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Effects of arabinoxylan on BDNF/TrkB/p-CREB signaling pathway in the prefrontal cortex and intestinal microbiome in post-stroke depressed rats

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Abstract

Aim To explore the effects of arabinoxylan on the BDNF/TrkB/p-CREB signaling pathway in the prefrontal cortex of post-stroke depressed rats, and to explore its neuronal protective effects through the microbial-gut-brain axis in the regulation of this pathway.

Methods The rat model of post-stroke depression (PSD) was established by middle cerebral artery occlusion (MCAO) combined with chronic unpredictable mild stimulation (CUMS). They were randomly divided into 5 groups (blank control, post-stroke depression, arabinoxylan, fluoxetine hydrochloride, fluoxetine hydrochloride combined arabinoxylan). The rats were treated differently for 28 days according to their grouping. Body mass, sugar and water consumption experiments and open-field experiments were used to evaluate the behavior of rats. The pathological changes were observed by H&E staining. The expression levels of amine neurotransmitters were detected by ELISA. The expression levels of BDNF mRNA and BDNF, TrkB and p-CREB were detected by RT-PCR and Western blot. The analysis of intestinal metagenomics was conducted by 16 S rDNA sequencing.

Results Compared with the post-stroke depression group, the body weight, activity and sugar water consumption rate of the arabinoxylan group were increased. The expression levels of 5-HT in the prefrontal cortex, colon and serum levels of 5-HT, DA and NE were increased. The expression levels of BDNF mRNA and BDNF, TrkB and P-CREB in the prefrontal cortex were also upregulated. The number of neurons in the prefrontal cortex increased; Colon mucosal injury and inflammatory cell infiltration decreased, the intestinal microbial diversity increased; The relative abundance of probiotics such as bifidobacterium, Christensenia, Dubosiella New York and ruminococcus increased. The relative abundance of Prevotella NK3B31 group was reduced. The level of 5-HT in the prefrontal cortex was negatively correlated with the abundance of Prevotellaceae NK3B31 group.

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Conclusion Arabinoxylan improved depressive-like behavior in rats and its neuroprotective role was achieved by promoting the growth of intestinal probiotics, improving the intestinal barrier, affecting the BDNF/TrkB/p-CREB signaling pathway, and increasing the expression levels of monoamine neurotransmitters 5-HT, DA and NE.

Keywords Post-stroke depression, Arabinoxylan, BDNF/TrkB/p-CREB signaling pathway, Gut microbes, Monoamine neurotransmitters, Behavior

Introduction

Post-stroke depression (PSD), as a common complication of affective disorders, has attracted the attention of the medical community in recent years due to its increasing incidence [1]. The main features of this disorder are depressed mood, loss of interest, and in severe cases, suicidal thoughts and behaviors [2]. The pathogenesis of PSD involves multiple and complex factors, such as neuroendocrine dysfunction, inflammation, alterations in neurotransmitters and neuroplasticity in the hippocampus and prefrontal cortex, among which the neurobiological and chemical transmitter theory and the microbial-gut-brain axis hypothesis have been gradually accepted [3]. The frontal lobe plays an important role in the pathogenesis of PSD, and it has been found that microstructural damage to the white matter of the frontal lobe may be associated with PSD [4]. As for monoamine neurotransmitters such as 5-hydroxytryptamine (5-HT), dopamine (DA) and denorepinephrine (NE), they transmit excitatory signals to the thalamus, basal ganglia, and frontal cortex through neuronal axons, and damage to these neural pathways leads to a decrease in the expression of neurotransmitters, which triggers symptoms of depression [5]. A recent study has found a reduction in brain-derived neurotrophic factor (BDNF) levels in PSD patients, which affects disease progression and prognosis [6].

It has been shown that the BDNF/TrkB/p-CREB signaling pathway is an important key pathway for neuronal cell regeneration, which is closely related to the pathogenesis of PSD [7]. BDNF plays a key role in the maturation of the nervous system and the maintenance of neuronal activity [8], its production is regulated by cAMP response element-binding (CREB) signaling, and the binding of the factor tyrosine kinase receptor (TrkB) initiates the BDNF/TrkB signaling, which in turn promotes the phosphorylation of CREB to form p-CREB, a process that enhances cellular antioxidant defenses and inhibits neuronal apoptosis, demonstrating its positive effects on antidepressants [9–11].

Recent studies have shown a strong correlation between the diversity and composition of the gut microbiota and PSD [12]. Rapid advances in gut macrogenomics have prompted more scholars to explore the microbial-gut-brain axis and neurological disease associations, revealing that gut microecology influences the course of PSD through multiple pathways such as metabolic, immune, endocrine and neuroendocrine mechanisms, neural transmission, neurogenesis, myelination, and axon formation [13]. Currently, 5-HT reuptake inhibitors such as fluoxetine are the mainstream drugs in depression treatment, but the side effects of long-term use are significant [14]. Probiotic supplementation has been documented to be beneficial in alleviating depressive symptoms [15]. Our previous research has reported that inulin as a probiotic, could alter gut microbiota to alleviate PSD-like behavior associated with the IGF-1-mediated BDNF/p-CREB signaling pathway [16]. Arabinoxylan, a naturally occurring intestinal microbial modulator, has been touted as a novel prebiotic with the ability to modulate the structure of the intestinal microbial community [17]. Previous work by our group has revealed that arabinoxylan in combination with medoxomil improves behavioral performance, gut microbial composition, and nigrostriatal dopaminergic neuron function in rats modeled with Parkinson's disease via the microbe-gut-brain axis pathway [18, 19]. However, the use of arabinoxylan in the treatment of PSD has not been explored.

This study aimed to verify the hypothesis that arabinoxylan exerts a neuronal protective effect by affecting the BDNF/TrkB/p-CREB signaling pathway in the prefrontal cortex through modulation of the gut microbiota, thereby alleviating the symptoms of PSD, to provide empirical support for the in-depth dissection of the antidepressant mechanism of arabinoxylan, and to provide a theoretical basis for a new strategy for PSD treatment based on prebiotics.

Materials and methods

Laboratory animals

A total of 30 7–8 weeks healthy SPF-grade SD male rats, weighing 180–200 g were purchased from Charles River Co. Ltd, Animal Laboratory Facility Certificate of Conformity No.: SYXK Gui 2022-0004, Quality Certificate of Conformity No.: 44,829,700,014,301. This study was approved by the Ethics Committee of the Science and Technology Department of the Youjiang Medical University for Nationalities (approval number: 2023060902). The experimental procedures in this study were conducted in strict compliance with the ethical requirements for laboratory animals and the relevant provisions of the Regulations of the People's Republic of China on the Management of Laboratory Animals, and humane care was provided in accordance with the principles of

the 3Rs (Replacement, Reduction, Refinement) for laboratory animals. The environment in which the rats were housed was strictly controlled. The rats were kept under (24 ± 2)℃, light time from 8:00 to 20:00, relative humidity of (50 ± 5)%. Feed and water were supplied after sterilization and the rats were fed equal proportions of feed and water according to their body weights to ensure the same food intake. The rats were acclimatized to the environment and reared in separate cages in the SPF-grade animal quarantine room, and then transferred to the SPF-grade laboratory after at least 1 week of rearing to confirm that they did not carry disease-causing microorganisms, and rats with a body mass of 240–260 g were selected for modeling [16].

PSD modeling and scoring of neuromotor function

Middle cerebral artery occlusion (MCAO) combined with chronic unpredictable mild stimulation (CUMS) was used for rat modeling [20]. According to our previous research [16], the rats were anesthetized with 5% isoflurane and fixed. The surgical area was shaved and sterilized. According to our previous research, a midline incision was made in the neck to separate the external carotid artery, internal carotid artery, and common carotid artery. The external carotid artery and the proximal end of the common carotid artery were ligated. A thread with a round head was inserted from the common carotid artery into the internal carotid artery and slowly advanced to the starting point of the occluded middle cerebral artery, at a depth of 20 ± 2 mm from the bifurcation of the common carotid artery. The internal arotid artery was ligated, and the thread was secured. Excess suture thread was removed after ligation, and the wound was sutured.

