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Antidepressant-like effects of *Lactobacillus plantarum* DP189 in a corticosterone-induced rat model of chronic stress

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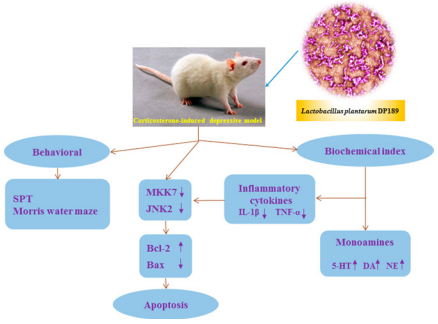
GRAPHICAL ABSTRACT



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ABSTRACT

Probiotic antidepressant effects demonstrated previously in clinical studies and animal models act via unknown mechanisms. Here we used a corticosterone injection-induced Sprague-Dawley rat chronic stress exposure model to investigate antidepressant-like effects of potential probiotic *Lactobacillus plantarum* DP189 (DP189) isolated from Chinese traditional fermented sauerkraut. After administration of DP189 (1.0×10^9CFU/d) suspension by gavage for 21 days, behavioral, histopathological and biochemical changes were assessed, including hippocampal neuronal apoptosis assessments via TUNEL staining and Western blot analysis. Behaviorally, DP189 treatment improved memory and spatial learning and reduced anhedonia, as measured using Morris water maze and sucrose preference tests, respectively. Histopathologically, DP189 treatment ameliorated hippocampal pathological changes and dramatically reduced TUNEL-positive cell numbers. Biochemically, DP189 decreased serum IL-1β and TNF-α levels, decreased hippocampal mitogen-activated protein kinase kinase 7 and c-Jun N-terminal kinase 2 levels, down-regulated pro-apoptosis protein Bax immunocontent and up-regulated anti-apoptosis protein Bcl-2 immunocontent. Collectively, these results suggest that DP189 treatment may prevent and/or alleviate depression-like behaviors and hippocampal neural injury induced by CORT.

1. Introduction

Major depression, also known as major depressive disorder (MDD), afflicts over 350 million people worldwide and is one of the most common mental disorders currently diagnosed [1]. MDD patients exhibit persistent sadness and lack of interest, while suffering greatly and functioning poorly at work, at school and at home. Analysis of treatment outcomes often reveals that patients experience serious side effects, such as constipation, dizziness and sleepiness, as well as high recurrence rates [2]. Of special concern is that effective treatments and

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biomarkers for monitoring treatment of patients with early-stage MDD are lacking; consequently, treatment of these patients may actually precipitate occurrence of major depression that frequently leads to suicide [3]. At the present time, MDD contributes greatly to the global disease burden as a leading cause of disability [4,5]. As such, effective adjunctive treatment strategies for preventing MDD during the early stage of depression will likely exert beneficial effects by slowing or preventing disease progression.

Recently, researchers have enumerated and analyzed numbers of bifidobacteria and lactobacilli in feces of depressed patients and healthy people and found that individuals with MDD harbored fewer bifidobacteria and/or lactobacilli organisms relative to healthy controls [6]. Such results have thus revealed the existence of a potential relationship between probiotics and depression. Meanwhile, oral probiotic treatments administered in several double-blind placebo-controlled trials were shown to significantly improve preclinical symptoms of individuals suffering from stress and depression; for example, significant improvement of sleep quality was associated with *L. gasseri* CP2305 treatment, significant reductions of depression and anxiety symptoms were observed with *L. rhamnosus* HN001 treatment, while improved cognitive performance and decreased kynurenine concentration were observed after *L. Plantarum* 299v administration [7–9]. In other studies, researchers have also explored the ability of probiotics to ameliorate depressive behavior in animal models through analysis of their effects on monoamine neurotransmitter and microbiota-gut-brain axis functions. Liu et al. administered *L. plantarum* PS128 to germ-free (GF) mice (a mouse depression model) and found that PS128 could significantly increase serotonin and dopamine levels [10]. The gut microbiome, especially its probiotic component, is critical for normal brain-gut axis function such that attenuation of excessive hypothalamic-pituitary-adrenal (HPA) axis responses may achieve antidepressant-like effects. For example, in one study *L. helveticus* NS8 administration alleviated adverse symptoms of anxiety and depression, as demonstrated using a maternal separation animal model [11]. As a second example, treatment with *B. pseudocatenulatum* CECT 7765 down-regulated MS-induced corticosterone production, as demonstrated using a chronic restraint stress depression animal model [12]. Although *Lactobacillus* has been shown to exert antidepressant-like effects that could potentially be harnessed for use in preventing and treating depressive disorders, its exact mechanism of action remains unclear.

In this study, we employed a corticosterone (CORT)-induced chronic stress exposure rat model in order to investigate potential antidepressant-like effects of *L. plantarum* DP189 (DP189), a probiotic isolated from Chinese traditional fermented sauerkraut. Effects of DP189 on behavior, hippocampal neuronal cell morphology and cellular apoptosis (explored using TUNEL and Western blot analysis) and on serum and hippocampal biochemical parameters were evaluated.

2. Materials and methods

2.1. Bacterial probiotic strain and culture conditions

L. plantarum strain DP189 (DP189), derived from a bacterial isolate obtained from homemade sauerkraut produced in northeastern China, was stored in the China Center for Type Culture Collection (CCTCC # M2019199). DP189 was grown in De Man Rogosa Sharpe (MRS) medium at 37 °C for 16 h, followed by harvesting of bacterial cells via centrifugation at 4500 × *g* for 8 min at 4 °C. Each pellet was washed twice, with each wash involving resuspension of cells in sterile phosphate buffered saline solution (PBS) (pH = 7.4) followed by centrifugation as mentioned above. Finally, pellets were resuspended in sterile saline then adjusted to 5.0 × 10^9 CFU/mL and stored at 4 °C until used experimentally.

