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Research report

Exosome derived from bone marrow derived mesenchymal stem cells prevents LPS-induced depressive like behaviors

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ABSTRACT

Major depressive disorder (MDD) is a multifaceted mental disorder marked by a spectrum of significant and persistent low mood symptoms. Its etiology involves genetic and environmental factors. In addition, the inflammatory process plays a crucial role in the pathophysiology of depression. Exosomes derived from bone marrow mesenchymal stem cells (BMSCs) have demonstrated significant effects in reducing proinflammatory cytokines. However, there is limited research on whether exosomes can prevent the occurrence of LPS-induced depression. This study aimed to investigate the role of BMSC-derived exosomes in LPS-induced depression and explore the underlying mechanisms. We administered exosomes to LPS-induced depression mice via the caudal vein and evaluated their effects on depressive-like behaviors. Our findings indicate that four injections of exosomes (200 μl at a concentration of 1.4×10^{11} particles/mL, administered every three days) significantly prevented depressive-like behaviors in LPS-induced depression mice. Further analyses revealed that exosome treatment reduced levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and increased anti-inflammatory cytokine IL-10. Additionally, exosome treatment markedly reduced oxidative stress in both the central and peripheral nervous systems of LPS-treated mice. Moreover, our data suggest that exosome treatment increased astrocyte proliferation and neurogenesis in the hippocampus of LPS mice. In summary, our results demonstrate the antidepressant effects of BMSC-derived exosomes in LPS-induced depression mice, suggesting a potential new therapeutic target for major depressive disorder (MDD).

1. Introduction

Major depressive disorder (MDD) is a debilitating neuropsychiatric condition characterized by persistent low mood, anhedonia, reduced energy, disturbances in appetite and sleep patterns, and an increased risk of suicidal behavior (Chen et al., 2024; Norkeviciene et al., 2022). According to World Health Organization projections, MDD is anticipated to emerge as the leading contributor to the global disease burden by 2030 (Li et al., 2021a). This disorder not only severely compromises patients' quality of life but also imposes substantial socioeconomic burdens worldwide (Mccarron et al., 2021; Liu et al., 2020; Zhdanava et al., 2021). Current therapeutic interventions for MDD, particularly in the context of postpartum depression, demonstrate limited efficacy and are primarily categorized into two classes: traditional antidepressants and novel rapid-acting agent. Traditional antidepressants are constrained by their delayed therapeutic onset, adverse side effects (headaches, nausea, vomiting, and sexual dysfunction) (Zhou et al., 2024). Conversely, novel rapid-acting agents are associated with central nervous system-related adverse effects (drowsiness, blurred vision, insomnia, dizziness, headaches, and tremors) and gastrointestinal complications (including nausea, vomiting, diarrhea, and constipation) (Hess et al., 2022). Importantly, neither pharmacological class demonstrates universal efficacy across the heterogeneous population of postpartum depression patients. These limitations underscore the critical need for developing effective preventive strategies against depression, which would significantly alleviate healthcare burdens and enhance social stability.

Emerging evidence has established a robust association between neuroinflammatory processes and the pathogenesis of depressive disorders (Hess et al., 2022). Neuroinflammation has consequently

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emerged as a pivotal focus in elucidating the etiological underpinnings of depression. Current research indicates that MDD patients exhibit elevated inflammatory states, triggering a cascade of biochemical alterations (Drevets et al., 2022; Islam et al., 2023). These immune signaling molecules can directly modulate cerebral activity and induce central nervous system (CNS) inflammatory responses through blood-brain barrier (BBB) disruption. This neuroinflammatory cascade manifests through multiple pathways: elevation of pro-inflammatory cytokines, activation of glial cells, infiltration of peripheral leukocytes, and neural tissue damage, collectively disrupting neural network integrity and neurotransmitter homeostasis, ultimately precipitating depressive-like behaviors (Campos et al., 2020; Afridi and Suk, 2021). Furthermore, neuroinflammation compromises neuroprotective mechanisms and neuronal repair capacity, exacerbating neurodegeneration and perpetuating the depressive pathology. Experimental evidence demonstrates that lipopolysaccharide (LPS) administration increases cerebral pro-inflammatory mediators, inducing anxiety- and depression-like behaviors in murine models (Guo et al., 2024; Wang et al., 2024). Clinical and preclinical studies have consistently identified elevated levels of specific pro-inflammatory factors in both MDD patients and various depression models, while antidepressant treatments have been shown to downregulate these inflammatory markers, suggesting an anti-inflammatory component to their therapeutic efficacy (Parente et al., 2024). Our previous investigations have further elucidated the intricate relationships between depression, neurogenesis, and oxidative stress, proposing that interventions targeting neuroinflammation and neurogenesis may represent a promising preventive strategy against depression (Liu et al., 2023a).

Exosomes, small membrane-bound extracellular vesicles ranging from 30 to 150 nanometers in diameter, are secreted by virtually all cell types (Huda et al., 2021; He et al., 2018). These nanovesicles encapsulate diverse cellular components, including DNA, RNA, lipids, metabolites, and cytoplasmic/cell surface proteins, exhibiting multifaceted biological properties such as antioxidant, anti-inflammatory, and anti-apoptotic effects (Kalluri and Lebleu, 2020). Recent advances have highlighted the therapeutic potential of mesenchymal stem cell (BMSC)-derived exosomes in addressing various brain disorders (Ni et al., 2019). Substantial evidence indicates that BMSC-derived exosome administration can significantly mitigate neuroinflammation while promoting neurogenesis (Zhang et al., 2015). Clinical investigations have consistently demonstrated the capacity of exosomes to restore inflammatory homeostasis and oxidative stress balance in depression model systems (Lyu et al., 2024). While these findings suggest potential therapeutic benefits of BMSC-exos in depression management, their preventive capabilities remain unexplored. Investigating the prophylactic potential of MSC-derived exosomes against depression represents a significant scientific endeavor with profound societal implications.

In the present study, we aimed to elucidate the functional significance of bone marrow mesenchymal stem cell (BMSC)-derived exosomes in a murine model of depressive-like behavior. Our experimental paradigm involved intravenous administration of MSC-derived exosomes via the tail vein, followed by LPS-induced depression modeling. Through this approach, we sought to investigate the preventive potential of MSC-derived exosomes against depression onset and elucidate the molecular mechanisms underlying their effects on cognitive and motor functions.