After surgery, the rats were transferred to a new cage equipped with fresh bedding, and a heater was used to maintain the ambient temperature at about 37 ℃, and the rats were allowed to eat and drink freely. Two hours after the operation, the rats were evaluated for neurological function and the plugs were removed. The evaluation criteria were set as follows: a score of 0 indicated no signs of neurological damage and free movement of the limbs; a score of 1 meant that the left front paw could not be fully extended, which was a slight neurological damage; a score of 2 meant that the rat rotated to the left side when walking, which was a moderate damage; a score of 3 indicated that the rat tilted to the left side, which was a severe damage; and a score of 4 indicated that the rat was unable to move on its own, had loss of consciousness, or was dead. Rats with scores between 1 and 3 were confirmed as successfully established the cerebral ischemia model, and after seven days of continued observation, the surviving individuals were selected and assigned to each group of the PSD model according to the random number method. And rats with scores of 0, 4 or postoperative death were not included in the model group.

SD rats with scores of 0 and 4 were executed by cervical dislocation under deep anesthesia to make the treatment consistent with euthanasia. The detailed steps of euthanasia were as follows: rats with unsatisfactory scores were re-positioned one by one in the induction box of the anesthesia equipment, and deep anesthesia was administered to the rats with a mixture of 2.0–2.5% isoflurane and oxygen for 5–10 min. After confirming that the rats were in a state of deep anesthesia, the rats were placed on the operating table, the root of the rat's tail was grasped with the right hand and lifted up, and the left thumb and forefinger were pressed downward forcefully on the head and neck of the rats, and the right hand grasped the root of the rat's tail and pulled upward forcefully toward the back, resulting in the cervical vertebrae being dislocated and the spinal cord was severed from the brainstem, and the experimental animals died immediately. The modeling was repeated with new rats to ensure that the number of surviving rats met the research needs of each group.

After the model was successfully constructed, in order to minimize the impact of other factors, individual caging and imposition of CUMS were implemented in all other groups to establish the PSD rat model as compared to the conventional group housing treatment in the blank control group. This stress protocol covered the following seven measures for 21 days, one of which was randomly selected to be implemented daily: (1) food deprivation for 24 h; (2) water source restriction for 24 h; (3) maintenance of cage tilt at a 45-degree angle for 24 h; (4) restriction of activity by means of a restraining tube for 6 h; (5) reversal of circadian rhythms for 24 h; (6) mild tail pinching for 1 min; and (7) use of moist bedding to cover the bottom of the cages for 24 h. The cages were then subjected to the CUMS protocol for 21 days.

Neurological function was scored and the wire bolus was removed 2 h after the rats were awake according to the Longa scoring scale [21]. The rats with unsatisfactory scores were executed by cervical dislocation under deep anesthesia, and the processing steps were in accordance with the requirements for euthanasia of experimental animals (Supplementary Fig. 1).

Grouping and drug administration

A total of 30 rats with body mass of 240–260 g were selected, sorted according to body mass, and then divided into blank control group and MCAO surgery group according to the random number table. The blank control group was given a normal food and water supply without surgical modeling treatment. The MCAO surgery group was firstly subjected to MCAO modeling, and the surviving rats after 7 days were selected for inclusion in the experiment, and then divided into the blank

control group (CON), the post-stroke depression group (PSD), the arabinogalactan group (AX), fluoxetine hydrochloride group (FLX) and fluoxetine hydrochloride combined with arabinoxylan group (FLX + AX) according to the random number table. Except for the CON group, in which 6 rats were housed in a combined cage, all other groups were housed in a single cage with one rat. A total of 30 rats died during MACO modeling. After 21 days of CUMS stress, the combined pharmacological interventions were started and administered for 28 days by gavage once a day, and the gavage dose was changed according to the change in body mass. The CON and PSD groups were gavaged with distilled water (10 mL/kg/d), the AX group with arabinogalactan (0.8 g/kg/d) (SEP20210506A01, Separate, China) and the FLX group with fluoxetine solution (2.33 mg/kg/d) (220404, Challage&young, China); FLA + AX group was given fluoxetine solution (2.33 mg/kg/d) combined with arabinogalactan (0.8 g/kg/d) by gavage.

Body mass monitoring and behavioral measurements

The body mass of the rats in each group was recorded before and after the start of the experimental modeling and before and after the drug intervention to analyze the changes in body mass.

The sucrose preference test (SPT) was performed 28 days after arabinoxylan ingestion. Rats were placed in different cages and exposed continuously for 12 h to two bottles, one containing sucrose water (1%) and one containing filtered water (water). Rats were deprived of water and food for 12 h before preference testing. During preference testing, rats were kept in individual cages and then had free access to 2 bottles containing sucrose solution (1% sucrose, 200 mL) and water (200 mL), respectively, for 12 h. The position of the two bottles was changed after 6 h to prevent possible effects of favoritism on drinking behavior. The consumption of sucrose solution and tap water was calculated by weighing the bottles. Sugar and water consumption rate values were calculated as sucrose intake (g)/total fluid intake (g)×100% [22].

The open field test (OFT) was performed 28 days after arabinoxylan intake. Rats were placed in an open field box (80 cm×80 cm×60 cm) and allowed to explore freely for 5 min, and the activity of the rats during the 5 min was recorded using an automatic video tracking system (Hancheng, China) [23].

Sequencing of 16s rDNA gene of rat intestinal microorganisms

The analysis of the gut microbiome was conducted using 16S DNA gene sequencing. Microbial genomic DNA was extracted using the magnetic bead method and its quality and quantity were verified. Polymerase chain reaction (PCR) was executed on MiniAmp thermal cycler (ABI, USA) to amplify V3 and V4 regions of the 16S rDNA gene: initial denaturation at 95 ℃ for 3 min, denaturation at 95 ℃ for 30 s, annealing at 55℃ for 30 s, extension at 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min. The PCR reaction mixture consisted of TransStart FastPfu (5×) buffer, dNTPs, forward and reverse primers, TransStart FastPfu DNA polymerase, template DNA, and distilled water. The primer sequences were: Forward 5'-CCT ACG GTG GCA GCAG- 3', Reverse 5'-GAC TAC ATG GGT ATC TAA TCC-3'. The PCR reaction was performed in triplicate [16]. After library construction was finished, high-throughput sequencing was executed using Miseq2000 High-throughput sequencing system (Illumina, USA) and the data was analyzed.

Enzyme-linked immunosorbent assay (ELISA)

Tissue samples were taken from rat hippocampus, homogenized with PBS and centrifuged at 5000*g* for 20 min. The supernatant was collected and the levels of neurotransmitters including 5-HT (E-EL-0033), NE (E-EL-0047), DA (E-EL-0046, Elabscience, China) were measured immediately using an ELISA kit; each sample (100 μL) was added to the ELISA plate in triplicate. A working curve was established using a reference standard, and biotinylated antibody, HRP coupler solution, substrate reagent and end solution were added sequentially according to the instructions. Neurotransmitter levels were measured by VICTOR Nivo multifunctional enzyme labeling instrument (Perkin Elmer, USA) at 450 nm.

Hematoxylin-eosin (H&E) staining

Fresh rat prefrontal cortex and colonic tissues were fixed using 4% paraformaldehyde in medium, and prefrontal cortex and colonic tissues from each group of rats were trimmed and dehydrated according to the pathological experimental testing procedures. The samples were embedded and sectioned using a paraffin slicer (ThermoFisher, USA). The samples were stained with hematoxylin (CR2211091) and eosin (CR2301051, Servicebio, China) and then sealed, and the final microscopic examination of qualified samples was carried out to observe the histopathological and morphological changes under the magnification of 200X light microscope. Morphological features of pyramidal cells, nuclei, and neurons were observed and recorded in prefrontal cortical tissue, and layers of mucosal tissue, nucleus structure, and morphological features of inflammatory cells were observed and recorded.

