Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Quercetin Improves Hippocampal Neurogenesis in Depression by Regulating the Level of Let-7e-5p in Microglia Exosomes

Ying Xie[1,*], Tongxuan Ouyang[1,*], Anli Xu[1], Qinglai Bian[1], Biran Zhu[1,2], Min Zhao[1,2]

[1]School of Basic Medical Sciences, Hubei University of Chinese Medicine, Wuhan, 430065, People's Republic of China; [2]Hubei Shizhen Laboratory, Wuhan, 430061, People's Republic of China

*These authors contributed equally to this work

Correspondence: Min Zhao; Biran Zhu, School of Basic Medical Sciences, Hubei University of Chinese Medicine, Huangjiahu West Road, Wuhan, Hubei Province, People's Republic of China, 430065, Tel +86 13437155626, Email zmin13@hbucm.edu.cn; zhubir@hotmail.com

Background: Adult hippocampal neurogenesis plays a beneficial role in the treatment of depression. The precise mechanism by which let-7e-5p functions as a potential marker for depression remains unclear. Quercetin, a flavonoid compound, exhibits antidepressant effects; however, further investigation is needed to elucidate its regulatory effect and mechanism on hippocampal neurogenesis.

Methods: Chronic unpredictable mild stress (CUMS) was employed to induce depressive-like signaling and cognitive impairment in mice, while quercetin was administered via oral gavage. The symptoms of the mice were evaluated using various signaling methods. The expression levels of microglia, neural stem cells, and let-7e-5p in the dentate gyrus (DG) area of hippocampus were assessed using pathological observation methods. The expression levels of let-7e-5p and the Wnt1/β-catenin signaling pathways in the hippocampal DG of mice were assessed using qRT-PCR and Western blotting, respectively. The exosomes from peripheral blood were isolated and identified, followed by the detection of expression levels for microglia markers CD11b and TMEM119. We isolated hippocampal neural stem cells (NSCs) and co-cultured them with exosomes secreted by BV2 cells under LPS stimulation to observe the proliferation of NSCs and the generation of new neurons. The targeting relationship between let-7e-5p and Wnt1 was ultimately confirmed through the utilization of a dual luciferase reporter assay.

Results: (1) Quercetin ameliorated depression-like behaviors in mice induced by CUMS and restored neurogenesis in the DG region of the hippocampus. (2) Quercetin suppressed the secretion of microglia-derived exosomes carrying let-7e-5p in the DG, which exerted effects on NSC. (3) let-7e-5p regulates depression-related neurogenesis through targeting the Wnt1/β-catenin signaling pathway.

Conclusion: The inhibitory effect of let-7e-5p in microglial exosomes on depression-associated neurogenesis is mediated through the blockade of the Wnt1/β-catenin signaling pathway, which can be effectively reversed by Quercetin treatment.