2. Materials and methods

2.1. Bone mesenchymal stem cells culture

Three-week-old C57BL/6 specific pathogen-free (SPF) mice were used for bone marrow mesenchymal stem cell (BMSCs) isolation. Mice were humanely sacrificed by cervical dislocation after anesthesia with 2 % isoflurane, followed by immersion in 75 % ethanol for 5 min to ensure aseptic conditions. Under a laminar flow hood, bilateral humeri were dissected, and adherent muscle tissues were meticulously removed using sterile forceps. The epiphyses of humeri were transected, and bone marrow was flushed out with 5 mL α-MEM medium (Gibco, USA) containing 10 % fetal bovine serum (FBS, Thermo Fisher) and 1 % penicillin-streptomycin (Gibco) using a 22 G needle. The cell suspension was gently pipetted to achieve uniform mixing, then passed through a 70-μm cell strainer (BD Falcon) to remove tissue debris. After discarding the medium, trypsin was added for digestion. The cell density was adjusted to 1×10^6 cells/mL, and the cells were seeded into culture dishes and incubated at 37°C in a 5 % CO_2 incubator. The medium was first changed after 8 h to remove non-adherent cells. When the third-passage cells reached 80 % confluence, bone marrow mesenchymal stem cells (BMSCs) were identified by flow cytometric analysis of CD90 + /CD45-/CD34- surface markers

2.2. Isolation, identification and Labeling of exosomes

To obtain exosomes derived from bone marrow mesenchymal stem cells (BMSCs), we cultured BMSCs to the third passage. When cell confluence reached 80 %, the culture medium was switched to serum-free medium, and cells were cultured for an additional 48 h. Following collection of the culture supernatant, exosomes were isolated and extracted using the Exosome Isolation Kit (Thermo Fisher Scientific). Subsequently, exosomes were concentrated using a Vivaspin® protein concentrator (Sartorius) at 10,000 ×g for 30 min. The isolated exosomes were then systematically characterized using the following approaches:Particle size analysis: The concentration and particle size distribution of exosomes were determined using a ZetaView nanoparticle tracking analyzer (Particle Metrix). Morphological assessment: The characteristic cup-shaped morphology of exosomes was visualized via transmission electron microscopy (TEM). Marker validation: Western blotting was performed to detect the expression of exosome-specific markers, including Alix and HSP70. For exosome labeling, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocar- bocyanine perchlorate (DiI)-a far-red fluorescent dye that specifically binds to the lipid bilayer of cell membranes and is widely used for specific exosome labeling—was employed. The labeling procedure was conducted as follows: DiI dye was mixed with the exosome suspension, followed by incubation in the dark at 37°C for 30 min. Free dye was subsequently removed by ultracentrifugation to obtain purified labeled exosomes. Labeling efficiency was verified using fluorescence microscopy. For in vivo tracking, DiI-labeled exosomes were administered via tail vein injection, and their in vivo distribution was assessed based on the spatial pattern of fluorescent signals, which indicated the cellular uptake sites of exosomes.

2.3. Tail vein injection

The mice were fixed using a mouse holder. The tails of the mice were wiped with 75 % alcohol to dilate the veins and expose the blood vessels. At the middle-two-thirds section of the tail, with the bevel of the needle facing upwards, the needle tip was inserted into the vein and then advanced parallel to the vessel. After ensuring that the needle tip was moving smoothly within the blood vessel, the needle was slowly pushed forward. Once all the exosomes had been injected, the injection site was pressed with a dry cotton ball to stop the bleeding.

2.4. Animals

Thirty-two C57BL/6 mice (male, seven week-old) were provided from Vital River Laboratory (Beijing, China). Four mice were housed in one cage under controlled breeding room conditions(temperature, 24 ± 1 ℃; humidity, 50 ± 1 %) with a 12/12 h light/dark cycle. The mice adapted to the living environment for 1 weeks before the experiment. The experimental mice were randomly divided into the following four groups: control group (Con, n = 8), model group (LPS,1 mg/kg, i.p., n = 8), LPS+Exosome group(EXO, 200ul 1.4 $\times 10^{11}$/mL, n = 8) and fluoxetine group (Flu,10 mg/kg, n = 8). The mice that received MSC-

exos were four injections in total.All animal procedures were approved by the Animal Care and Use Committee of the Minzu University of China (ECMUC2019001AO).

2.5. Drug administration and animal experimental procedures

Exosomes were administered via injection at three-day intervals, with a total of four injections. Commencing on the eighth day subsequent to the initial exosome injection, lipopolysaccharide (LPS) was introduced, and this was followed by consecutive daily injections over a period of seven days. The comprehensive protocol is delineated in Fig. 2A. Subsequently, a series of behavioral assays were carried out to assess the motor performance of the mice.

2.6. Behavioral studies

OFT(Open Field Test)

The open field test (OFT) is one of the most classic paradigms in neurobehavioral research, which evaluates depression-like behaviors in mice by quantifying their movement trajectories and spatial preference in a novel open environment. The OFT was conducted as the first behavioral assay in this study following a previously described with modifications (Liu et al., 2023b). An open field apparatus (50 cm×50 cm×50 cm) was used to performing the test. The base was divided into nine squares on average. Mice were placed in the experimental room for 1 h to adapt the new environment and each mouse was placed in the center squares of the base. The mice was allowed move freely for 6 min and the movement track of mice were recorded by video tracking system. The total distance in the last 5 min was recorded using an open field system (Tai meng Software Co. Ltd., Sichuan). After each animal test, the bottom and side walls of the apparatus were wiped with 70 % ethanol to eliminate residues of urine, feces, and odor."

NSFT(Novelty Suppressed Feeding Test)

The Novelty Suppressed Feeding Test (NSFT) can evaluate depression-related symptoms in mice by observing the latency of animals to approach food in a novel environment. NSFT was performed in the open field apparatus (50 cm x 50 cm x 50 cm) with small pieces of food on the centure following a previously described with modifications (Luo et al., 2024). Mice were fasted for 24 h before the test and placed in one corner of the apparatus bottom during the experiment. After allowing free movement, the time taken for mice to pick up the food was recorded via a camera system. The experiment lasted five minutes, and if the mice failed to explore the food within five minutes, the latency time was recorded as five minutes.It should be noted that the apparatus were wipe clean before next mice experiments.

TST(Tail suspension test)

Tail suspension test (TST) is a classic method for reflecting depressive states by recording the immobility time of animals. The experiment is conducted in a tail suspension chamber following a previously described with modifications (Fu et al., 2023), where the mouse is suspended approximately 30 cm above the ground by fixing the distal 2–4 cm of its tail with adhesive tape. Immobility is defined as the state when the mouse ceases struggling. The behavior is observed for 6 min, and the immobility time during the last 5 min is recorded using a video tracking system. It is noteworthy that the experimental environment must be kept quiet with uniform lighting. Additionally, the apparatus should be cleaned after each trial to prevent odor interference with subsequent animals.

FST(Forced Swimming Test)

The forced swimming test (FST) is a classic tool in depression research, evaluating antidepressant effects by quantifying "behavioral despair." FST was performed following a previously described with modifications (Du et al., 2023). Each mice was placed into a glass cylinder (50 cm×10 cm) containing a depth of 25 cm water at a temperature 24 + 1 °C. Mice are placed in the inescapable water tank, and the duration during which they cease struggling (only making slight movements to keep their heads above water) is recorded as immobility time. The observation lasts for 6 min, with the last 5 min of immobility time recorded using a video tracking system. After the experiment, the animals are immediately dried to prevent hypothermic stress, and a heating pad is used if necessary.