Western blotting

The hippocampal tissues of three rats were randomly selected from each group, and 40 mg of rat hippocampal tissue was extracted respectively, put into a mortar and

pestle, added the appropriate amount of protein lysate and protease inhibitor, ground at low temperature, collected the homogenate, centrifuged at 12,000 rpm/min, 4 ℃ for 20 min, and collect the supernatant, which was divided and put into a refrigerator at − 80 ℃ for preservation; the BCA method was used for quantification of the proteins. After protein electrophoresis using the DYY-6 C electrophoresis instrument (Bio-rad, USA), membrane transfer and closure were performed, and rabbit anti-rat primary antibodies against BDNF (25699-1-AP), TrkB (3129-1-AP), p-CREB (81871-1-RR), Tubulin (80713-RR, Proteintech, China) were incubated. Finally, sheep anti-rabbit secondary antibody (511203, Zhengneng, China) was added for incubation and visualization.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the prefrontal cortex of rats by lysis with Trizol reagent, reverse transcribed into cDNA using SYBR qPCR master mix (22208-01, Tolobio, China) and amplified by PCR. The relative expression level of BDNF mRNA was calculated using the 2-ΔΔCt method using the ALL-in-one RT Easy Mix for qPCR (22107, Tolobio, China) kit for PCR reaction on an amplifier (ThermoFisher, USA) with β-actin as an internal reference. The primer sequences were: BDNF-Forward 5'-TTG GGG CAG ACG AGA AAG C-3', BDNF-Reverse 5'-CCA GCA GAA AGA GCA GAG GAG-3'; β-ACTIN-Forward 5'-TGT CAC CAA CTG GGA CGA TA-3', β-ACTIN-Reverse 5'-GGG GTG TTG AAG GTC TCA AA-3'.

Statistical analysis

SPSS 27.0 and GraphPad v10 software were used for data analysis. Data normality was assessed by the Shapiro-wilk test and homogeneity of variance by Levene's test. Measurement data were described as mean ± standard deviation. Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by a post hoc Fisher's least significant difference test. If the variance was homogeneous, Tukey's multiple comparison test was used for comparison between the two groups; if the variance was not homogeneous, Tamhane's T-method was conducted. Changes in body weight over time were analyzed using repeated measures ANOVA. Spearman correlation analysis was performed to assess the correlation between gut microbiota abundance values and 5-HT levels. $P < 0.05$ indicated that the difference was statistically significant.

Results

AX improved body mass in PSD rats

Changes in body mass of rats were monitored according to the time points of the experimental process. On day 0, there was no significant difference in the body mass of the rats in each group ($n = 6$ for each group, $P > 0.05$); on day 7, there was no significant difference in the body mass of the rats in each group ($P > 0.05$); on day 28, the body mass of the rats in the PSD group decreased compared with the CON group ($P < 0.01$); on day 56, the body mass of the PSD group decreased compared with the CON group ($P < 0.01$); the body mass of the AX group increased compared with the PSD group ($P < 0.05$); there was no significant difference in body mass in the FLX group and FLX + AX group compared with the AX group ($P > 0.05$) (Fig. 1).

AX changed the behavior of PSD rats

The rate of sugar and water consumption was used to reflect the rats' desire for pleasurable stimuli. On day 56, sugar water consumption rate decreased in the PSD group compared with the CON group ($P < 0.01$); sugar water consumption rate increased in the AX group compared with the PSD group ($P < 0.05$); sugar water consumption rate increased in the FLX group compared with the AX group ($P < 0.05$); and sugar water consumption rate increased in the FLX + AX group compared with the FLX group ($P < 0.01$); the use of placebo did not significantly change the sugar water consumption rate of rats in the CON group and the PSD group ($P > 0.05$).

OFT was used to assess the exercise of rats, with higher activity representing a lesser degree of depression. On day 56, compared with the CON group, the PSD group showed a decrease in activity ($P < 0.01$); the AX group showed an increase in activity compared with the PSD group ($P < 0.05$); the FLX group showed an increase in activity compared with the AX group ($P < 0.05$); and the FLX + AX group showed an increase in activity compared with the FLX group ($P < 0.05$); the use of placebo did not have a significant effect ($P > 0.05$) (Fig. 2).

AX increased the expression level of 5-HT in the prefrontal cortex and colon of PSD rats

The results of 5-HT levels detection in prefrontal cortex of rats in each group showed that 5-HT expression levels decreased in the PSD group compared with the CON group ($P < 0.01$); 5-HT expression levels increased in the AX group compared with the PSD group ($P < 0.05$); and there was no significant difference in 5-HT expression in the AX group compared with the FLX group and the FLX + AX group ($P > 0.05$). The results of 5-HT expression level detection in the colon of rats in each group showed that the 5-HT expression level in the PSD group decreased compared with that in the CON group ($P < 0.01$); the 5-HT expression level in the AX group increased compared with that in the PSD group ($P < 0.05$); there was no significant difference in the 5-HT expression level in the AX group compared with that in the FLX group ($P > 0.05$); the 5-HT expression level in the FLX

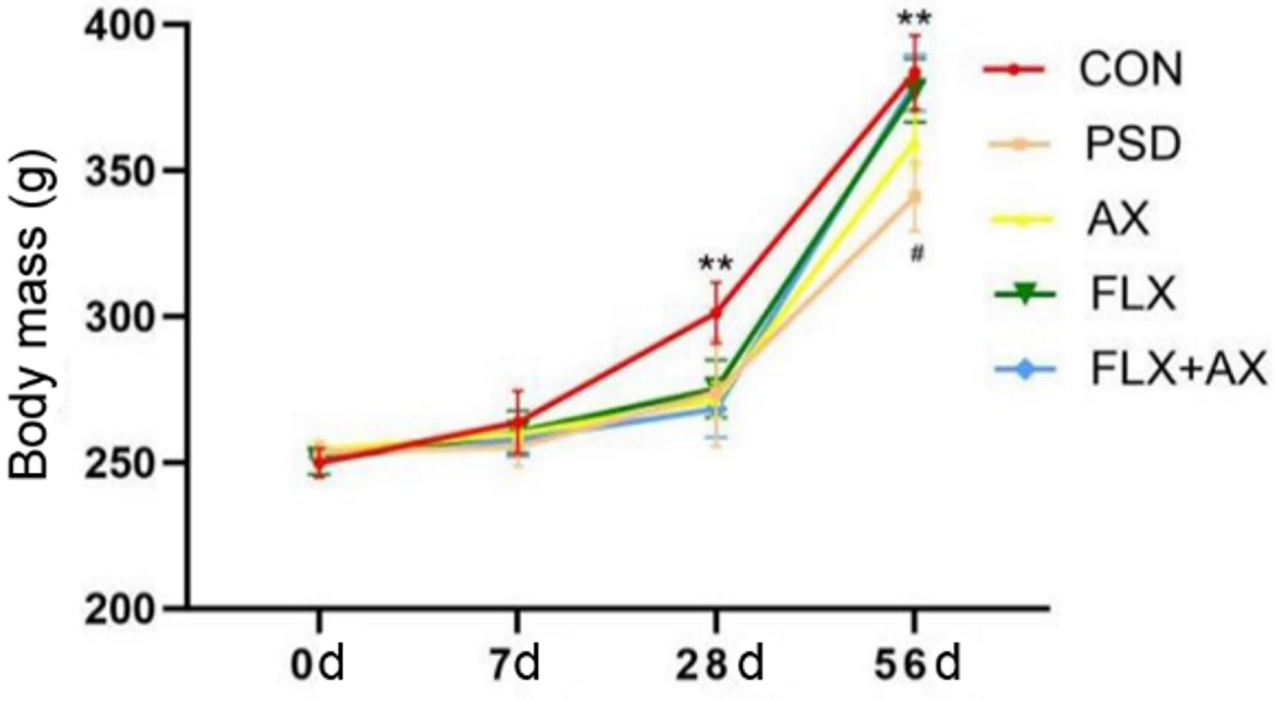

Fig. 1 Changes in body mass of rats in each group. $^{**}P < 0.01$ for PSD group compared with CON group; $^{\#}P < 0.05$ for AX group compared with PSD group