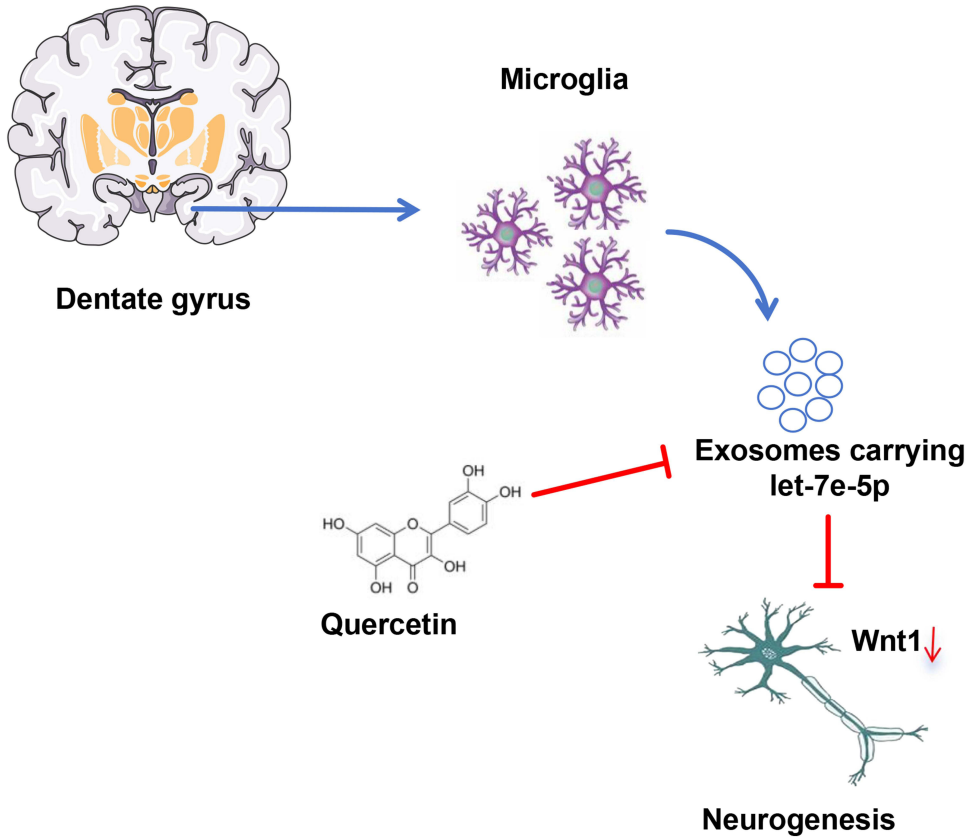
Keywords: depression, neurogenesis, quercetin, microglia, exosome, let-7e-5p, Wnt1/β-catenin

Introduction

Depression is widely recognized as a significant global public health issue and has been characterized as an "unseen burden" that exerts a profound impact on both individuals and society. The prevalence of depression in China is also significant, with many patients not receiving timely and effective treatment due to the neglect they face and the limited availability of mental health care resources.[1] With the growing focus on depression, a range of antidepressant medications have been utilized in clinical practice. However, there is an increased risk of depression recurrence following discontinuation of these medications.[2] The identification of safe, efficacious, and non-dependent antidepressant medications is imperative.

Received: 11 October 2024
Accepted: 18 March 2025
Published: 24 March 2025

Graphical Abstract



The pathogenesis of depression remains inconclusive at present. On one hand, this is due to the intricate anatomical structure and functioning of the human brain. On the other hand, depression is highly idiopathic, with increased risk associated with external stressors, hormonal changes, and medication side effects.[3] The precise mechanisms underlying depression remain unclear; however, it is believed that modulation of neurogenesis holds potential for alleviating depression.[4] The atrophy of certain brain structures is a characteristic feature of depression, with the hippocampus being particularly affected.[5] Defects in hippocampal neurogenesis are considered to be one of the key indicators of depression.[6] The modulation of dentate gyrus (DG) function by increased adult hippocampal neurogenesis may be essential for the efficacy of antidepressants.[7] The findings of study have demonstrated that the augmentation of adult hippocampal neurogenesis can significantly ameliorate symptoms resembling depression and facilitate the restoration of cognitive impairments.[8] Therefore, the identification of novel targets that promote neurogenesis in the hippocampus may offer a fresh avenue for the advancement of strategies targeting depression.

Exosomes are a type of extracellular vesicles secreted by eukaryotic cells, playing crucial roles in intercellular communication pathways.[9] The size of exosomes varies slightly depending on the observation techniques employed. By electron microscopy, the diameter of exosomes measures 30–100 nm, whereas nanoparticle tracking analysis (NTA) reveals a slightly larger range of 80–160 nm.[10] Exosomes transport lipids, proteins, mRNA, and microRNAs (miRNAs) to target cells for the purpose of regulating gene expression levels within those cells.[9] The interaction between neurons and glial cells is crucial for the maintenance of brain function, with exosomes playing a pivotal role in facilitating communication between these two cell types.[11] The findings of multiple studies have demonstrated that microglia-derived exosomes carry a diverse range of miRNAs and play a crucial role in the pathological progression of various diseases of the central nervous system.[12–14] The regulation of miR-146a-5p levels in