2.2. Animal model of chronic stress exposure and design of experiments

Sprague-Dawley rats (males, 220-250 g) were obtained from Yisi Laboratory Animal Technology Co. Ltd, Changchun, China. Rats were acclimatized for 7 days by housing them under humidity-, temperature-, and light-controlled laboratory conditions (50 % –60 % relative humidity, temperature of 23 ± 2 °C; 12 h/12 h light/dark cycle) and fed ad libitum. All animal experiments were conducted according to guidelines established by the Animal Care Committee of Jilin Academy of Agricultural Sciences. All procedures involving animal experimentation were approved by the Animal Care and Ethic Committee of Jilin Academy of Agricultural Sciences.

A CORT-induced chronic stress exposure rat model was used in this this study [13,14]. Briefly, rats were randomly assigned to four groups (n = 10 per group): control group, CORT group, fluoxetine group and DP189 group. Rats were housed and maintained in cages containing 3-4 rats per cage, with all rats within a cage belonging to the same control or experimental group. Rats in the CORT, fluoxetine and DP189 groups received daily subcutaneous CORT injections (40 mg/kg) for 21 days [13]. Rats in fluoxetine and DP189 groups were pretreated respectively by gavage with fluoxetine (10 mg/kg) [15] or *L. plantarum* DP189 (1.0 × 10^9 CFU/d) [10] 6 h prior to each CORT injection, while control group rats received parallel injections with the same corresponding volumes of saline. CORT was purchased from Sigma–Aldrich and fluoxetine hydrochloride was purchased from Suzhou Zhonghua Pharmaceutical Industry Co. Ltd., China. A detailed diagram outlining the experimental design of treatments and behavioral, histopathological and biochemical analyses is presented in Fig. 1. After rats received the 21-day course of treatments, they were subjected to behavioral testing. At completion of behavioral testing, all rats were anesthetized using sodium pentobarbital (2 %, 30 mg/kg weight) followed by collection of

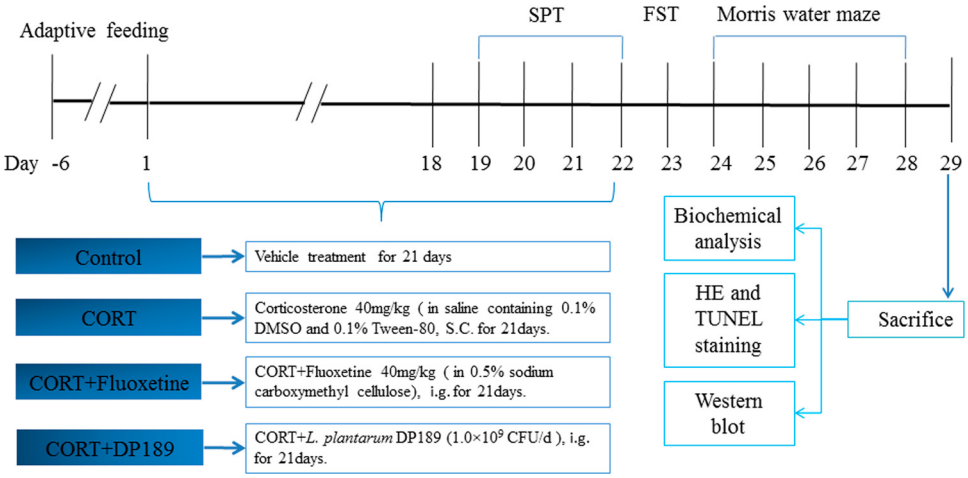

Fig. 1. Experimental design. This study aimed to evaluate the effect of *L. plantarum* DP189 on chronic corticosterone-induced depression-like behavior in rats. Fluoxetine was taken as the standard medicine. Behavioral tests, including sucrose preference test (SPT), forced swim test (FST) and Morris water maze (MWM), were conducted following the end of the dosing schedule. After behavioral tests, serum and hippocampus were collected and stored at -80.0 °C for further analysis.

serum samples for pro-inflammatory cytokine level determinations. Next, quick decapitation was conducted then the hippocampus was rapidly removed and placed on ice; one portion of each hippocampus was snap-frozen in liquid nitrogen as soon as possible and another hippocampal portion was fixed in buffered formalin (10 %, neutral pH) for hematoxylin and eosin (H&E) and TUNEL staining. Serum and hippocampal specimens were stored at −80 °C.

2.3. Behavioral tests

2.3.1. Sucrose preference test

Sucrose preference testing (SPT) was carried out according to the protocol reported by Wei et al [16] with minor modifications. Rats were trained to select a 1 % (w/v) sucrose solution instead of water 72 h prior to testing. During training, rats in each cage first received 24 h access to sucrose solution (two bottles/cage) followed by replacement of one bottle with a bottle of tap water and access to both bottles (sucrose and water) for a second 24 h. Next, rats were housed in individual cages with no food or drink for 18 h then they were provided 5 h access to one bottle each of sucrose solution and tap water, with bottle positions randomly placed in cages and intakes of tap water and sucrose solution recorded. Sucrose preference was determined by calculating the percentage of sucrose consumed of the total volume of sucrose and tap water consumption combined.

2.3.2. Forced swim test

To evaluate the effect of DP189 on despair behavior, rats were subjected to a forced swim test (FST) as described in previously [17,18]. Rats were placed individually in a plastic cylinder (diameter 30 cm, height 50 cm) that was filled to a height of 25 cm with water (25 ± 2 °C). Duration of immobility was recorded during the last 5 min of the 6-min test session. Rats floating in the water without struggling while maintaining only their heads above water were recorded as immobile.