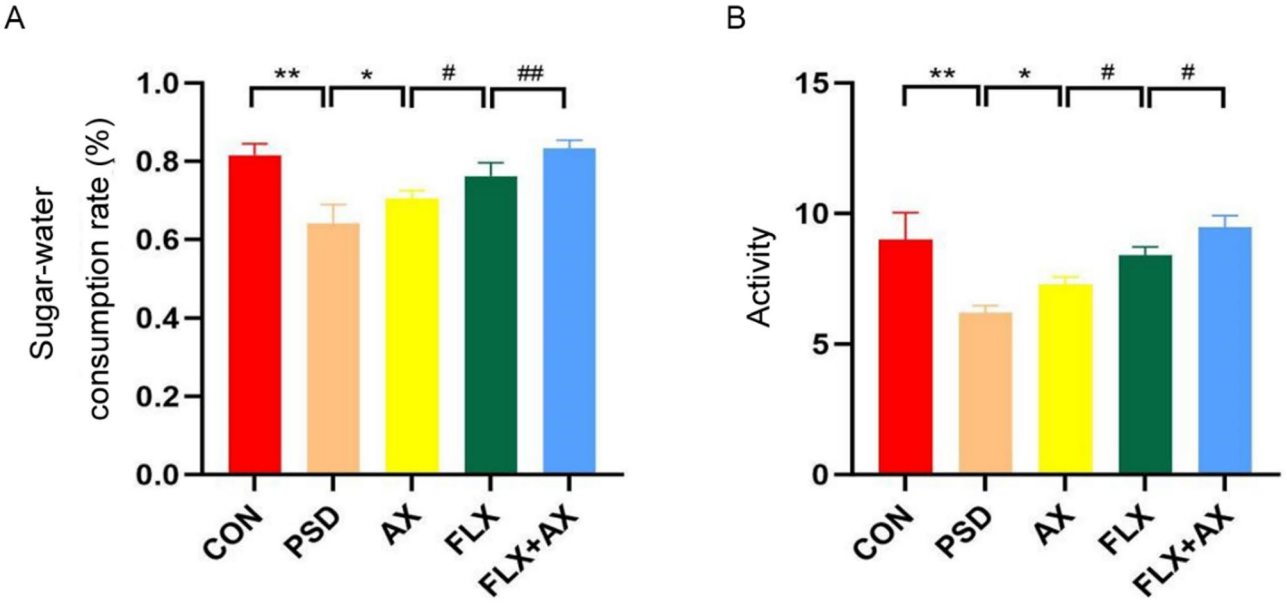

Fig. 2 Behavioral changes of rats in each group. **A** Plot of sugar-water consumption rate of rats in each group; **B** Plot of activity of rats in each group; compared with PSD group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with FLX group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$

group increased compared with that in the FLX + AX group ($P < 0.05$); the 5-HT expression level in the AX group increased compared with that in the FLX + AX group ($P < 0.05$) (Fig. 3).

AX increased the expression levels of serum 5-HT, NE and DA in PSD rats

The results of serum 5-HT levels detection of rats in each group showed that the 5-HT expression level decreased in PSD group compared with CON group ($P < 0.01$); the 5-HT expression level increased in AX group compared

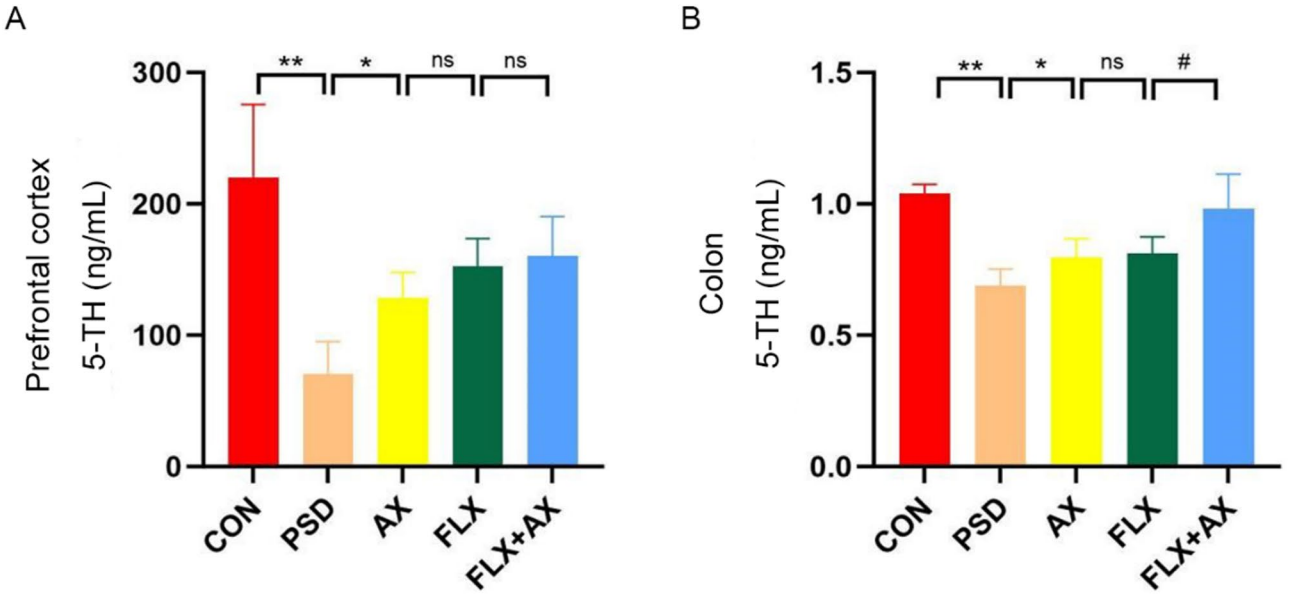

Fig. 3 5-HT expression levels in the prefrontal cortex and colon of rats in each group. Compared with the PSD group, *$P < 0.05$, **$P < 0.01$; compared with the FLX group, #$P < 0.05$

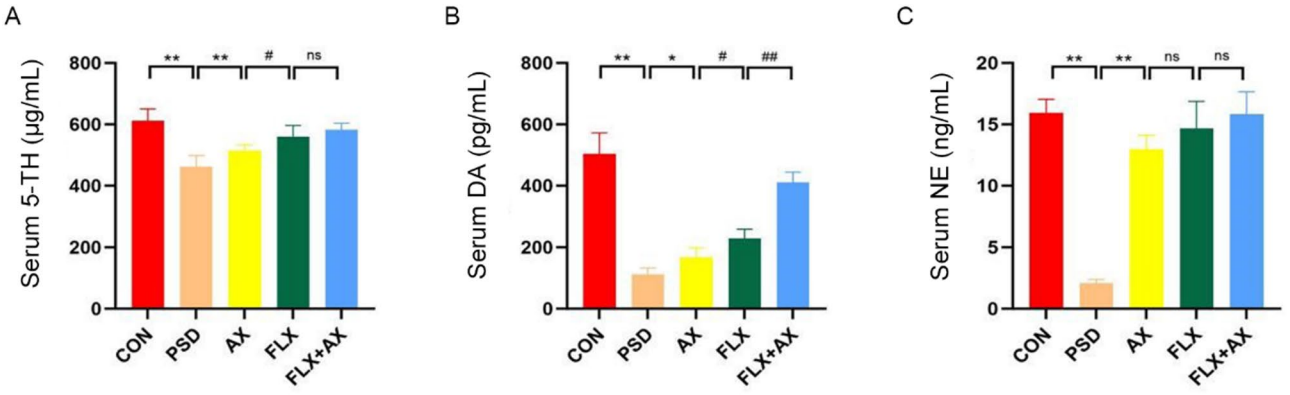

Fig. 4 Expression levels of serum 5-HT, NE and DA in rats of each group. **A** Serum 5-HT expression levels in each group of rats; **B** Serum DA expression levels in each group of rats; **C** Serum DA expression levels in each group of rats; compared with the PSD group, *$P < 0.05$, **$P < 0.01$; compared with the AX group, #$P < 0.05$, ##$P < 0.01$

with PSD group ($P < 0.01$); there was no significant difference in the 5-HT expression level in FLX group compared with AX group ($P > 0.05$); and no significant difference in the 5-HT expression level in FLX + AX group compared with FLX group ($P > 0.05$). There was no significant difference in the expression level of 5-HT in the FLX + AX group compared with that in the FLX group ($P > 0.05$). Measurement of DA level in serum of rats in each group showed that DA expression level decreased in PSD group compared with CON group ($P < 0.01$); DA expression level increased in AX group compared with PSD group ($P < 0.01$); DA expression level increased in FLX group compared with AX group ($P < 0.05$); DA expression level increased in FLX + AX group compared with FLX group ($P < 0.01$). Measurement of serum NE levels in each group of rats showed that the NE expression level decreased in the PSD group compared with the CON group ($P < 0.01$); the NE expression level increased in the AX group compared with the PSD group ($P < 0.05$); and there was no significant difference in the NE expression level of the AX group and the FLX + AX group compared with the FLX group ($P > 0.05$) (Fig 4).