2.7. Quantitative real-time polymerase chain reaction

According to our previous report (Liu et al., 2023a), the total RNA was extracted from hippocampal tissue and prefrontal tissue by using Trizol reagent (Thermo Fisher, MA, USA) according to the instructions of the manufacturer. The cDNA was synthesized by using Reverse Transcription Kit (GenStar, Beijing, China), and then PCR was performed on LightCycler® 96 system (Roche, Switzerland). β-actin was used as an internal control to normalize the expression levels of the target gene. The relative quantification of the transcripts was calculated by using the $2^{-\Delta\Delta CT}$ approach. Primers were made by sangon Biotech, Shanghai, listed in Table 1.

2.8. Immunofluorescence analysis

The brains fixed with 4 % paraformaldehyde were embedded in optimal cutting temperature compound, and cut into 35-μm-thick sections. For immunofluorescence staining, the antigen retrieval of sections was washed 3 times with 1x PBS(5 min each) buffer and then blocked blocking solution (Beyotime, Shanghai, China, P0260) for 15 mins at room temperature. Subsequently, the sections were incubated overnight at 4°C with primary antibodies against Iba-1(FUJIFILM Wako, 019–19741,1:1000),GFAP(Proteintech,16825–1-AP,1:300), DCX(Cell Signaling Technology, #4604, 1:800) and washed with PBS for 3 times (5 mins each) then incubated with a secondary antibody for 2 h at room temperature. Finally, the DAPI was added to stain the nuclei. The fluorescence signals were captured via Leica TCS SP8 (Leica, Solms, Germany) and the number of Iba-1 cells and GFAP cells in PFC sections of each animal was calculated by Image Pro Plus 6.0. The specific operations are as follows: Import the image and separate the GFAP and Iba-1 channel. Enhance the fluorescence signal by adjusting parameters in "Brightness/Contrast". Subsequently, apply Gaussian Blur with a radius of 0.8 pixels to reduce background noise. Use the "Threshold" tool to manually adjust the threshold at 150 to retain GFAP-positive and Iba-1 -positiveregions and remove non-specific staining.

2.9. Detection of oxidative markers

As previously described (Liu et al., 2022), we determined the levels of oxidative markers (malondialdehyde, superoxide dismutase, total antioxidant capacity, and nitric oxide) in serum and hippocampal tissue using the enzymatic colorimetric method. The detection procedures were carried out in accordance with the instructions provided by the manufacturer(Nanjing Jiancheng Bioengineering Institute).

Table 1
All Primer Sequences for qRT-PCR.

Genes	Primers (5'-3')	
IL−1β	Forward	AGCTGGAGAGTGTGGATCCC
	Reverse	CCTGTCTTGGCCGAGGACTA
TNF-α	Forward	GGCTTTCCGAATTCACTGGAG
	Reverse	CCCCGGCCTTCCAAATAAA
IL−6	Forward	GAAATGCCACCTTTTGACAGTG
	Reverse	TGGATGCTCTCATCAGGACAG
IL−10	Forward	GCTCTTACTGACTGGCATGAG
	Reverse	CGCAGCTCTAGGAGCATGTG
β-Actin	Forward	AAGCCCTGGATGAAGAAACAG
	Reverse	TGGGAACCAATCTCGTAGGTC

2.10. Statistical analysis

All data were presented as means ± SEM and analysed by GraphPad prism 9.0. *$p < 0.05$,** $p < 0.01$,*** $p < 0.001$ were set as the significant difference.The difference among groups were asssessed by one-way analysis of variance(ANOVA) followed by Tukey test.

3. Results

3.1. Characterization of BMSC and BMS-derived exos

Following sorting and purification, BMSCs exhibited normal proliferation with a fibroblastic, colony-forming growth pattern (Fig. 1A). Flow cytometric analysis confirmed the phenotypic characteristics of these cells, which were positive for CD44 and CD90 ($CD44^+$, $CD90^+$) and negative for CD34 ($CD34^-$) (Fig. 1B–D). BMSC-derived exosomes were extracted using a commercial kit. Under a transmission electron microscope, the BMSC-exos showed a typical double-layer membrane and cup holder-like structure with a particle size of approximately 50 nm (Fig. 1E). The exosomal marker proteins Alix and HSP70 were highly expressed (Fig. 1F). In additional, we observed that exosomes can cross the blood-brain barrier and enter the hippocampal tissue by labeling exosomes with Dil(Fig. 1G,H).

3.2. Exosome prevented LPS-induced depressive-like behaviors in mice

To investigate the antidepressant effects of exosome on LPS-induced depressive-like behaviors of mice, we performed a series of behavioral tests including OFT, NSFT, TST and FST. As shown in Fig. 2B, there is no significant differences in body weight among the groups during drug administration. The result indicated that exosome treatment had no effect on the physical growth and development of the mice. OFT is a commonly used experimental paradigm for assessing the behavioral responses of laboratory mice in a novel environment, thereby inferring their psychological states such as anxiety, depression, exploratory behavior, and locomotor activity. Compared with the control group, the LPS challenge decreased the motor function, while the supplementation of exosomes reversed this change ($P < 0.01$, $P < 0.001$,Fig. 2C,D). During TST, Fig. 2E, the immobility time of LPS-treated group in the TST was greatly longer than that of the control group, the exosome administration decreased the immobility time, similar to the fluoxetine hydrochloride treatment group($P < 0.001$,Fig. 2E).In the NSFT,the time in LPS-treated group to find food in the central area was largely increased, and significantly decreased by treatment of exosome and fluoxetine hydrochloride ($P < 0.05$, $P < 0.01$, Fig. 2F,G). In the FST, the immobility time was increased in LPS-treated group, the immobility time was decreased by treatment of exosome and fluoxetine hydrochloride ($P < 0.01$, $P < 0.001$,Fig. 2H,I). Taken together, these results indicate the exosome prevents LPS-induced depressive-like behaviors.

3.3. Exosome ameliorated neuronal damage by alleviating neuroinflammation

As previously described, the prefrontal cortex (PFC) and hippocampus are two key brain regions central to emotional regulation.Lipopolysaccharide (LPS), a potent inducer of systemic inflammation, triggers excessive immune activation and cytokine hyperproduction under conditions of high-dose or sustained exposure. In this study, we explored the impact of exosomes on the production of pro-inflammatory cytokines within the PFC and hippocampal regions in a murine model of LPS-induced neuroinflammation. In prefrontal cortical tissues,LPS treatment significantly increased the expression levels of pro-inflammatory cytokines (*il-1β*、*Tnf-α*、*il*-6), while markedly decreasing the anti-inflammatory factor *il-10*. Exosome and fluoxetine hydrochloride treatment significantly reversed these changes ($P < 0.05$, $P < 0.01$, $P < 0.001$, Fig. 3A-D). In the hippocampal tissues, exosome and fluoxetine hydrochloride treatment significantly reduced the expression levels of pro-inflammatory factors (*il-1β*、*Tnf-α*、*il*-6). For *il-10*, our results showed that fluoxetine can significantly increase the expression of anti-inflammatory factor *il-10*. In additional, After exosome treatment, the expression of the anti-inflammatory factor *il-10* was increased, but without statistical significance. ($P < 0.05$, $P < 0.01$, Fig. 3E-H).These results show that exosome ameliorate the neuroinflammation in LPS challenged mice by reducing the levels of pro-