microglia-derived exosomes has been reported to have an impact on hippocampal neurogenesis in depression.[15] These findings suggest that targeting miRNAs within microglial exosomes could be a potential approach for antidepressant treatment. The miRNAs, which are endogenous non-coding RNAs with a length of 20–22 nucleotides, exert regulatory control over the expression levels of target genes by binding to the 3' untranslated regions (UTRs) of mRNAs, leading to their degradation or translational inhibition.[16] The expression of circulating miRNA-let-7e-5p has been found to be up-regulated in the plasma of patients with major depression and bipolar disorder, indicating that circulating let-7e-5p holds potential as a diagnostic biomarker for these conditions.[17] However, the impact of regulating the expression level of let-7e-5p in microglia exosomes on hippocampal neurogenesis remains uncertain.

The flavonoid Quercetin exhibits diverse biological activities, including anti-inflammatory, antioxidant, antitumor, and anti-aging effects. A mounting body of research has substantiated the neuroprotective efficacy of Quercetin.[18–20] Notably, Quercetin also exhibits antidepressant effects, mitigating depression-like behaviors induced by chronic unpredictable mild stress (CUMS) in mice.[21] The findings of Du et al demonstrated that Quercetin effectively ameliorated depression-like behavior and cognitive impairment in mice through its anti-neuroinflammatory properties.[22] These studies suggest that Quercetin holds significant potential for the development of novel antidepressant drugs. However, the potential modulatory effect of Quercetin on hippocampal neurogenesis in depression remains unclear. Therefore, this study aimed to investigate the effects of Quercetin on depression-like behavior and hippocampal DG neurogenesis in CUMS-induced mice in vivo, followed by further exploration of its mechanism in vitro. The objective is to provide valuable data supporting Quercetin as a potential candidate drug for the treatment of depression and cognitive dysfunction.

Materials and Methods

Depression Model and Experimental Groupings

36 male C57BL/6 mice (8 weeks old, weighing 18–22 g) were obtained from the Experimental Animal Research Center of Hubei Province. After one week of adaptive feeding, the mice were randomly allocated into four groups (n = 9): control, model, low-dose of Quercetin (Que-L), and high-dose of Quercetin (Que-H). Except for the control group, depression models were induced in the other groups of mice using the CUMS method. The specific modeling methods were as follows: The mice, housed in isolation and subjected to various stress stimuli, including food deprivation (12 h), water deprivation (12 h), light and dark cycle inversion (24 h), cold water swimming (4°C, 5 min), hot water swimming (45°C, 5 min), tail clamp (1 min), cage tilt (45°, 12 h), wet cushion exposure (12 h). A different stimulus was randomly administered each day for a total of 21 d. The mice received daily intragastric administration of the drug for a period of 21 d following daily stimulation. The doses of quercetin administered via gavage were 10 mg/kg and 40 mg/kg for the low and high groups, respectively. Both the control group and the model group received an equivalent volume of normal saline via gavage. The study was approved by the Research Ethics Committee of Bestcell Model Biological Center (Approval number: BSMS2024-06-26B). The mice were anesthetized via intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg) or decapitation. The animal experiments in this study were conducted in strict compliance with The Animal Research: Reporting of in vivo Experiments (ARRIVE) guidelines. The welfare of experimental animals in this study followed the Laboratory animal-Guideline for ethical review of animal welfare issued by the Ministry of Science and Technology of China.

Open Field Test (OFT)

At the end of the drug administration, mice were subjected to OFT. The experimental box used in the OFT was an open field with a height of 35 cm and a bottom side length of 50 cm. The mice were placed in the central grid of the open field while the video was captured and timed for 5 min. The activity state of the mice was assessed using a computerized tracking analysis system, and the time spent in the central area and the number of times that the mice crossed the central grid within 5min were counted.

Forced Swimming Test (FST)

Before the formal experiment, the mice underwent a 10-minute training session for adaptive swimming. For the FST, mice were placed in a transparent plastic cylinder filled with clear water at a depth of 15 cm and maintained at a temperature of 23 to 25 °C. After acclimating the mice for 2 min, the duration of immobility over the subsequent 4 min was recorded.