AX increased the expression level of BDNF mRNA in the prefrontal cortex of PSD rats

On day 56, the BDNF mRNA expression level decreased in the PSD group compared with the CON group ($P < 0.01$); the BDNF mRNA expression level increased in the AX group compared with the PSD group ($P < 0.05$); the BDNF mRNA expression level increased in the FLX group compared with the AX group ($P < 0.05$); There

was no significant difference in mRNA expression levels between the FLX + AX group and the FLX group. (Fig. 5).

AX increases the expression levels of BDNF, TrkB, and p-CREB in the prefrontal cortex of PSD rats

Neurons in the prefrontal cortex play an important role in cognition and control of emotions, and apoptosis of neuronal cells may lead to psychiatric disorders. A decrease in neurons can lead to depression and produce neuropathological changes, while high expression of BDNF can promote neuronal repair and alleviate depressive behaviors. Our experimental results showed that the expression levels of BDNF, TrKB, and p-CREB were decreased in the PSD group compared to the CON group ($P<0.05$); the expression levels of BDNF, TrKB, and p-CREB were increased in the AX group compared to the PSD group ($P<0.05$); there was no significant difference in the protein expression of BDNF, TrKB, and p-CREB in the FLX group compared to the AX group ($P>0.05$); there was no significant difference in BDNF expression in the FLX + AX group compared with the FLX group ($P>0.05$), TrKB expression was increased in

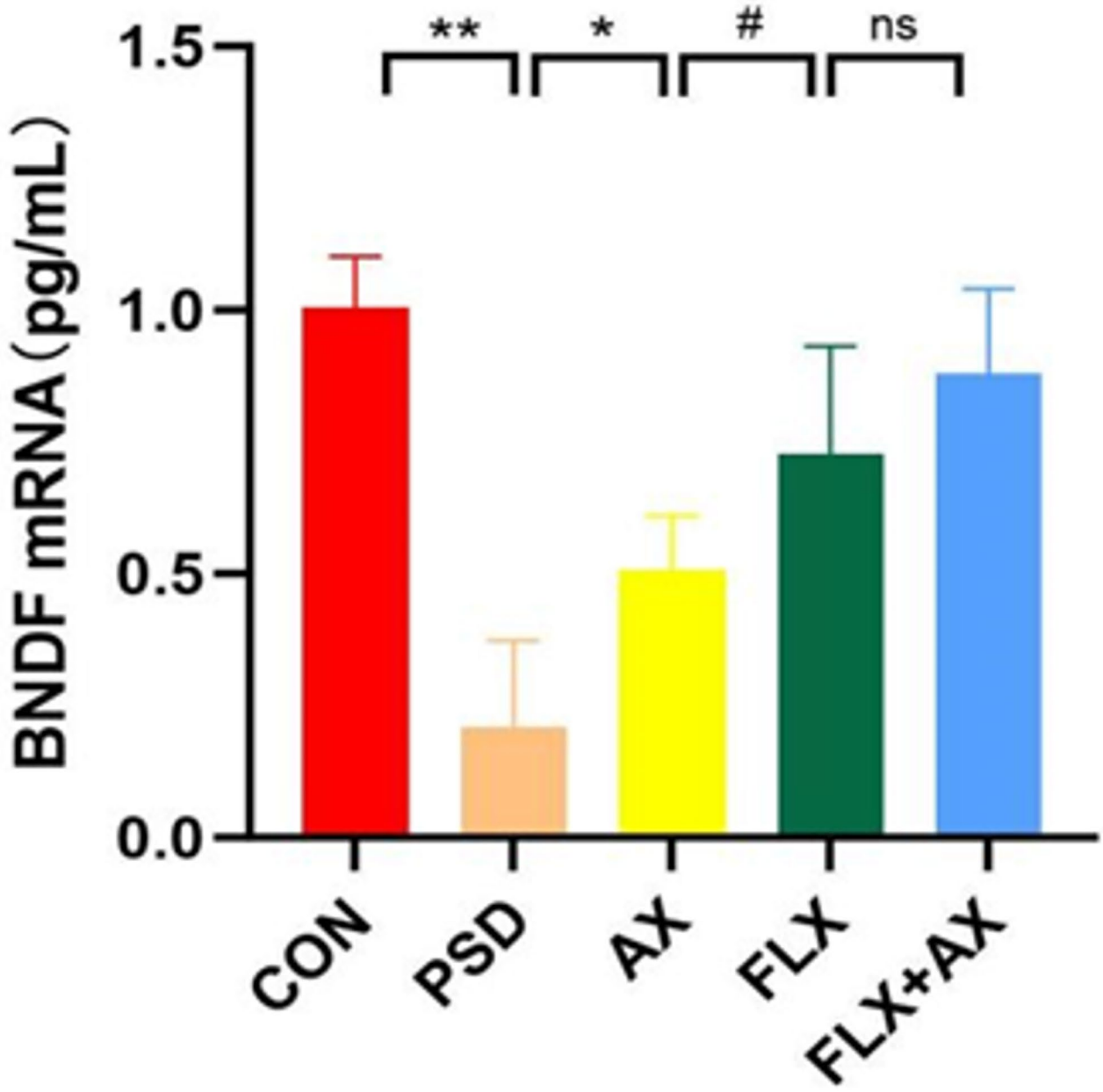

Fig. 5 BDNF mRNA expression levels in the prefrontal cortex of rats in each group. Compared with the PSD group, *$P<0.05$, **$P<0.01$; compared with the AX group, #$P<0.05$

the FLX + AX group ($P < 0.01$), and p-CREB expression was increased in the FLX + AX group ($P < 0.05$) (Fig. 6).

Pathologic changes of prefrontal cortex and colon in PSD rats

The vertebral cells in the prefrontal cortex of rats in the CON group were neatly arranged, structurally intact, with well-defined nuclei, deep staining, a higher number of neuronal cells, and no inflammatory reaction; in the PSD group, the vertebral cells in the prefrontal cortex of the PSD group were haphazardly arranged, with varying morphology, separated nuclei and plasmas, blurred boundaries, structurally incomplete, and the neuronal cells were reduced in size, significantly decreased in number, and showed a pike shape or a triangular shape. Compared with the PSD group, the pyramidal cells in each treatment group (AX, FLX, and FLX + AX groups) were relatively neatly arranged, structurally intact, with intact nuclei visible, a small amount of cellular crumpling, and no inflammatory reaction and decrease in number of cells detected (Fig. 7A). Compared with the CON group, there was necrotic shedding of epithelial cells with deeply stained and solidly shrunken nuclei in the mucosal layer of the colon of rats in the PSD group. Compared with the PSD group, the colonic mucosal injury of rats in each treatment group was alleviated to different degrees, and the inflammatory cell infiltration was reduced (Fig. 7B).