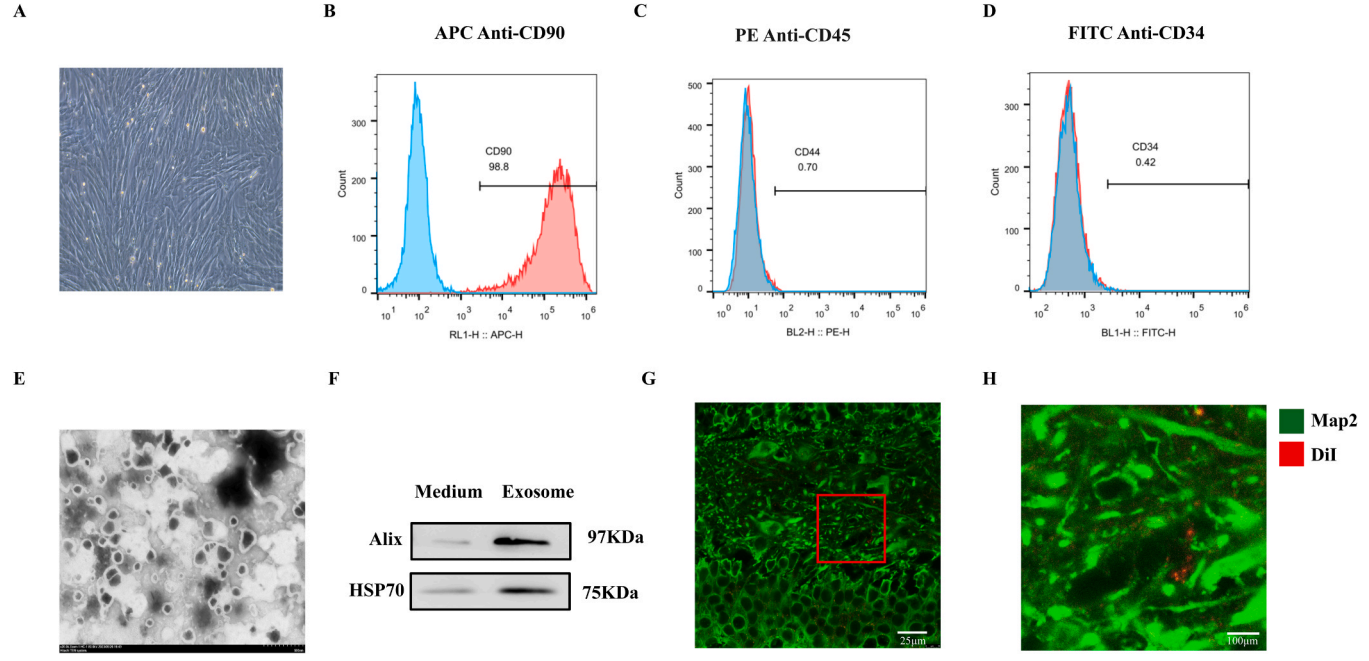

Fig. 1. Extract and culture of mouse BMSCs and identification and label of BMSC-exos. (A). The images of mouse BMSCs at passage 4. (B,C,D.) Flow cytometric analysis of surface marker gene expressions of mouse BMSCs, characterized as CD90-positive and CD44-, CD34-negative. (E). Exosome morphology under transmission electron microscope.(F). Western blot photos of exosome markers. (G,H). Immunofluorescence micrographs of co-staining with MAP2 and Dil.

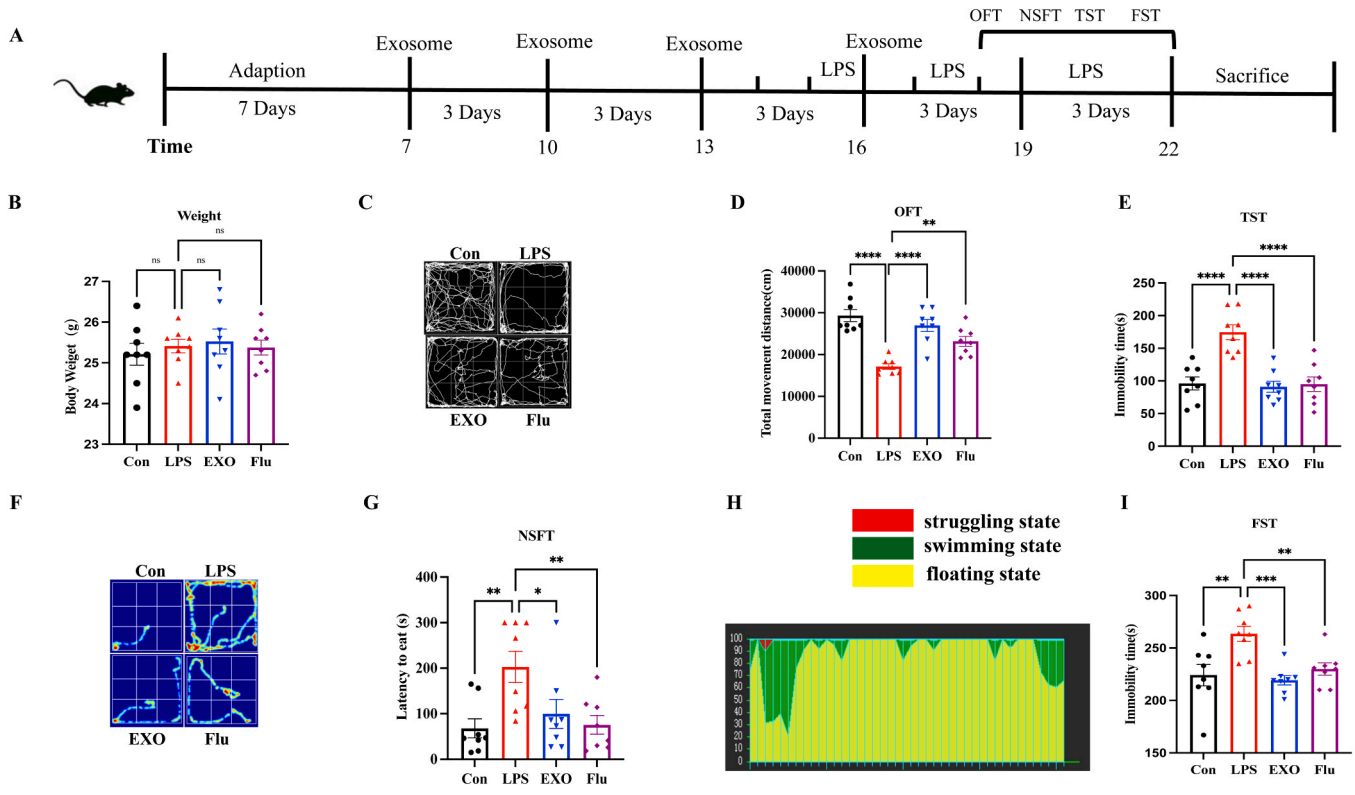

Fig. 2. Assessment of the effect of exosomes on LPS-induced depressive-like behaviors. (A) Flow of experimental design. (B) Measurement of body weight.(C) Schematic diagram of the movement trajectory of WT mice. (D) OFT. (E) TST. (F) Trajectory diagram of food finding by mice in the LPS-treated group. (G) NSFT. (H) Time proportion of three states of exosome-treated mice in the forced swimming test. (I) FST. Data are presented as means ± SEM, with n = 8 in each group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