2.3.3. Morris water maze

To assess effects of treatments on spatial learning and memory, Morris water maze tests were conducted using previously reported methods [19,20] with minor modifications. The maze (ZH-morris, Anhui Zhenghua Biological Instrument Equipment Co. Ltd, Huaibei, China) used here was a circular pool (diameter 200 cm, 50 cm height) filled with water (25 ± 1 °C) to a depth of 30 cm. After dividing the pool into 4 equal quadrants, boundaries of quadrants were marked with external cues to help rats orient themselves while in the pool. A 15 cm diameter invisible escape hatch was placed in the center of one quadrant and submerged under the water surface by 2 cm. Swimming paths of rats were recorded using a video recorder that was physically suspended above the center of the pool.

Two spatial learning trials were performed daily for 4 successive days. Rats were randomly assigned access to the water maze at predetermined locations in order to avoid egocentric bias with regard to orientation. Rats were required to swim to and reach the invisible platform within 60 s under the guidance of externally placed spatial cues before they were allowed to rest there for 10 s. Rats that failed to reach the platform after 60 s were placed onto the platform to rest for 10 s. The two trials conducted each day were separated by 1 min intervals to ensure the possibility of observing a short-term memory trace generated during the earlier trial that day. Memory was consolidated by repeating the first-day schedule during subsequent days. During the trials, escape latency (EL) time to hidden platform discovery was recorded. On the fifth day of testing, the platform was removed and the numbers of crossings over the previous hidden platform location within 60 s were recorded.

2.4. H&E staining of hippocampus sections

Hippocampal tissue was fixed in 10 % neutral formalin, paraffinized, then sectioned into 5-μm-thick slices that were stained with H&E stain. Histopathological changes throughout the entire hippocampus (and in particular its CA1 sub-region) were observed at 100 × and 400 × using an optical microscope (Olympus, Tokyo, Japan). Due to the fact that the CA1 sub-region is the most sensitive hippocampal area to pathological effects of lesion activity, this hippocampal sub-region (especially its hippocampal pyramidal-shaped cells) received special scrutiny in our assessment of hippocampal pathological changes.

2.5. Biochemical analyses

Serum levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), as well as hippocampal supernatant levels of 5-hydroxytryptamine (5-HT), dopamine (DA) and norepinephrine (NE), were measured using ELISA kits from Shanghai Jianglai Biotechnology Co. Ltd. (Jianglai, Shanghai, China) according to the manufacturer's instructions. All experimental data were statistically analyzed via ANOVA analysis and post-hoc tests; all statistical analyses were conducted using SPSS software.

2.6. TUNEL staining for detection of apoptosis

Evaluation of hippocampal neuronal cell apoptosis using TUNEL staining was conducted according to the manufacturer's instructions provided with the In Situ Cell Death Detection Kit-POD (Roche, Basel, Switzerland). Briefly, hippocampal tissue was dehydrated, embedded, sliced, then placed in a 50 μL volume of TUNEL reaction mixture followed by incubation in a humidified atmosphere for 1 h at 37 °C in the dark. Finally, hippocampal tissues were stained with DAPI at 37 °C for 10 min, with three sections stained for each hippocampus. The entire hippocampus, with special emphasis on its CA1 sub-region, was observed at 100× and 400 × under a fluorescence microscope (Olympus, Tokyo, Japan). Numbers of apoptotic cells were analyzed using Image J software and the ratio of TUNEL-positive cells to DAPI-positively stained cells was used to evaluate the degree of apoptosis. Testing was conducted by a blinded observer.

2.7. Western blot analysis

Hippocampal tissues were first suspended in buffer (complete RIPA buffer, 1:10, Solarbio, Beijing, China) then homogenized to lyse the cells. Protein concentrations of hippocampal lysates were determined using a Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Soluble protein (50 μg) was loaded onto sodium dodecyl sulfate (SDS)-polyacrylamide gels (10 %) then electrophoresed. Separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes then membranes were blocked for 2 h followed by incubation with anti-mitogen-activated protein kinase kinase 7 (anti-MKK7, ab52618), anti-c-Jun N-terminal kinase 2 (anti-JNK2, ab76125), anti-Bcl-2 (ab194583), anti-Bax (ab32503) or anti-β-actin (Sangon Biotech, Shanghai, China) antibodies. After 3 washes to remove antibodies, membranes were incubated with horseradish peroxidase-conjugated secondary antibody at 37 °C for 1 h then were washed and developed using an enhanced chemiluminescence (ECL) kit. Protein bands adsorbed to membranes were visualized using a Clinx Science Instruments imaging system (Clinx, Shanghai, China). Levels of proteins were normalized to β-actin protein levels. Gray analysis of Western blot bands was conducted using image-processing software (Image J, National Institutes of Health, Bethesda, MD, USA). The results for each protein were expressed as a fold-change in protein band intensity value relative to the corresponding band intensity of of the control group.