Sucrose Preference Test (SPT)

The mice underwent sucrose adaptation training one day prior to the experiment, followed by a 24-hour fasting and water deprivation. Drinking water bottles containing 1% sucrose solution and regular drinking water were placed in the cages, and after 8 h, the bottles were removed. The liquid volume in each bottle was measured before and after the experiment, allowing for calculation of the sucrose preference rate.

Morris Water Maze (MWM) Test

The experimental system for the Morris water maze consisted of a circular pool and an automated image acquisition and processing system. The circular pool was divided into four quadrants of equal size, and a platform was placed in the third quadrant, 1cm away from the water surface. The mice were placed in a randomly selected quadrant of the water maze, and the escape latency, which refers to the time taken by the mice to find the platform within 1 min, was recorded. If a mouse failed to locate the platform within 1 min, it was guided to the platform and allowed to stay there for 30s in order to familiarize itself with its position before proceeding with subsequent experiments. The mice require a 4-day training period in navigation, with daily fixed-time training sessions. Each mouse undergoes 4 training trials per day, entering the water from different quadrants. The space exploration experiment was carried out on the 5th day, the platform was withdrawn, and the first quadrant farthest from the platform position was selected as the water entry point of mice. The number of times mice accessed the escape platform and their escape latency within a one-minute timeframe were recorded.

Primary Neural Stem Cells (NSC) Isolation and Culture

The C57BL/6 mice were harvested within 24 h of birth, and bilateral hippocampal tissues were promptly dissected after decapitation and placed in DMEM/F12 basal medium (Gibco, Carlsbad, CA, USA) at 4°C. Afterwards, the vessels and meninges were dissected, and the hippocampal tissue was finely chopped and blown into a single-cell suspension. The cells were filtered through a 70 μm strainer (Millipore Corp., Billerica, MA, USA) and cultured in DMEM/F12 complete medium [DEME/F12 basal medium supplemented with 20 μg/L epidermal growth factor (Gibco), 20 μg/L basic fibroblast growth factor (Gibco), and 2% B27 (Gibco)]. The media was refreshed on the third day, and the cells were passaged on the sixth day. For subsequent experiments, neural stem cells from the third passage were selected.

BV2 Cells Culture and Processing

Mouse microglia BV2 were provided by the China Center for Type Culture Collection and cultured in MEM (Provell, Wuhan, China) containing 10% FBS (Gibco). The BV2 cells in the logarithmic growth phase were seeded into 96-well plates and subjected to treatment with 1 mg/L of lipopolysaccharide (LPS) along with varying concentrations (0, 10, 20, 30, 40, 50, and 60 μM) of quercetin for a duration of 12 h. The control group consisted of normal cultured BV2 cells without any experimental intervention. The CCK-8 method was employed to determine the optimal concentration of quercetin for BV2 cell intervention. Additionally, BV2 cells were categorized into a control group, LPS group, LPS + Quercetin group, LPS + Quercetin + let-7e-5p mimics group, and LPS + Quercetin + NC group.

Cell Transfection

The BV2 cells in the logarithmic growth phase were seeded in 12-well plates and cultured overnight at 37 °C under a 5% CO_2 atmosphere. The culture medium was replaced with serum-free DMEM two hours prior to transfection. The opti-MEM (Gibco) was used to respectively dilute the let-7-5p mimics (Ribobio, Guangzhou, China), NC, and

Lipofectamine^{TM} 2000 (Invitrogen, Carlsbad, CA, USA). The mimics diluent or NC diluent was then mixed with the transfection reagent blender and left at room temperature for 20 min. Afterwards, the mixture was added to the cell culture plate and gently shaken for blending. The cells were subsequently cultured for 48 h before conducting further assays.

