells in autoimmunity. *Int J Mol Sci*. 2018;19(3). doi:10.3390/ijms19030730
37. Schiweck C, Valles-Colomer M, Arolt V, et al. Depression and suicidality: a link to premature T helper cell aging and increased Th17 cells. *Brain Behav Immun*. 2020;87:603–609. doi:10.1016/j.bbi.2020.02.005
38. Grosse L, Hoogenboezem T, Ambrée O, et al. Deficiencies of the T and natural killer cell system in major depressive disorder: t regulatory cell defects are associated with inflammatory monocyte activation. *Brain Behav Immun*. 2016;54:38–44. doi:10.1016/j.bbi.2015.12.003
39. Beurel E, Medina-Rodriguez EM, Jope RS, Dantzer R. Targeting the adaptive immune system in depression: focus on T helper 17 cells. *Pharmacol Rev*. 2022;74(2):373–386. doi:10.1124/pharmrev.120.000256
40. Yang L, Anderson DE, Baecher-Allan C, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature*. 2008;454(7202):350–352. doi:10.1038/nature07021
41. Tran GT, Hodgkinson SJ, Carter NM, et al. IL-5 promotes induction of antigen-specific CD4+CD25+ T regulatory cells that suppress autoimmunity. *Blood*. 2012;119(19):4441–4450. doi:10.1182/blood-2011-12-396101
42. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014;13(6):668–677. doi:10.1016/j.autrev.2013.12.004
43. Komatsu N, Okamoto K, Sawa S, et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nat Med*. 2014;20(1):62–68. doi:10.1038/nm.3432
44. Ghosh R, Mitra P, Kumar P, Goyal T, Sharma P. T helper cells in depression: central role of Th17 cells. *Crit Rev Clin Lab Sci*. 2022;59(1):19–39. doi:10.1080/10408363.2021.1965535
45. Brockmann L, Soukou S, Steglich B, et al. Molecular and functional heterogeneity of IL-10-producing CD4(+) T cells. *Nat Commun*. 2018;9(1):5457. doi:10.1038/s41467-018-07581-4
46. Choi G, Park YJ, Cho M, et al. A critical role for Th17 cell-derived TGF-β1 in regulating the stability and pathogenicity of autoimmune Th17 cells. *Exp Mol Med*. 2021;53(5):993–1004. doi:10.1038/s12276-021-00632-9
47. Foley ÉM, Parkinson JT, Mitchell RE, Turner L, Khandaker GM. Peripheral blood cellular immunophenotype in depression: a systematic review and meta-analysis. *Mol Psychiatry*. 2023;28(3):1004–1019. doi:10.1038/s41380-022-01919-7

48. Yang J, Li H, Hao Z, et al. Mitigation effects of selenium nanoparticles on depression-like behavior induced by fluoride in mice via the JAK2-STAT3 pathway. *ACS Appl Mater Interfaces*. 2022;14(3):3685–3700. doi:10.1021/acsami.1c18417
49. Chen Y, Zhang Y, Wang J, et al. Anti-neuroinflammation effects of transcutaneous auricular vagus nerve stimulation against depression-like behaviors via hypothalamic α7nAchR / JAK2 / STAT3 / NF-κB pathway in rats exposed to chronic unpredictable mild stress. *CNS Neurosci Ther*. 2023;29(9):2634–2644. doi:10.1111/cns.14207
50. Yang Y, Tan X, Xu J, et al. Luteolin alleviates neuroinflammation via downregulating the TLR4/TRAF6/NF-κB pathway after intracerebral hemorrhage. *Biomed Pharmacother*. 2020;126:110044. doi:10.1016/j.biopha.2020.110044
51. Xu X, Lu YN, Cheng JH, et al. Ginsenoside Rh2 reduces depression in offspring of mice with maternal toxoplasma infection during pregnancy by inhibiting microglial activation via the HMGB1/TLR4/NF-κB signaling pathway. *J Ginseng Res*. 2022;46(1):62–70. doi:10.1016/j.jgr.2021.04.003
52. Wang S, Zhang X, Zhai L, et al. Atorvastatin attenuates cognitive deficits and neuroinflammation induced by Aβ(1–42) Involving modulation of TLR4/TRAF6/NF-κB pathway. *J Mol Neurosci*. 2018;64(3):363–373. doi:10.1007/s12031-018-1032-3
53. Medina-Rodriguez EM, Rice KC, Beurel E, Jope RS. (+)-naloxone blocks toll-like receptor 4 to ameliorate deleterious effects of stress on male mouse behaviors. *Brain Behav Immun*. 2020;90:226–234. doi:10.1016/j.bbi.2020.08.022