2.8. Statistical analysis

SPSS version 15.0 software (SPSS Inc, Chicago, IL, USA) was used

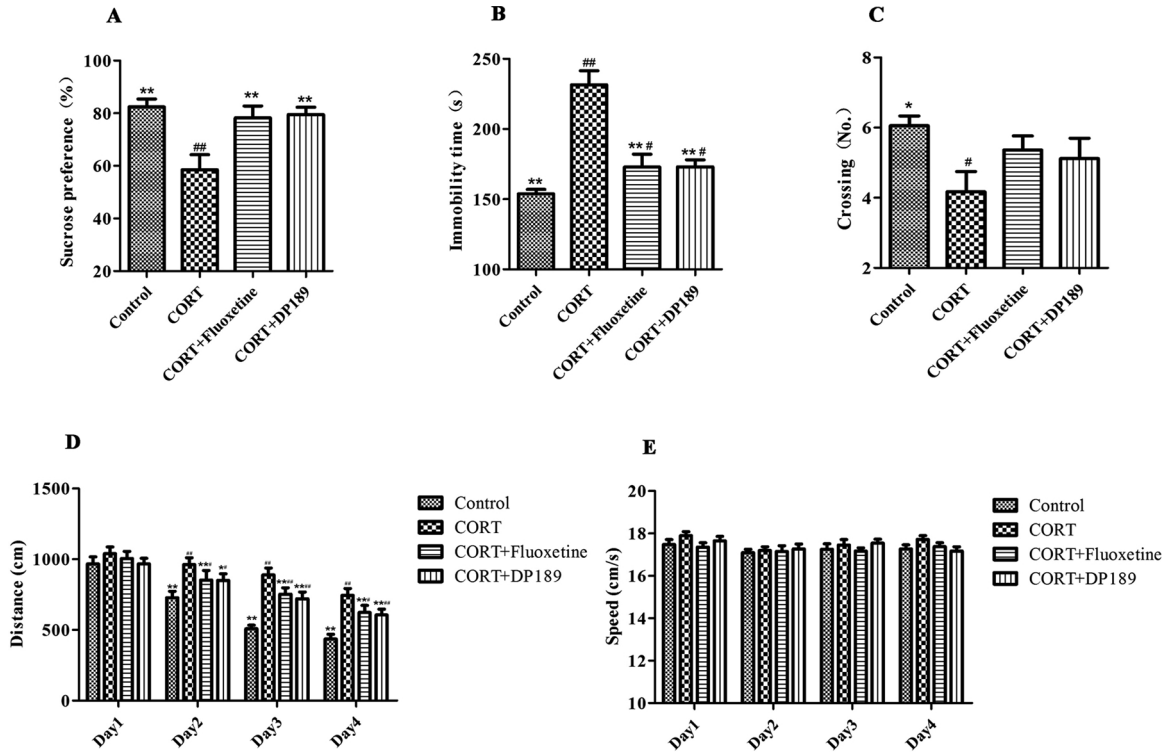

Fig. 2. Effects of *L. plantarum* DP189 on CORT-induced depression-like behaviors alterations in rats. The behavioral tests that were performed included sucrose preference test (A), forced swim test (B) and morris water maze (C-E). All the values were given as mean ± SD (n = 10), ## P < 0.01, # P < 0.05 vs Control; ** P < 0.01, * P < 0.05 vs CORT.

for statistical analysis [21]. All values were expressed as means ± standard deviation values (means ± SD) for each group and one-way analysis of variance (ANOVA) was conducted for comparisons involving more than two groups. Statistical significance determinations were based on $p < 0.05$ and $p < 0.01$ cutoffs.

3. Results

3.1. L. plantarum DP189 effects on depressive-like behavior, spatial learning and memory

Sucrose consumption was measured to assess the effect of probiotics DP189 and fluoxetine on the anhedonic-like phenotype induced by CORT in the depressed rat model ($F(3,36) = 6.743$, $P < 0.05$). As shown in Fig. 2A, sucrose consumption of CORT-exposed rats was significantly less than that of DP189- ($P = 0.008$) and fluoxetine- ($P = 0.01$) treated rat groups.

Results of the forced swim test (Fig. 2B) demonstrated that chronic corticosterone treatment significantly increased the duration of immobile period of the CORT group as compared to that of the control group ($F(3,36) = 20.846$, $P = 0.000$). By contrast, treatments with DP189 ($P = 0.001$) or fluoxetine ($P = 0.001$) significantly attenuated symptoms of behavioral despair as compared with corresponding results for the CORT group.

Regarding Morris water maze results, escape latency a (EL) times for reaching the platform were shorter in all groups (Table 1); however, CORT group rats spent much more time searching for the platform on days 2 ($F(3,36) = 11.430$, $P = 0.000$), 3 ($F(3,36) = 13.29$, $P = 0.000$) and 4 ($F(3,36) = 27.378$, $P = 0.000$) than did control group rats, which may reflect memory deficits resulting from repeated CORT injections. Meanwhile, escape latenc times of DP189 group rats on days 3 ($P = 0.01$) and 4 ($P = 0.000$) were notably shorter relative to corresponding CORT group escape latency times while total swimming distance to the platform decreased progressively during the training period (Fig. 2D). The DP189 group showed a shorter path length compared to the CORT group beginning on the second day ($F(3,36) = 10.752$, $P = 0.02$), while no significant differences in swimming speed were observed among the four groups (Fig. 2E). The abovementioned results indicate that rat spatial learning improved after DP198 pretreatment. Moreover, spatial memory test results for day 5 ($F(3,36) = 2.693$, $P = 0.195$) showed increases in time spent by DP189 rats in crossing the location previously occupied by the hidden platform during test period (Fig. 2C).

3.2. Effect of L. plantarum DP189 on histopathological changes of the hippocampus

Histological changes of hippocampal neuron morphological structures in rats are shown in Fig. 3. In controls, the pyramidal-shaped cells in the CA1 sub-region were regularly shaped, clearly defined and tightly arranged, with rounded vesicular nuclei and prominent nucleoli. Conversely, neuronal cells of the CORT group were small and more deeply stained, revealing irregular, polygonally shaped and shrunken pyramidal cells with evidence of karyopyknosis. These changes show that the pyramidal-shaped cells had been injured, resulting in morphological changes in cell structure. Conversely, most pyramidal cells in the fluoxetine and DP189 groups were intact, with few cells exhibiting karyopyknosis.

3.3. L. plantarum DP189 increased hippocampal monoamines levels

Changes in hippocampal monoamines composition with various treatments are shown in Fig. 4. The four groups exhibited significant differences in levels of both 5-HT ($F(3,34) = 1.257$, $P < 0.05$) and DA ($F(3,35) = 4.873$, $P < 0.05$) levels. After DP189 administration, 5-HT, DA and NE contents in hippocampal supernatants increased to 3.18 ± 0.43, 0.08 ± 0.02 and 0.293 ± 0.08 ng/mL respectively. This result indicates that *L. plantarum* DP189 may inhibit CORT-induced

Table 1
Effect of *L. plantarum* DP189 on the escape latency time of rats in Morris water maze.