Exosomes Extraction and Identification

The mice were anesthetized via intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg), and peripheral blood was collected from the eyeballs. Subsequently, the collected blood samples were centrifuged at a speed of 3000 r/min for 10 min to obtain serum. The supernatants were obtained by centrifuging mouse serum or BV2 cell culture medium at 12000 r/min for 30 min at 4 °C. The supernatant was passed through a 0.22 μm strainer, followed by centrifugation at 15000 r/min for 90 min at 4 °C to eliminate the supernatant. Subsequently, the exosomes precipitate was resuspended in 200 μL phosphate buffer solution (PBS, Servicebio, Wuhan, China). Finally, exosomes were identified using the following methods: (1) Western blotting was performed to analyze protein expression levels of exosome markers CD63 and CD81 (Abcam, Cambridge, MA, USA); (2) Nanoparticle tracking analysis (NTA) was used to determine particle size and concentration of exosomes; (3) Scanning electron microscopy was employed for observing the morphology of exosomes.

Exosome Uptake Assay

The third-generation NSCs were seeded in 24-well plates with coverslips and cultured for 24 h. Exosomes were labeled with PKH-67 (MedChemExpress, Monmouth Junction, NJ, USA), and the protein concentration of exosomes was determined using the BCA protein assay kit (Solarbio, Beijing, China). The exosomes from each group were subsequently added to individual well of the cell plate at a concentration of 50 μg/mL, and the cells were cultured for an additional 24 h. Cell slides were fixed with 4% paraformaldehyde for 15 min and incubated for 5 min by dropping DAPI (Beyotime, Shanghai, China) onto the glass slides. After sealing, the uptake of exosomes by cells was visualized using a fluorescence microscope (Nikon, Tokyo, Japan).

5-Ethynyl-2'- Deoxyuridine (EdU) Incorporation Assay

The NSCs were fixed by adding 4% paraformaldehyde to the slide, followed by incubation with an osmotic agent (0.5% Triton X-100) for 15 min. Subsequently, the cells were stained with EdU dyeing solution and incubated at room temperature in the dark for 30 min. Finally, Hoechst 33342 dyeing solution was added to stain the cell nucleus. The cell staining was observed using a fluorescence microscope.

Immunofluorescence Staining

After sacrificing the mice, the brain tissues were promptly isolated and fixed in 4% paraformaldehyde for a duration of 24 h. Tissue samples were removed for dehydration and then prepared into paraffin sections, which were deparaffinized for antigen repair. The sections were treated with goat serum (Boster, Wuhan, China) and blocked for 30 min at room temperature. Antibodies against ionized calcium binding adapter molecule 1 (Iba-1, 1:100; Abcam) or Nestin (1:100; Proteintech, Wuhan, China) were then added dropwise and incubated overnight at 4 °C. Fluorescently labeled IgG (1:100; Boster) was subsequently added dropwise and incubated for 1 h at room temperature. After a 5-minute incubation in the dark with DAPI (Beyotime), tissue staining was observed using a fluorescence microscope.

For immunofluorescence staining of NSCs, the cell samples were fixed with 4% paraformaldehyde on the slides, and 0.5% Triton X-100 was added at room temperature for 20 min to disrupt the cell membrane. After blocking the slides with normal goat serum for 30 min, the doublecortin (DCX, 1:100; Bioss, Beijing, China) antibody was added and incubated overnight at 4 °C. Subsequently, fluorescently labeled IgG was added and incubated at room temperature for 1 h. DAPI was added and incubated in the dark for 5 min before being observed using a fluorescence microscope.

Fluorescence in Situ Hybridization (FISH) Assay

The paraffin sections of mouse brain tissue were removed, and digested with 20 μg/mL proteinase K for 30 min at 37 ° C after antigen repair. Following this, the sections were treated with prehybridization solution and incubated at 37°C for 1 h. The hybridization solution containing let-7e-5p probe (5'-AACTATACAACCTCCTA CCTCA-3') was then added dropwise and incubated overnight at 37°C. After the sections were washed, Iba-1 or Nestin antibodies were introduced for immunofluorescence staining. Subsequently, the localization and expression of let-7e-5p in tissues were observed using a fluorescence microscope.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The mouse hippocampal DG tissues, exosomes, or NSCs were collected and lysed using Trizol reagent (Ambion, Austin, TX, USA) for RNA extraction. The RNA was reverse transcribed into cDNA using the HiScript II Q Select RT SuperMix for qPCR kit (Vazyme, Nanjing, China). The qRT-PCR was performed using SYBR Green Master Mix (Vazyme), following the response procedures: initial denaturation at 95 °C for 10 min, denaturation at 95 °C for 15s, annealing and extension at 60 °C for 60s, repeated for a total of 40 cycles. The expression level of let-7e-5p was quantified using the $2^{-\Delta\Delta Ct}$ method. The sequence primers utilized in the experiments were as follows: let-7e-5p loop primer: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACAACTATAC-3', forward primer: 5'-TGCGCTGAGGTAGG AGGTTG TA-3'; U6 forward primer: 5'- CGCTTCGGCAGCACATATAC-3', reverse primer: 5'-AAATATGGAACGCTTCACGA-3'.