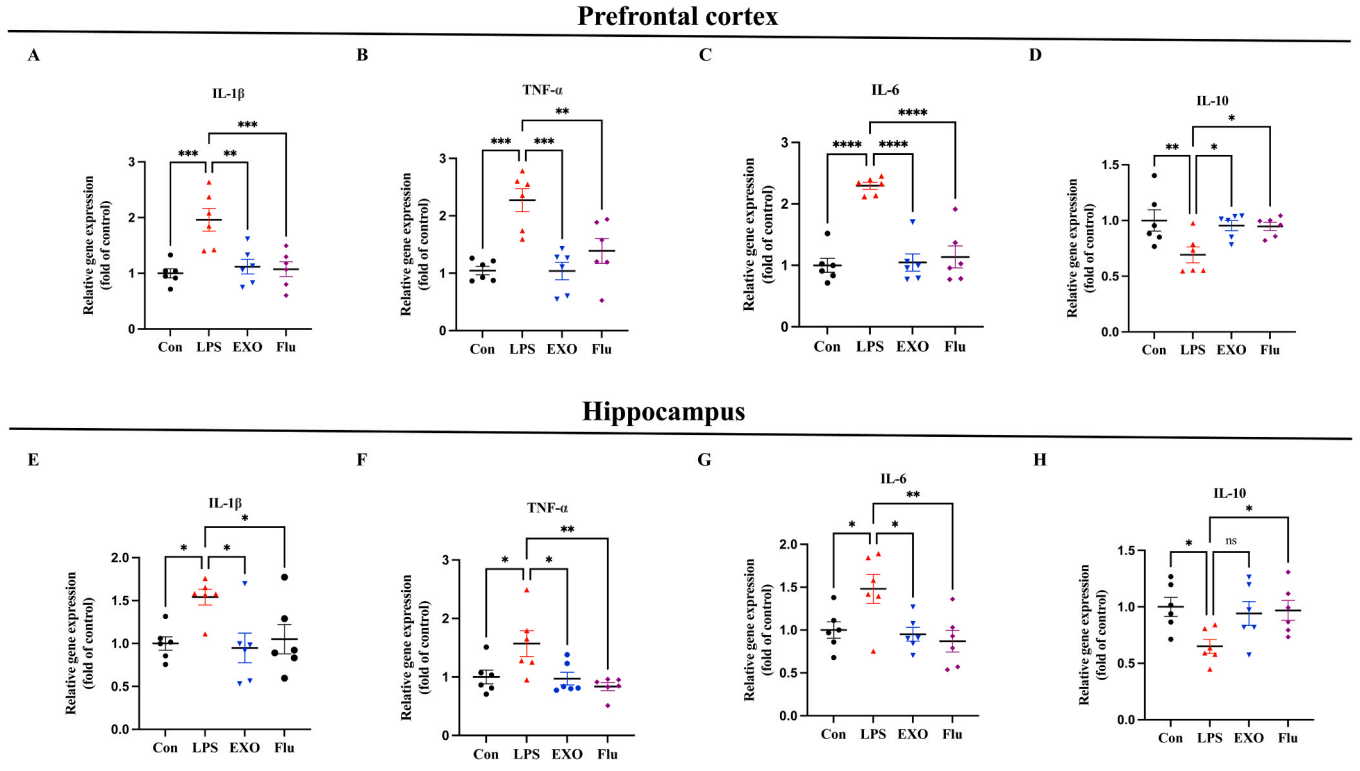

Fig. 3. The examination on mRNA expression of inflammation-related factors in the hippocampus and prefrontal cortex. (A,B,C,D) The inflammation-related factors (*il-1β, Tnf-α, il-6 and il-10*)quantified by Quantitative PCR in the hippocampus. (E,F,G,H) The inflammation-related factors(*il-1β, Tnf-α, il-6 and il-10*)quantified by Quantitative PCR in the prefrontal cortex. Data are presented as means ± SEM, with n = ~~5~~ 6 in each group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

inflammatory cytokines in the PFC and hippocampus.

3.4. Exosomes reversed the LPS-induced increase in microglia number, possibly by reducing glial cell activation

When inflammatory signals emerge, microglia are the first cells to respond in the central nervous system. They are like the "immune cells" of the nervous system, rapidly transitioning from a resting state to an activated state. Additionally, neuroinflammation triggers astrocyte activation, and inflammatory signals promoting astrocyte proliferation. For this reason, we stained the two types of glial cells with the microglial marker Iba-1 and the astrocyte marker GFAP on prefrontal cortical tissue (Fig. 4A,B) and hippocampal tissue(Fig. 4E,F) For prefrontal cortical tissue, the results demonstrated that the number of Iba-1 labeled microglia in the lipopolysaccharide (LPS)-treated group increased significantly compared with the control group. However, treatment with exosomes and fluoxetine markedly inhibited this significant increase in the number of microglia ($P < 0.01$, $P < 0.01$, Fig. 4C). Moreover, the results of astrocyte immunofluorescence staining were similar. LPS stimulation elevated the expression level of the related marker GFAP, while treatment with exosomes and fluoxetine inhibited this elevation ($P < 0.01$, $P < 0.01$, Fig. 4D). In addition, consistent with the observations in the prefrontal cortex, we drew the same conclusion. LPS treatment increased the number of glial cells in the hippocampal tissue, while supplementation with EXO or Flu reduced this increase in glial cells ($P < 0.01$, $P < 0.001$, Fig. 4G, H)." These results indicate that exosomes exert a preventive effect on the occurrence of depression by reducing the number of glial cells induced by neuroinflammation in the prefrontal cortex or hippocampal tissue of LPS challenged mice.