Groups	1st day	2nd day	3rd day	4th day
Control	62.12 ± 7.97	38.46 ± 4.16**	29.37 ± 3.36**	21.91 ± 3.87**
CORT	61.17 ± 6.57	57.18 ± 3.83##	51.83 ± 4.76##	47.98 ± 3.72##
CORT + Fluoxetine	58.88 ± 6.97	49.37 ± 3.53#	36.45 ± 4.15**	27.37 ± 3.36**
CORT + DP189	60.38 ± 7.12	48.12 ± 4.98*#	38.93 ± 4.82**	28.45 ± 4.32**

Data are represented as mean ± SD.
$P < 0.01$, # $P < 0.05$ vs Control; ** $P < 0.01$,* $P < 0.05$ vs CORT.

hippocampal declines in monoamine levels.

3.4. L. plantarum DP189 decreased pro-inflammatory cytokine levels in serum

As shown in Fig. 5, repeated injections of CORT significantly increased serum pro-inflammatory cytokine TNF-α ($F(3,33) = 14.933$, $P = 0.000$) and IL-1β ($F(3,32) = 4.713$, $P = 0.007$) levels as compared with the control group. However, after 3 weeks of DP189 treatment, serum IL-1β ($P = 0.027$) and TNF-α ($P = 0.003$) levels decreased markedly compared with CORT group levels. Meanwhile, the DP189 group showed greater reductions of pro-inflammatory cytokine levels than did the fluoxetine group.

3.5. L. plantarum DP189 attenuated apoptosis of hippocampal cells

TUNEL staining was used to further illuminate DP189 effects on apoptosis of hippocampal neurons. As shown in Fig. 6, percentages of TUNEL-positive cells exhibiting green fluorescence approached 36.61 % of total cells in the CORT group as compared to only 19.89 % in the DP189 group. Therefore, it is clear that DP189-treated rats exhibited less hippocampal apoptosis of neurons than did the CORT group ($F(3,8) = 4.713$, $P = 0.027$).

3.6. The effect of L. plantarum DP189 on MKK7, JNK2, Bcl-2 and Bax immunocontent

Levels of key proteins within the JNK signal pathway, as detected via Western blot analysis, are shown in Fig. 7A, where MKK7 ($F(3,8) = 55.347$, $P = 0.001$) and JNK2 ($F(3,8) = 12.517$, $P = 0.001$) immunocontents were significantly down-regulated after DP189 treatment as compared to corresponding CORT-only group results ($P < 0.01$). Additionally, DP189 effects on immunocontent of major Bcl-2 family members Bcl-2 and Bax were also investigated, since Bcl-2 family members are known to play critical regulatory roles in controlling apoptosis. The results indicated that high CORT-induced Bax immunocontent levels were markedly decreased by DP189 treatment ($F(3,8) = 33.318$, $P = 0.000$); by contrast, CORT-induced down-regulation of Bcl-2 ($F(3,8) = 53.496$, $P = 0.000$) immunocontent was restored by DP189 (Fig. 7B). Therefore, the above mentioned results

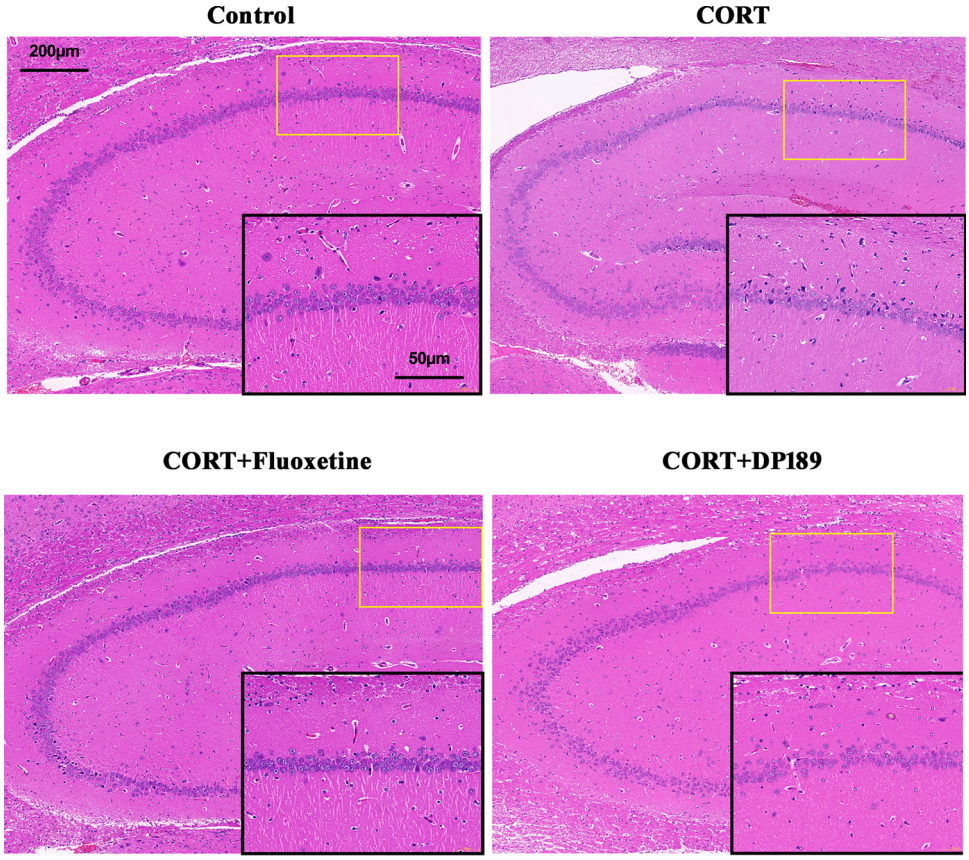

Fig. 3. *L. plantarum* DP189 attenuates CORT-induced hippocampal histopathological change. The entire hippocampus (and in particular its CA1 sub-region) was stained with H&E to assess the morphological changes of hippocampus. (H&E staining, 100 × and 400 × magnification).