Western Blotting

The RIPA lysate (Meilunbio, Dalian, China) was utilized for the extraction of total proteins from mouse hippocampal DG tissues, exosomes and NSCs, while nuclear proteins were extracted from both tissues and cells using a nuclear and cytoplasmic protein extraction kit (KeyGEN BioTECH, Nanjing, Jiangsu). After determining the protein concentration using the BCA kit (GBCBIO, Guangzhou, China), the proteins were denatured in a boiling water bath and then separated by electrophoresis. Subsequently, the gel was removed, the target band was cut, and the protein was transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). The PVDF membranes were immersed in a blocking solution containing 5% skim milk powder for 2 h at room temperature. Primary antibodies, including CD11b (1:2000; Abclonal, Wuhan, China), TMEM119 (1:1000; Abclonal), Wnt1 (1:1000; Affinity, Cincinnati, OH, USA), glycogen synthase kinase-3β (GSK-3β, 1:2000; Proteintech), β-catenin (1:5000; Proteintech), GAPDH (1:1000; Goodhere, Hangzhou, Zhejiang) and Histone H3 (1:2000; Proteintech), were added to the membranes which were then incubated overnight at 4°C. The HRP-labeled secondary antibody (1: 10000; Boster) were added and incubated at room temperature for 2 h. Subsequently, the chemiluminescence reagent was applied onto the film, which was then scanned after development to quantitatively analyze the gray value of protein bands.

Dual-Luciferase Reporter Assay

The target sequence of let-7e-5p binding to Wnt1 and its mutant sequence were amplified initially. Subsequently, the amplified target fragment was ligated with the vector pYr-MirTarget to construct recombinant plasmids, which were named as Wnt1 wild-type (Wnt1-WT) and Wnt1 mutant-type (Wnt1-MUT). The logarithmically growing 293 cells were transfected with the recombinant plasmid and let-7e-5p mimics or NC, followed by a 48-hour incubation period. Subsequently, the cells were lysed and the supernatant was collected. The dual luciferase reporter gene detection kit (Beyotime) was employed to ascertain the firefly fluorescence value and renilla fluorescence value in the cells, thereby validating the targeted regulatory relationship between let-7e-5p and Wnt1.

Statistical Analysis

The data in this study were analyzed and plotted using SPSS 22.0 and GraphPad Prism 5.0. The results are presented as the mean ± standard deviation. One-way ANOVA followed by LSD's post hoc test was employed to assess differences among multiple groups. A significance level of $P<0.05$ was considered statistically significant.

Results

Quercetin Improved Depression-Like Behavior and Cognitive Impairment in CUMS-Induced Mice

To investigate the impact of quercetin on depression, we observed the behavioral alterations in mice following CUMS induction and oral administration of Quercetin for a duration of 3 weeks (Figure 1A). The results of the OFT indicate that mice in the model group exhibit repetitive and limited exploration behavior within the open field, accompanied by