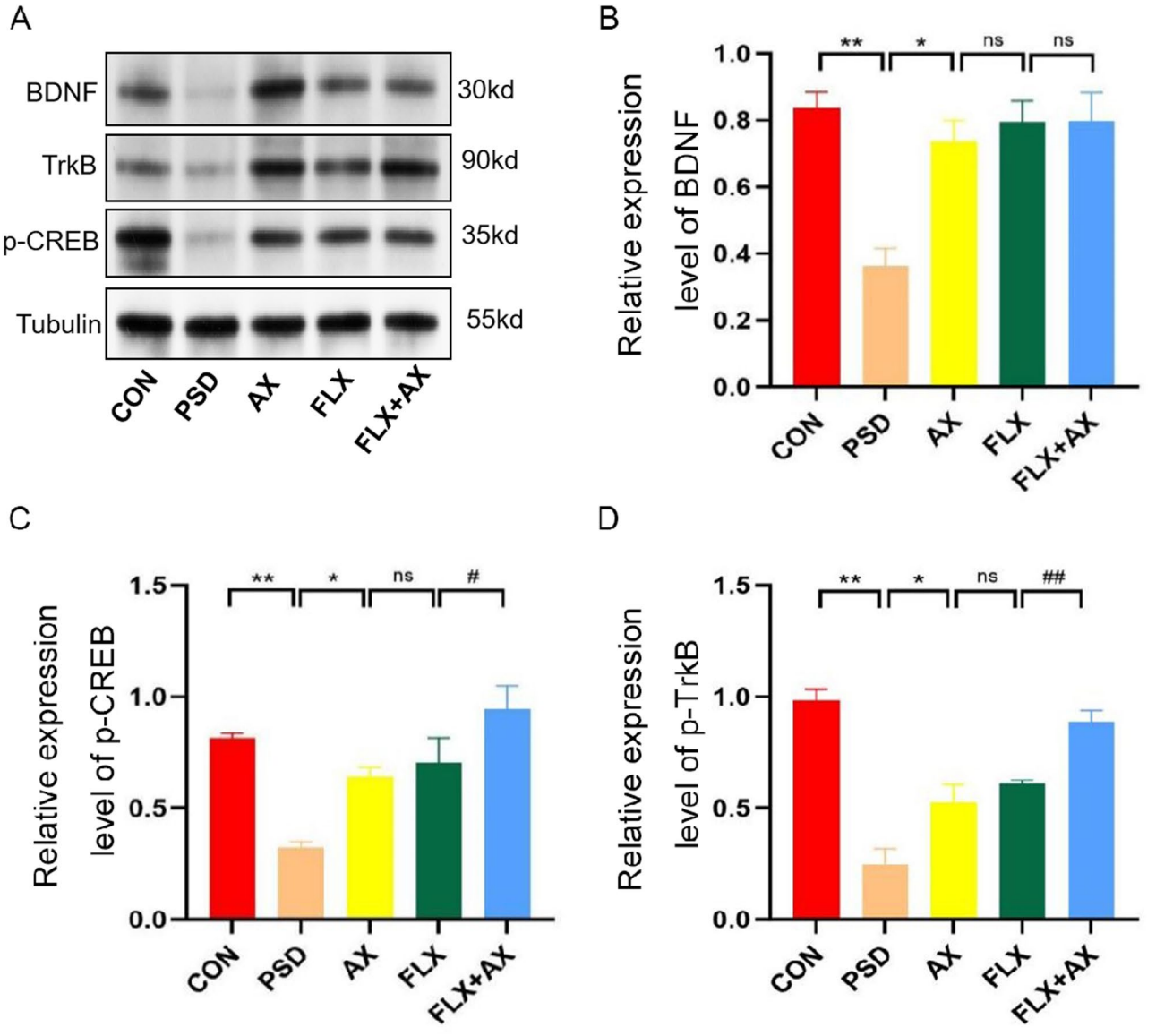

Fig. 6 Protein expression levels in the prefrontal cortex of rats in each group. **A** Protein expression level of rat prefrontal cortex in each group; **B** BDNF protein expression level of rat prefrontal cortex in each group; **C** TrkB protein expression level of rat prefrontal cortex in each group; **D** p-CREB expression level of rat prefrontal cortex in each group; compared with the PSD group, *$P < 0.05$, **$P < 0.01$; compared with the FLX group, #$P < 0.05$, ##$P < 0.01$

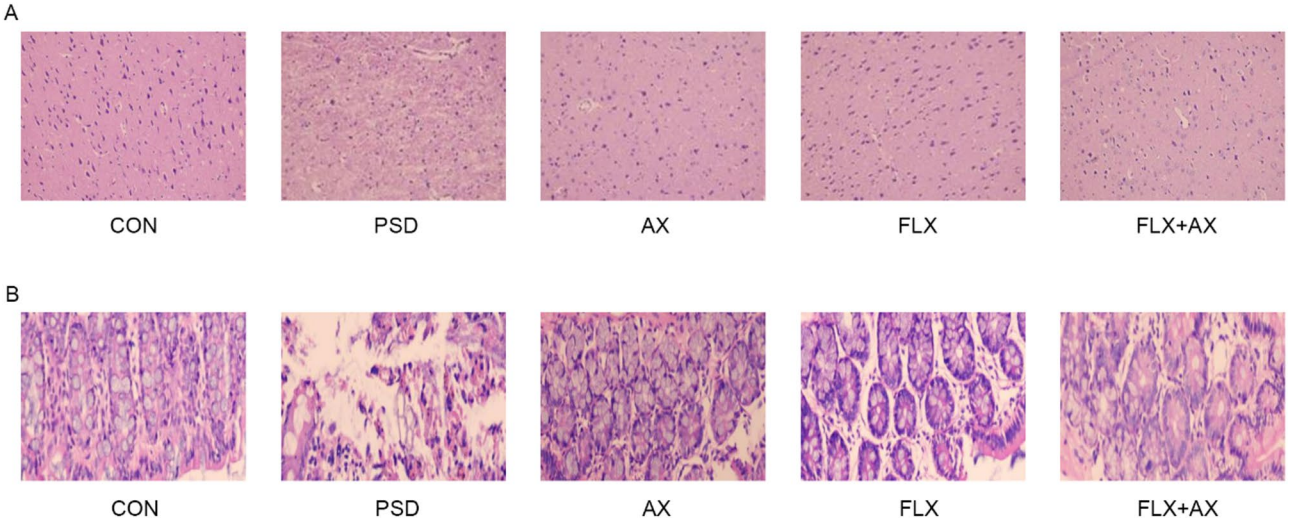

Fig. 7 Pathologic changes in the prefrontal cortex and colon of rats in each group. **A** H&E staining (×200) of the prefrontal cortex of rats in each group; **B** H&E staining (×200) of the colon of rats in each group

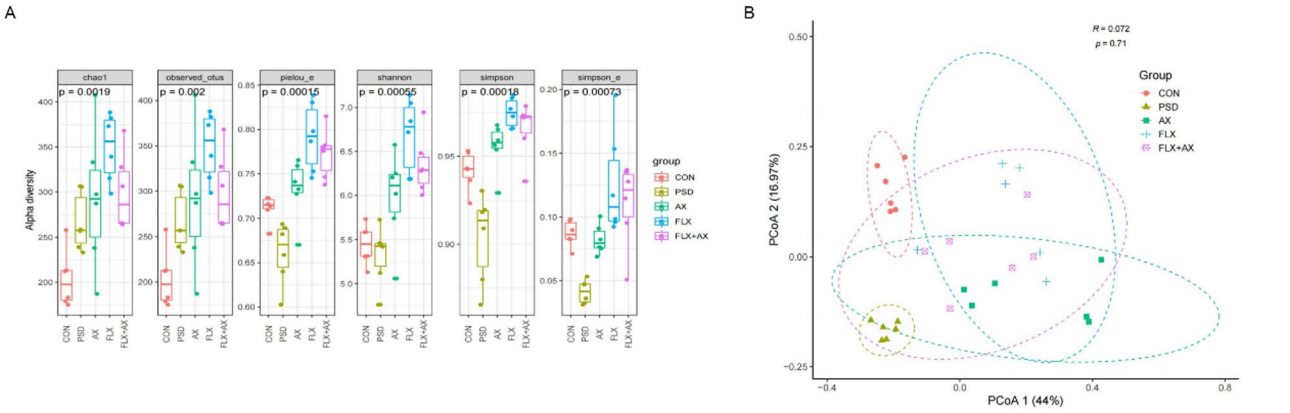

Fig. 8 Microbial diversity of rat gut. **A** microbial α-diversity (based on raw abundance, $n=6$): expressed as Simpson index; Note: $^{*}P<0.05$, $^{**}P<0.01$ compared with PSD group; **B** microbial β-diversity: each point in the graph is a sample, and the different colors indicate groups with different experimental designs; the closer the distance between the points indicates that the samples are more similar. PCoA was analyzed using the distance matrix calculated from the species composition of the samples, and the horizontal and vertical axes indicate the contribution of the first and second principal components, respectively

AX altered the diversity and abundance of intestinal flora in PSD rats

The difference in species diversity in the PSD group was mainly reflected in the Simpson index (Fig. 8A), and the rats in the PSD group showed a decrease in the Alpha diversity of intestinal flora species compared to the rats in the CON group ($P<0.01$); the AX group showed an increase in the Alpha diversity of intestinal flora species compared to the rats in the PSD group ($P<0.05$). Altered microbial beta diversity was derived from principal component analysis of the relative abundance data of different groups of the intestinal flora (Fig. 8B). The data showed that the samples within the CON, PSD and AX groups were spaced far apart and had different sample characteristics; the CON, PSD and AX groups were far apart in spatial dimensions, and the samples between the groups could be significantly differentiated on the first and second principal components; whereas, the samples within the AX, FLX, and FLX + AX groups were spaced close to each other, and had similar sample characteristics; and the samples within the CON group, FLX group, and FLX + AX group are closer together and have similar sample characteristics.