3.5. Exosome prevented oxidative stress damage induced by LPS

Oxidative stress is intricately associated with neuroinflammation. As previously described, neuroinflammation activates glial cells, and activated glial cells release a large number of inflammatory mediators,that in turn exacerbate oxidative stress. Therefore, we measured the oxidative stress related factors in peripheral serum and hippocampal tissues, such as malondialdehyde (MDA), superoxide dismutase (SOD), Total Antioxidant Capacity(T-AOC), and nitric oxide (NO). In peripheral serum, MDA and NO were significantly increased in the LPS treated

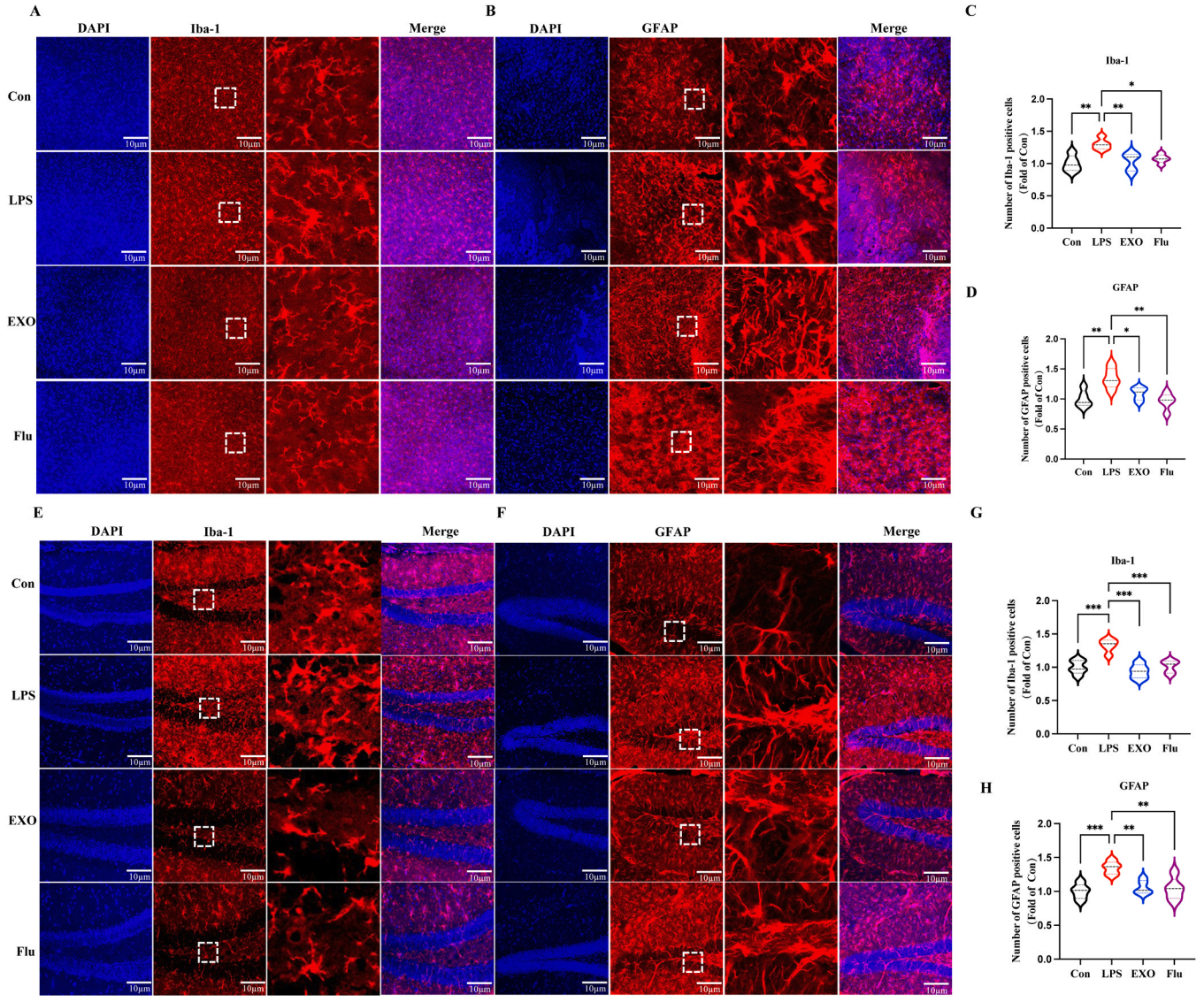

Fig. 4. BMSC-exos inhibited the proliferation and activation of microglia and astrocytes in the prefrontal cortex of LPS-treated mice. (A)Fluorescence detection of microglia marker Iba-1 in the prefrontal cortex. (B) Fluorescence detection of astrocytes marker GFAP in the prefrontal cortex. (C) Quantification of the number of GFAP positive cells. (D) Quantification of the number of Iba-1 positive cells. Data are presented as means ± SEM, with n = 5 in each group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

group. However, treatment with exosomes and fluoxetine hydrochloride markedly prevented this increasion compared with the control group., Regarding SOD and T-AOC, the LPS treatment could inhibit this index, while treatment with exosomes and fluoxetine hydrochloride significantly blocked this inhibition($P < 0.05$,$P < 0.01$,$P < 0.001$,Fig. 5A-D). In addition, consistent with the observations in the prefrontal cortex, we drew the same conclusion in hippocampal tissues. After LPS treatment, the contents of DDA and NO were abnormally increased, while the contents of SOD and T-AOC were abnormally decreased. Exosome supplementation restored the contents of SOD and T-AOC in hippocampal tissues and reduced the contents of DDA and NO($P < 0.05$,$P < 0.01$, $P < 0.001$,Fig. 5E-H).These results indicate that exosomes alleviate the oxidative damage caused by LPS stimulation in the peripheral serum and hippocampus of LPS-challenged mice.

3.6. Exosome inhibited the neuron damage induced by LPS

The pathogenesis of depression is closely linked to impaired neurogenesis in key brain regions, most notably the hippocampus (Fan et al., 2022). Doublecortin (DCX), predominantly expressed in newly generated neurons during the phases of migration and differentiation, serves as a pivotal biomarker for evaluating the level of neurogenesis (Franjic et al., 2022). In both depressive patients and animal models, a marked reduction in the quantity of DCX-positive newly generated neurons within brain regions like the hippocampus is frequently observed, which strongly implies the impairment of neurogenesis. In this study, to assess the depressive status of mice, we employed immunofluorescence techniques to quantify the number of doublecortin (DCX)-positive nascent neurons in the hippocampal region(Fig. 6A). The results revealed that there was a significant decline in the neuronal count in mice subjected to lipopolysaccharide (LPS) treatment. However, supplementation with either exosomes or fluoxetine hydrochloride effectively mitigated this decrease ($P < 0.01$,Fig. 6B). These results suggest that exosomes confer a protective effect on hippocampal neurons, potentially playing a role in preventing the development of depression.

4. Discussion

Our study provides comprehensive evidence supporting the preventive efficacy of bone marrow mesenchymal stem cell-derived exosomes (BMSC-exos) against LPS-induced depressive-like behaviors in mice. The results demonstrate that BMSC-exos exert their prophylactic effects through multifaceted mechanisms, including anti-inflammatory modulation, regulation of glial cell activation, mitigation of oxidative stress, and preservation of neurogenesis. These findings establish, for the first time, that supplementation with BMSC-exos represents a novel and promising strategy for preventing inflammation-associated depression.