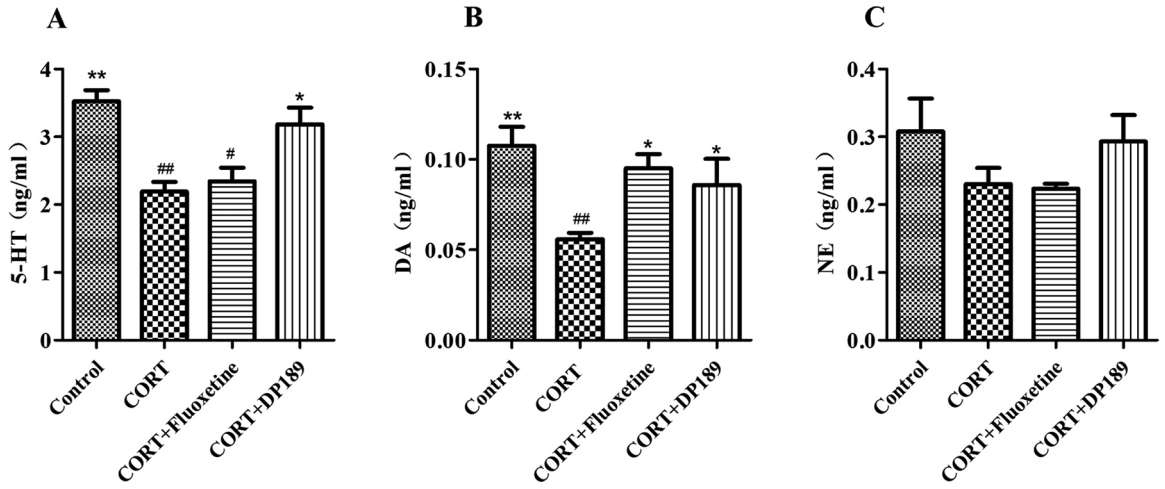

Fig. 4. Effect of *L. plantarum* DP189 on the monoamines level in hippocampus. The changes of monoamines were detected by ELISA. A: 5-hydroxytryptamine, B: Dopamine, C: Norepinephrine. All the values were given as mean ± SD (n = 10), $^{\#\#}$ P < 0.01, $^{\#}$ P < 0.05 vs Control; ** P < 0.01, * P < 0.05 vs CORT.

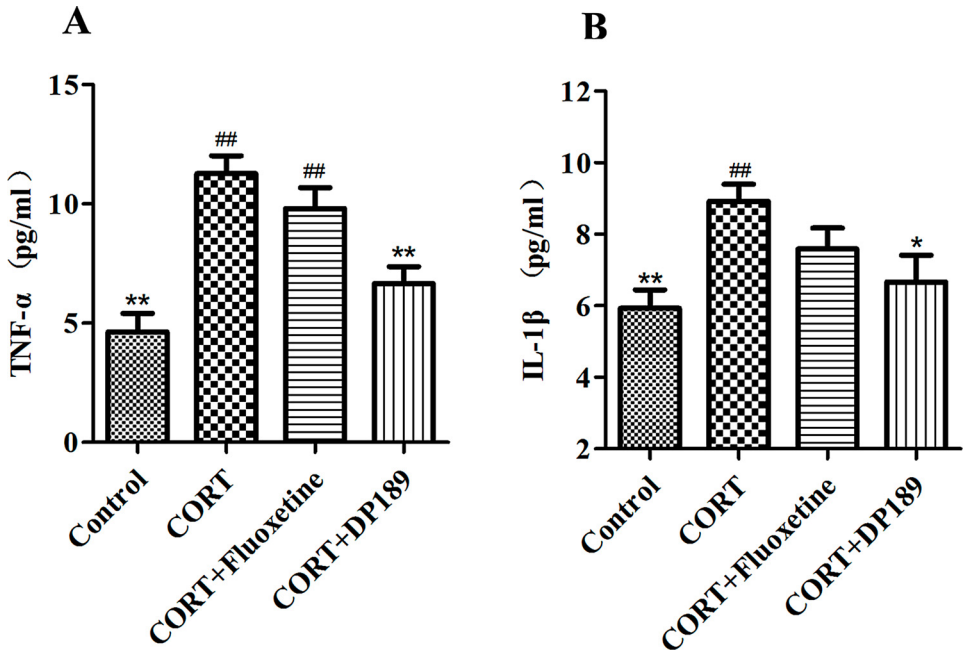

Fig. 5. Effect of *L. plantarum* DP189 on the level of pro-inflammatory cytokines in serum. ELISA was used to detect the change of Pro-inflammatory cytokines. A: TNF-α; B: IL-1β. All the values were given as mean ± SD (n = 10), $^{\#\#}$ P < 0.01, $^{\#}$ P < 0.05 vs Control; ** P < 0.01, * P < 0.05 vs CORT.

collectively suggest that DP189 may attenuate hippocampal neuronal apoptosis by inhibiting both JNK pathway activation and Bax/Bcl-2-mediated apoptosis.

4. Discussion

This study confirmed and extended previous results and demonstrated that CORT administration induced depression-like behavior and cognitive (memory) deficits in rats after 21 days of CORT administration [13,14]. These behavioral and cognitive abnormalities were accompanied by biochemical changes that included decreased hippocampal levels of 5-HT, DA and NE, histopathological alterations of hippocampal morphology and serum increases of pro-inflammatory cytokine levels. Notably, most abnormalities induced by CORT administration were alleviated by DP189 supplementation, thus supporting our hypothesis that DP189 supplementation can exert antidepressant-like effects by alleviating behavioral, cognitive and biochemical abnormalities induced by CORT.