AX altered the structural characteristics of gut microbes in PSD rats

Previous analyses have shown that AX intake alters the diversity and abundance of gut flora in PSD rats. LEFSE analysis was performed on the CON, PSD, and AX groups, and LDA scores were utilized to assess the magnitude of the effect of different species on the degree of intergroup differences. The results showed that the CON

group had 19 species of dominant flora as Bacteroides, Lachnospiraceae, Parabacteroides, etc. (LDA > 2); the PSD group had 5 species of dominant flora as PrevoteL-Laceae, Ruminococcaceae, etc. (LDA > 2); the AX group had 15 species of dominant bacteria, for Bifidobacterium, Dubosiella, Romboutsia, Helicobacter, etc. (LDA > 2) (Fig. 9A). The phylogenetic branching tree showed that the significantly different groups of bacteria that played an important role in the CON group were f_Bacteroidaceae, f_Muribaculaceae, f_Tannerellaceae, f_Deferribacteraceae; and the significantly different groups of bacteria that played important roles in the PSD group were f_ Bacillaceae, f_Lactobacillaceae; the significantly different groups of bacteria that played an important role in the AX group were f_Bifidobacteriaceae, f_Staphylococcaceae, f_Helicobacteraceae, f_ Erysipelotrichaceae, f_Ruminococcaceae, f_Enterococcaceae, f_Corynebacteriaceae, f_Streptococcaceae, f_Aerococcaceae, f_ Enterobacteriaceae (Fig. 9B).

AX intake alters species abundance of gut microbial genera in PSD rats

At the microbial genus level, AX intake produced significant differences in microbial diversity, and the gut microbial genera that received the most significant effects of AX intake compared to the PSD group were screened (Fig. 10), including Bifidobacterium, Christensenellaceae R-7 group, Dubosiella, Prevotellaceae NK3B31 group, and Ruiuminococcaceae NK4A214 group, etc.; the relative abundance of Prevotellaceae NK3B31 group was increased in the PSD group compared with the CON group ($P < 0.01$); and after 28 days of AX intake, the Prevotellaceae NK3B31 group relative abundance was significantly decreased ($P < 0.05$); moreover, compared with the PSD group, AX intake increased the relative abundance of Bifidobacterium, Christensenellaceae R-7 group, Dubosiella, Ruiuminococcaceae NK4A214 group and other probiotics relative abundance ($P < 0.05$).

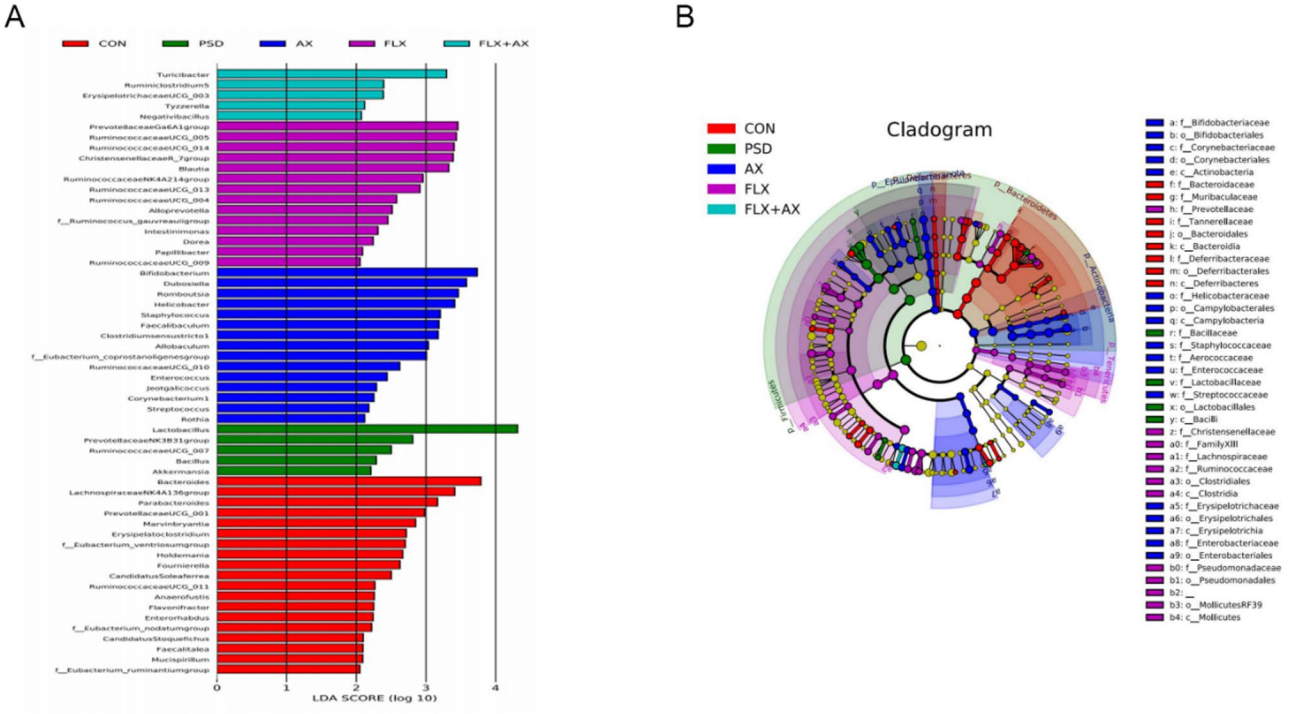

Fig. 9 Lineage-specific effect size (LEFSE) and phylogenetic tree branching. **A** Species with significant differences in abundance across groups are shown in the LDA value distribution histograms, and the length of the histograms represents the effect size (i.e., LDA Score) of the differing species. Vertical coordinates show the species with significant differences between groups, while horizontal coordinates visualize the logarithmic score values of LDA analysis for each species in a bar chart. Species are sorted according to the size of the score, the longer the length indicates the more significant difference of the species, and the color of the bar graph indicates the corresponding sample group of the species; **B** the circles radiating from inside to outside represent the taxonomic levels from phylum to genus (the innermost circle is the boundary), each small circle on different taxonomic levels represents a classification under the level, and the size of the diameter of the small circle represents the relative abundance of the size of the circle Color: Species with no significant differences are uniformly colored in yellow, and species with significant differences follow the group. The name of the fungus is preceded by k_ for: kingdom, p_ for: phylum, c_ for: class, o_ for: order, f_ for: family, g_ for: genus, s_ for: species

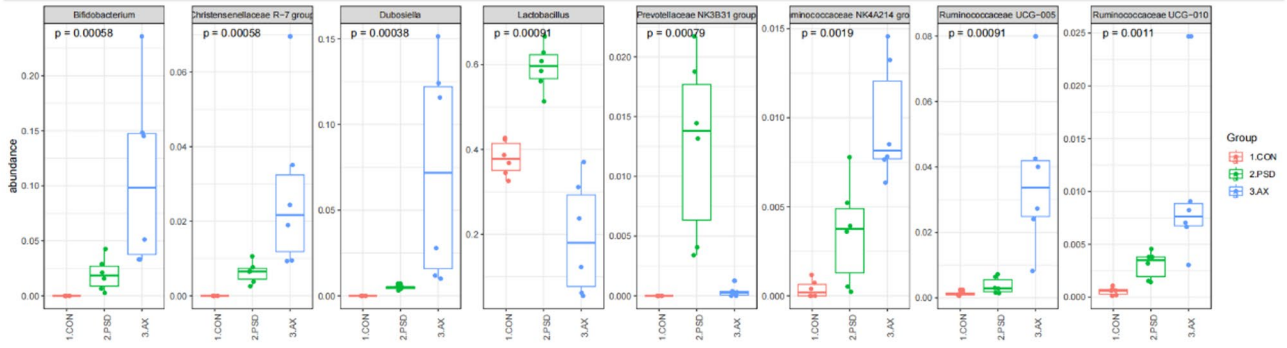

Fig. 10 Box line plot of the abundance of gut microbial genera in rats of each group