54. Bousman CA, Stevenson JM, Ramsey LB, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A genotypes and serotonin reuptake inhibitor antidepressants. *Clin Pharmacol Ther*. 2023;114(1):51–68. doi:10.1002/cpt.2903
55. Su P, Wu M, Yin X, et al. Modified Xiaoyao San reverses lipopolysaccharide-induced depression-like behavior through suppressing microglia M1 polarization via enhancing autophagy involved in PI3K/Akt/mTOR pathway in mice. *J Ethnopharmacol*. 2023;315:116659. doi:10.1016/j.jep.2023.116659
56. Cao H, Yang D, Nie K, et al. Hesperidin may improve depressive symptoms by binding NLRP3 and influencing the pyroptosis pathway in a rat model. *Eur J Pharmacol*. 2023;952:175670. doi:10.1016/j.ejphar.2023.175670
57. Zheng JY, Li XX, Lin WY, et al. Huang-Lian-Jie-Du decoction alleviates depressive-like behaviors in dextran sulfate sodium-induced colitis mice via Trem2/Dap12 pathway. *J Ethnopharmacol*. 2023;315:116658. doi:10.1016/j.jep.2023.116658
58. Zhijun Y, Xiaoying H, Ping Y, Xiaomei Z, Xiaogang L. Morroniside improves neurological function in rats with cerebral hemorrhage by inhibiting inflammatory response. *Chin J Tissue Eng Res*. 2018;22(08):1217–1222.
59. Xia L, Tiejun Z, Yali F. The protective effect of morroniside on ischemia-reperfusion injury in diabetic rats through AMPKα / PGC-1α / GLUT4 pathway. *Chin J Immunol*. 2018;34(07):990–995.
60. Saini Y, Wenjiao Z, Liangyuan L, et al. Inhibitory effect of loganin on microglial activation. *Shandong Sci*. 2021;34(05):34–41.
61. Siyun C, Jun O, Chenqian B, et al. Effects of curcumin pretreatment on renal function and expression of TLR-4 / NF-κB pathway protein in renal tissue of mice with cisplatin-induced acute kidney injury. *J Zhengzhou Univ*. 2022;57(05):694–698.
62. Gao S, Zhang W, Zhao Q, et al. Curcumin ameliorates atherosclerosis in apolipoprotein E deficient asthmatic mice by regulating the balance of Th2/Treg cells. *Phytomedicine*. 2019;52:129–135. doi:10.1016/j.phymed.2018.09.194
63. Kang S, You HJ, Ju Y, et al. Butyl-fructooligosaccharides modulate gut microbiota in healthy mice and ameliorate ulcerative colitis in a DSS-induced model. *Food Funct*. 2022;13(4):1834–1845. doi:10.1039/D1FO03337A
64. Blackwell J, Saxena S, Petersen I, et al. Depression in individuals who subsequently develop inflammatory bowel disease: a population-based nested case-control study. *Gut*. 2021;70(9):1642–1648. doi:10.1136/gutjnl-2020-322308
65. Xiaoming W. Effects of morroniside on depression-like behavior and neuroinflammation in depression model rats. *Clin J Chinese Med*. 2020;12(34):19–21.
66. Kou Y, Li Z, Yang T, et al. Therapeutic potential of plant iridoids in depression: a review. *Pharm Biol*. 2022;60(1):2167–2181. doi:10.1080/13880209.2022.2136206
67. Guo YX, Xia CY, Yan Y, et al. Loganin improves chronic unpredictable mild stress-induced depressive-like behaviors and neurochemical dysfunction. *J Ethnopharmacol*. 2023;308:116288. doi:10.1016/j.jep.2023.116288
68. Zhang Y, Li L, Zhang J. Curcumin in antidepressant treatments: an overview of potential mechanisms, pre-clinical/clinical trials and ongoing challenges. *Basic Clin Pharmacol Toxicol*. 2020;127(4):243–253. doi:10.1111/bcpt.13455
69. Liao D, Lv C, Cao L, et al. Curcumin attenuates chronic unpredictable mild stress-induced depressive-like behaviors via restoring changes in oxidative stress and the activation of Nrf2 signaling pathway in rats. *Oxid Med Cell Longev*. 2020;2020:9268083. doi:10.1155/2020/9268083
70. Weifeng G, Xiaolan C, Lei S, Jianxiang L, Lankun Z, Yunzhi M. Expert consensus on integrated traditional Chinese and western medicine diagnosis and treatment of depression. *Chin J Integr Tradit West Med*. 2020;40(02):141–148.