Changes in the monoamine neurotransmitter system are key features of depression, anxiety and other neuropsychiatric disorders. Previously, targeted metabonomics studies of mouse models of depression revealed significant changes in neurotransmitter levels. Concurrently, other research has demonstrated that *L. plantarum* PS128 administration could improve anxiety- and depressive-like symptoms and regulate levels of neurochemicals known to be associated with emotional disorders [10]. Meanwhile, probiotic effects observed using a maternal separation rat depression model indicated that probiotic treatment could restore levels of monoamines in brain areas known to be associated with depression [22]. Taken together, these findings provide evidence that ingested probiotics may improve the ability of rats to cope with stress. Interestingly, several researchers have shown that the absence of an intestinal microbiome may alter several brain neurotransmitters within important pathways involved in the interaction between intestinal flora and the brain, such as 5-HT, DA, NE, etc. Consistent with their results, our results here showed that DP189 significantly increased 5-HT, DA and NE levels in the hippocampus.

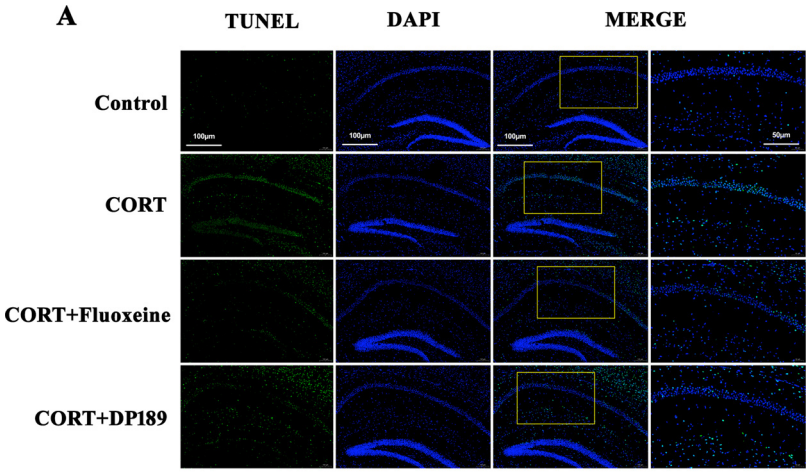

Fig. 6. Effects of *L. plantarum* DP189 on apoptosis of hippocampus. A: The apoptosis of hippocampal neuron was detected by TUNEL staining (100 × and 400 × magnification). B: Quantification of TUNEL staining. The histogram shows the relative proportion of TUNEL-positive cells in different groups, Values were expressed as mean ± SD, $^{\#\#}$ P < 0.01, $^{\#}$ P < 0.05 vs Control; ** P < 0.01, * P < 0.05 vs CORT.