Neuroinflammation plays a pivotal role in depression pathogenesis (Wang et al., 2022). LPS administration triggered a systemic inflammatory cascade, characterized by excessive release of pro-inflammatory cytokines, which disrupt neurotransmitter metabolism, synaptic plasticity, and neuroendocrine functions (Salvesen et al., 2017). Among these cytokines, interleukin-1β (il-1β)a pleiotropic inflammatory mediator critical for cerebral inflammatory responses—was significantly elevated in hippocampal and cortical tissues. Notably, BMSC-exos administration effectively attenuated il-1β levels and restored inflammatory homeostasis, creating a favorable microenvironment for neural recovery. This aligns with emerging evidence that exosomal cargo, including anti-inflammatory miRNAs and cytokine-binding proteins, can neutralize pro-inflammatory signaling cascades (Kalluri and Lebleu, 2020; Ni et al., 2019).

Previous studies have shown that LPS treatment can induce a centralinflammatory response associated with proinflammatory cytokine production, glial cell activation (Li et al., 2021b). Glial cell dysregulation further contributes to depression progression (Lu et al., 2025; Zhang et al., 2024). Under LPS challenge, hyperactivated microglia and

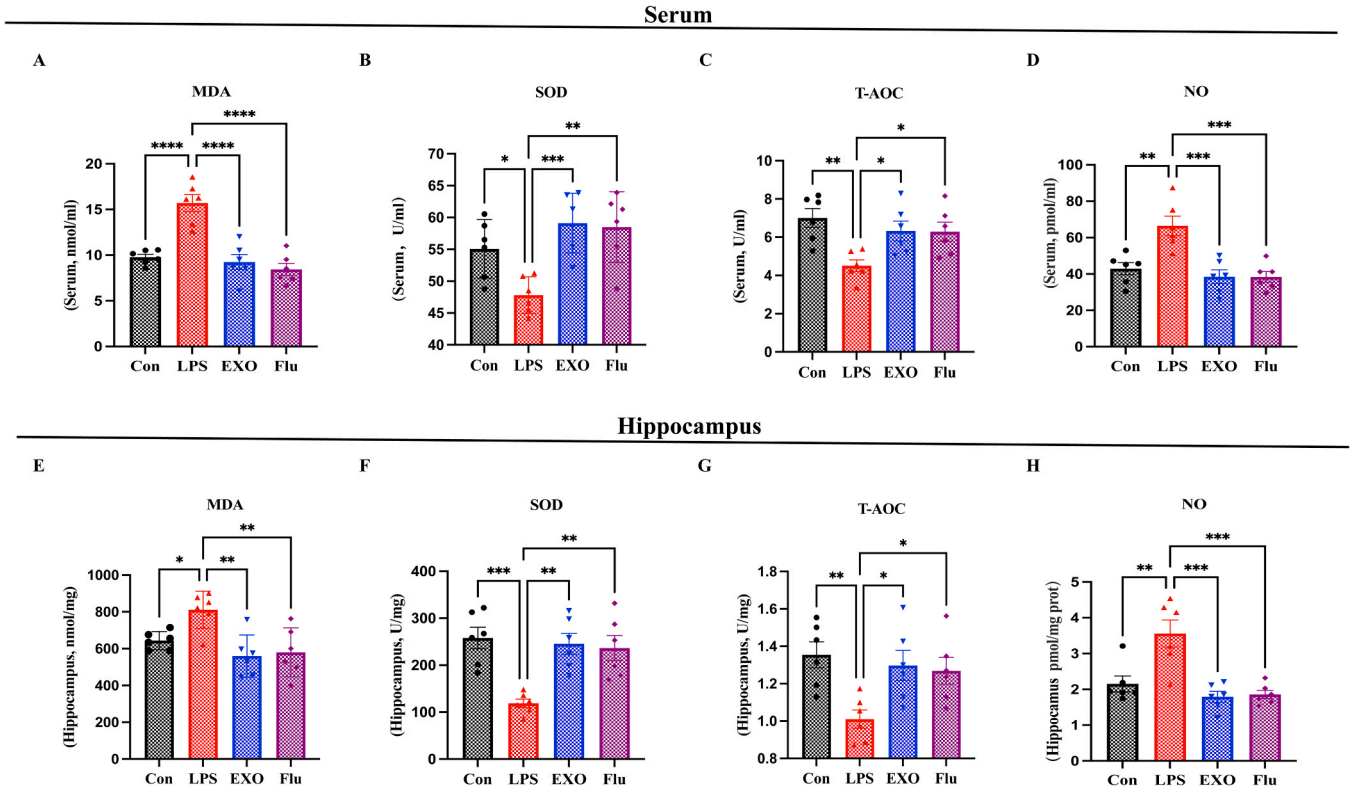

Fig. 5. BMSC-exos prevents oxidative stress in serum and hippocampus. (A,B,C,D) BMSC-exos prevents abnormal expression of oxidative stress-related factors such as MDA, SOD, NO,T-AOC in the serum. (E,F,G,H) BMSC-exos prevents abnormal expression of oxidative stress-related factors such as MDA, SOD, NO,T-AOC in the hippocampus. Data are presented as means ± SEM, with n = 5 in each group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

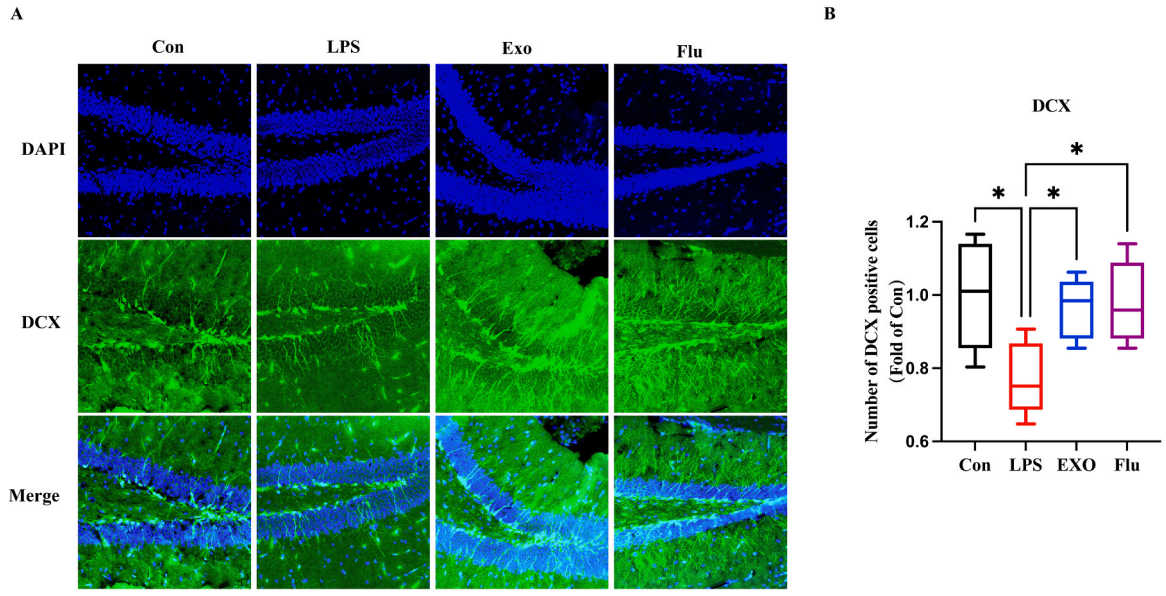

Fig. 6. BMSC-exos promotes neurogenesis in the hippocampus. (A)Fluorescence detection of neonatal neurons marker DCX in the hippocampus. (B) BMSC-exos prevents the loss of DCX cell induced by LPS. Data are presented as means ± SEM, with n = 5 in each group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