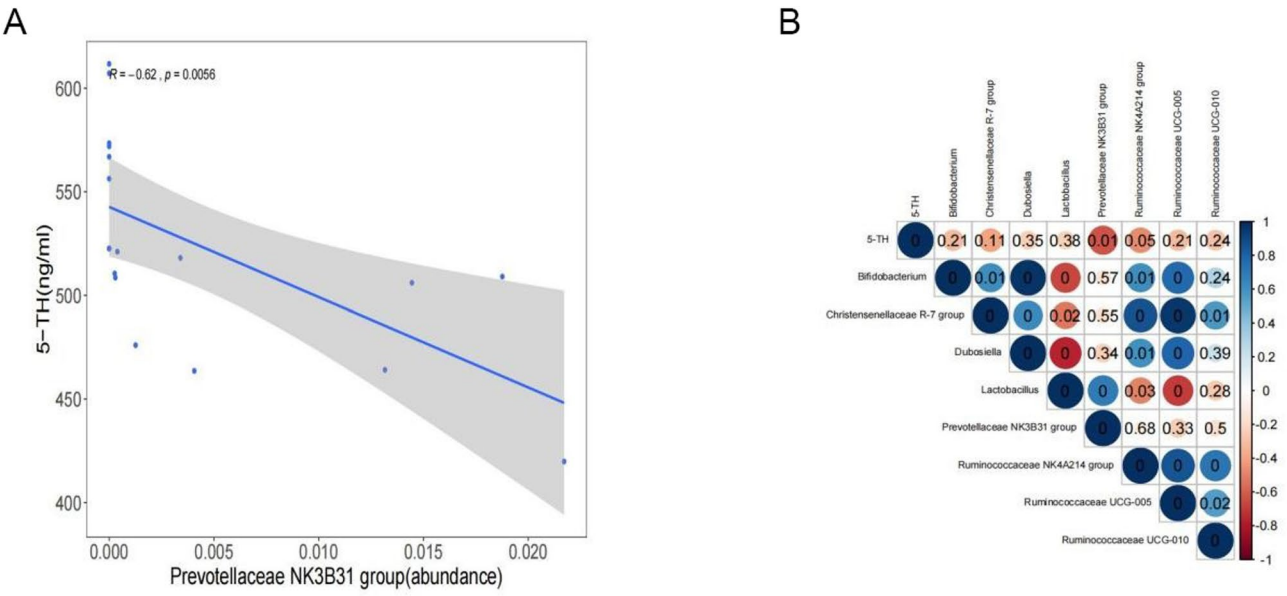

Fig. 11 Correlation analysis between gut microorganisms and prefrontal cortex 5-HT levels. **A** Regression analysis between Prevotella and prefrontal cortex 5-HT levels; **B** Correlation analysis between microbial differences and prefrontal cortex 5-HT levels, with the size of the circle representing the correlation value. Blue color represents positive correlation and red color represents negative correlation

Correlation analysis of rat intestinal microorganisms and the level of prefrontal cortex 5-HT

Correlation analysis of the genera and the level of prefrontal cortex 5-HT with all significant differences among CON, PSD, and AX groups showed that Prevotellaceae NK3B31group had a significant negative correlation with the level of prefrontal cortex 5-HT (R=-0.62, P<0.01) (Fig. 11A), and the rest of the genera had a negative correlation with the prefrontal cortex 5-HT level but the differences were not statistically significant (P>0.05). (Fig. 11B).

Discussion

PSD is an affective disorder syndrome that commonly occurs after a stroke, and its core symptoms involve depressed mood and diminished interest [24]. Patients suffer from impaired cognitive function and an increased risk of falls, and they have a poor prognosis and even suicidal tendencies, posing a serious health burden to society [25]. In this study, we found AX improved body mass and positively changed the behaviors of PSD rats to show anti-depression impact, which pointed out a potential anti-depression role of a natural novel prebiotic.

Monoamine neurotransmitters, such as 5-HT, DA, and NE, regulate a wide range of physiological and endocrine functions, and a decrease in the levels of these neurotransmitters is closely associated with the development of depression, and a decrease in their concentration is not only a sign of increased depressive symptoms, but also one of the key indicators for clinical diagnosis of depression. Fluoxetine, a widely used antidepressant, acts by selectively inhibiting the reuptake of 5-HT, and despite its remarkable efficacy, the accompanying side effects cannot be ignored [26]. In this research, not only did AX increase the expression level of 5-HT in the prefrontal cortex and colon of PSD rats, but also it upregulated the

expression levels of serum 5-HT, NE and DA in PSD rats, which exerted beneficial roles and less side effects as a novel antidepressant.

Recent studies have revealed a link between the microbial-gut-brain axis and PSD, suggesting that probiotic supplementation may be a novel strategy for intervening in neurological disorders such as depression [27]. A previous study reported a decrease in beneficial bacteria (*Lactobacillus*) and an increase in harmful bacteria (*Ruminococcus*, Oscillospiraceae) after PSD [28]. In this study, using fecal macro-genome sequencing technology, it was confirmed that arabinoxylan could effectively promote the proliferation of intestinal probiotic flora and reinforce intestinal barrier function. Arabinoxylan could promote the proliferation and diversity of the intestinal microbiota, because it was not digested by human digestive enzymes, after reaching the the colon, it was broken down by specific pentosan hydrolysis, which in turn promoted the growth of beneficial flora, and showed a positive effect on the health of the posterior section of the colon, studies have found that arabinoxylan promotes the proliferation of bifidobacteria, which points to the potential probiotic. In addition, by analyzing the expression levels of 5-HT in the prefrontal cortex and colonic tissues and 5-HT, DA, and NE in the blood, the results showed that arabinogalactan could significantly elevate the content of these neurotransmitters and effectively attenuate the depression-like behavioral performance of rats in the model of PSD.

The onset and development of PSD are closely associated with neuronal damage and often result in low expression of BDNF/TrkB/p-CREB pathway proteins [11]. The BDNF/TrkB/p-CREB signaling pathway plays an important role in the modulation of synaptic structure and is a key mechanism driving neuronal cell proliferation and maturation [9, 29]. Among them, BDNF, a prominent biomarker in depression research, actively promotes neuronal generation and development [30, 31]. TrkB receptors, as molecules that bind exclusively to BDNF, are embedded in the process of learning and memory formation [32]. When BDNF binds to TrkB, it activates a series of intracellular signaling pathways that accelerate neuronal maturation and expansion [33]. In addition, CREB, as a key antidepressant, can regulate oxidative stress and reduce neuronal apoptosis through TrkB-mediated phosphorylation to the p-CREB form, thus demonstrating its antidepressant effects [34, 35]. In the present study, we found that AX upregulated the expression levels of BDNF, TrkB, and p-CREB in the prefrontal cortex of PSD rats which indicated that AX might present its antidepression effects via modulating BDNF/TrkB/p-CREB signaling pathway.

This research has some limitations. First, the expression levels of inflammatory markers relevant to PSD, such as TNF-α, IL-6 and IL-1β need to be tested in the subsequent research. Second, the comparison between arabinoxylan and other prebiotics needs to be conducted in the future to determine its relative efficacy. Third, the duration of the study was relatively short which could not assess the long-term effects of arabinoxylan. Last but not least, the small sample size was also a major limitation in the animal behavioral assessment.

In summary, arabinoxylan ingestion showed positive effects on the depression-like behavior of rats in the PSD model, as evidenced by an increase in body mass, an increase in activity in the open field experiment, and an increase in sugar and water consumption. This mechanism of action is hypothesized to be related to the promotion of the growth of probiotics in the intestine, reinforcement of the intestinal barrier function, up-regulation of the BDNF/TrkB/p-CREB signaling pathway, and increase in the expression of monoamine neurotransmitters, which play a role in the protection of neurons.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12868-025-00964-6.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

The study was conceived and designed by Bin-yu Bin and Xue-bin Li. Data collection and assembly were conducted by Liu Huang and Lin Lin, while data analysis and interpretation were performed by Jun Zhou, Wei-juan Yan, and Jie Wang. All authors contributed to the manuscript writing and provided final approval of the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval

This study was approved by the Ethics Committee of the Science and Technology Department of the Youjiang Medical University for Nationalities (approval number: 2023060902). The experimental procedures in this study were conducted in strict compliance with the ethical requirements for laboratory animals and the relevant provisions of the Regulations of the People's Republic of China on the Management of Laboratory Animals, and humane care was provided in accordance with the principles of the 3Rs (Replacement, Reduction, Refinement) for laboratory animals.

Consent to participate

Not applicable.

Competing interests
The authors declare no competing interests.

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