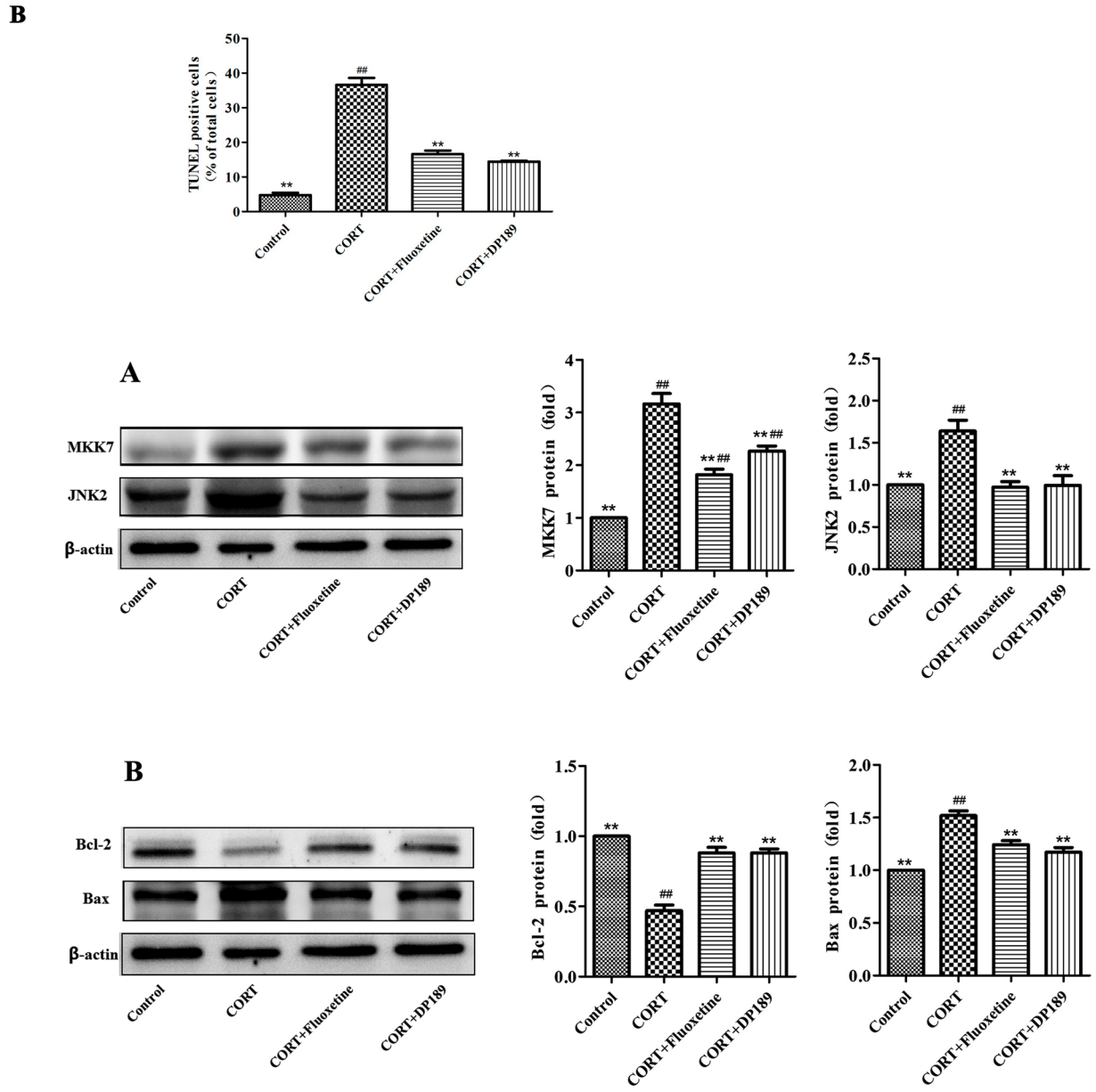

Fig. 7. Effects of *L. plantarum* DP189 on immunocontent of MKK7, JNK2, Bcl-2 and Bax protein. MKK7 and JNK2 (A), Bcl-2 and Bax (B) protein were measured by western blot analysis. Data were expressed as mean ± SD. β-actin was used as control. Experiments were done in triplicate. $^{\#\#}$ P < 0.01, $^{\#}$ P < 0.05 vs Control; ** P < 0.01, * P < 0.05 vs CORT.

Currently, many researchers are actively studying the influence of intestinal flora profiles on health. In turn, probiotics as a source of intestinal symbiotic microorganisms have been closely studied for effects on a variety of diseases linked to dysregulation of intestinal flora. Notably, several clinical and animal experiments have shown that probiotic treatments may improve symptoms of neuropsychiatric disorders by inducing changes in intestinal microbiota [23,24]. Indeed, the absence of commensal microbiota has been shown to greatly impact expression profiles of monoamine neurotransmitter-related genes. Based on these findings, numerous researchers are investigating the mechanisms by which gut symbionts regulate monoamine neurotransmitter expression, while also working to elucidate relationships between gut flora and neuropsychiatric disorders [25].

As another key research avenue, associations between depression and elevated levels of central and peripheral nervous system inflammatory chemokines and other inflammatory mediators are under intense study using both human and animal depression models [26]. Specifically, several studies have shown serum IL-1β and TNF-α concentrations to be directly correlated with patient depression severity [17,27,28]. Meanwhile, animal studies have demonstrated that preclinical IL-1β decreases were correlated with alleviation of depression-associated behaviors [18]. Importantly, beneficial effects of probiotics for relieving neurological and psychiatric symptoms have been linked to reduced proinflammatory cytokine levels [29]. In our study, treatment with *L. plantarum* DP189 significantly reduced levels of IL-1β and TNF-α relative to CORT group levels; our results mirror previously reported results demonstrating protective effects of probiotic against certain types of disorders, specifically depression and anxiety disorders that had been previously linked to microbiota-gut-inflammasome-brain axis dysregulation [30]. Here, DP189 treatment may have acted via the same mechanism; our Western blot results revealed that DP189 effects for alleviating neuronal apoptosis and inflammation in the CORT-induced chronic stress exposure model mainly depended on down-regulation of MKK7and JNK2 immunocontent. Therefore, our results and those of previous studies collectively demonstrate that therapeutic strategies which alleviate apoptosis and inflammation hold considerable promise for depression treatment [31].

Notably, previous research has confirmed the JNK signaling pathway to be the main modulatory pathway involved in neurodegenerative disease-associated neuronal cell apoptosis [32]. For example, pretreatment with paeoniflorin was shown in one study to significantly reduce neuronal loss and tributyltin in chloride (TBTC)-induced cell damage by decreasing immunocontent of pMKK4 and p-JNK proteins [33]. Meanwhile, another study demonstrated that puerarin may attenuate neuronal apoptosis in a high glucose-induced depression model by reducing p38 and JNK phosphorylation [34]. In our study, we found that immunocontent levels of MKK7 and JNK2 in DP189-treated rats were significantly lower than respective CORT group levels, with DP189 group TUNEL-positive cell numbers markedly reduced relative to CORT group numbers ($P < 0.01$). We therefore postulate that DP189 may regulate neuronal cell apoptosis by down-regulating immunocontent of MKK7 and JNK2 proteins through the JNK signaling pathway (Fig. 6A). In this scenario, mitochondrial translocation of activated JNK may directly induce mitochondria-dependent apoptotic signaling via regulation of targeted cytoplasmic Bcl-2 and Bax protein activities [35]. Notably, effects of activities of Bcl-2 family proteins on hippocampal neuronal apoptosis and processes involved in neuronal damage have been confirmed in several studies [36,37]; here, opposing dynamic expression tendencies of Bax and Bcl-2 immunocontent were observed between DP189 and CORT groups, with DP189 likely exerting effects on MKK7 and JNK2 immunocontent that ultimately reduced apoptosis. Taken together, the abovementioned experimental results suggest that DP189 has a neuroprotective effect on CORT-induced hippocampal apoptosis and injury that are due, at least in part, to inhibition of JNK signaling pathway activation and Bax/Bcl-2-mediated apoptosis.

In conclusion, *L. plantarum* DP189 can alleviate CORT-induced depression by ameliorating depression-like behaviors, increasing neurotransmitters in brain tissue, reducing serum levels of inflammatory factors and by regulating hippocampal neural apoptosis. Collectively, these results highlight *L. plantarum* DP189 as a new probiotic candidate with potential beneficial value for prevention and treatment of depression.

Declaration of Competing Interest

The authors declare they have no conflicts of interest.

CRediT authorship contribution statement

Yujuan Zhao: Investigation, Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Ge Yang:** Investigation. **Zijian Zhao:** Investigation, Writing - original draft. **Chao Wang:** Investigation. **Cuicui Duan:** Investigation. **Lei Gao:** Data curation. **Shengyu Li:** Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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