astrocytes disrupt neuro-glial network equilibrium, impairing synaptic support and neuromodulatory functions. GFAP is a specific marker protein for astrocytes, and Iba-1 is a marker protein for microglia. Following LPS treatment, the increased expression of these two proteins suggests that astrocytes and microglia may be activated. Activated glial cells release abundant proinflammatory factors, oxygen free radicals, which further exacerbate neuroinflammation. In addition, one study has demonstrated that exo exhibited antidepressant effects by suppressing M1 microglial neuroinflammation (Li et al., 2024). This result is consistent with our conclusion and strongly supports that exosomes exert an antidepressant effect by regulating microglial neuroinflammation. Our findings reveal that BMSC-exos treatment normalized glial activation states in the prefrontal cortex and hippocampus, restoring neuro-glial homeostasis. This regulatory effect may be attributed to exosome-mediated modulation of NF-κB inflammasome pathways, which are central to glial activation dynamics (He et al., 2018; Fan et al., 2018).

Oxidative stress, another hallmark of depression, was markedly alleviated by BMSC-exos (Tavakolizadeh et al., 2018). LPS-induced oxidative-antioxidant imbalance generated excessive reactive oxygen species (ROS), causing neuronal lipid peroxidation and DNA damage (Loffredo et al., 2020; He et al., 2023). BMSC-exos administration enhanced antioxidant enzyme activity (superoxide dismutase, catalase) and reduced ROS levels in both serum and hippocampal tissues. These effects likely stem from exosomal transfer of antioxidant molecules (glutathione peroxidase, miR-21–5p), which directly scavenge free radicals and reinforce endogenous defense systems (Zhang et al., 2015).

The persistence of MDD is closely associated with reduced neurogenesis in the dentate gyrus (DG) of the hippocampus, and inflammatory responses serve as a key factor disrupting this neuroregenerative process. LPS treatment reduces the number of DCX- positive cells in the hippocampal dentate gyrus (Fan et al., 2018). Furthermore, BMSC-exos demonstrated neuroprotective properties by preserving hippocampal neurogenesis—a process severely impaired in depression. LPS-induced suppression of doublecortin (DCX)-positive newborn neurons was reversed by exosome treatment, consistent with previous reports linking exosomal BDNF and GDNF to enhanced neuronal survival and synaptic plasticity (Inoue et al., 2016; Liao et al., 2019). These observations underscore the dual capacity of BMSC-exos to mitigate neuronal damage while promoting regenerative processes.

Despite these advances, several challenges must be addressed to translate BMSC-Exos into clinical applications. First, standardization of exosome isolation protocols and scalable production methods is imperative to ensure batch consistency and therapeutic efficacy. Second, the specific bioactive components within BMSC-exos (non-coding RNAs, proteins) responsible for their antidepressant effects require precise identification through proteomic and transcriptomic analyses. Third, optimizing delivery routes (intranasal) to enhance blood-brain barrier penetration and bioavailability warrants further investigation. Addressing these questions will bridge the gap between preclinical findings and clinical implementation.

5. Conclusion

In conclusion, our study elucidates that BMSC-derived exosomes prevent LPS-induced depressive-like behaviors by orchestrating anti-inflammatory, glial-modulatory, antioxidant, and neurogenic responses. Mechanistically, exosomes suppress neuroinflammation through inhibition of microglial hyperactivation(Fig. 7). These findings not only advance our understanding of exosome-mediated depression prevention but also provide a scientific foundation for their clinical translation. Future research should focus on refining exosome engineering techniques and validating their therapeutic potential in heterogeneous patient populations, ultimately paving the way for novel, mechanism-based interventions in depression management.

CRediT authorship contribution statement

Juan-li Hu: Supervision, Data curation. **Xue-Jun Yan:** Supervision, Data curation. **Hu Pan:** Supervision. **Hua Liu:** Writing – original draft, Project administration. **Yong Cheng:** Funding acquisition, Conceptualization. **Xiao Mao:** Supervision, Software.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

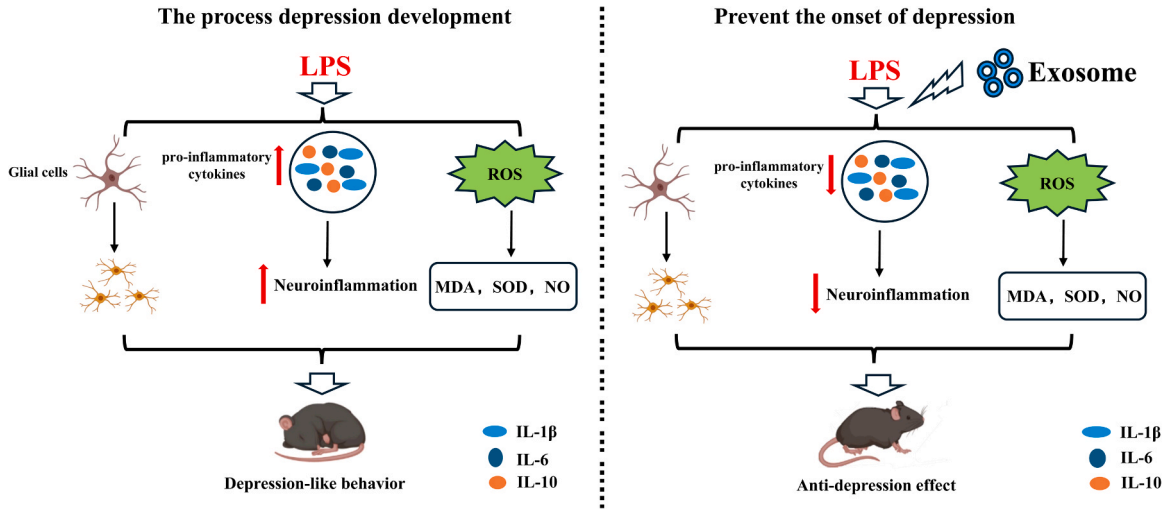

Fig. 7. Potential molecular mechanism of BMSC-exos prevents depression-like behaviror caused by LPS.

the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainresbull.2025.111527.

Data availability

The data that has been used is confidential.

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