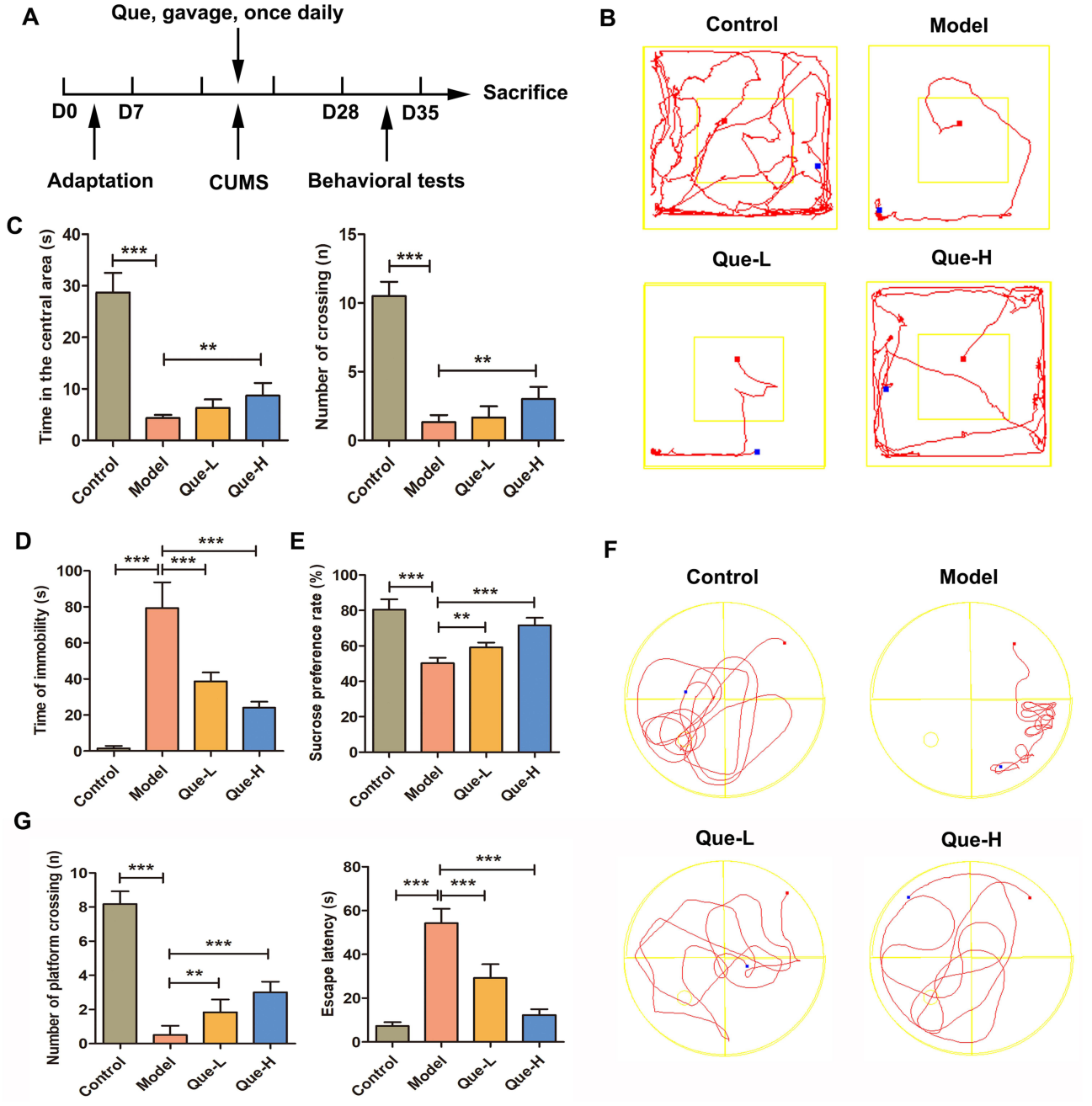


Figure 1 Effect of Quercetin on depression-like behavior and cognitive impairment in CUMS-induced mice. (**A**) Schematic diagram of the animal experiment procedure. (**B**) The trajectory diagram of mice in the open field test. (**C**) The duration of time spent in the central area of the open field and the number of crossings by mice. (**D**) The immobility time of mice during forced swimming test. (**E**) The sucrose preference rate of mice in the sucrose preference test. (**F**) The trajectory diagram of mice in the Morris water maze test. (**G**) The number of times mice crossed the escape platform and the time of escape latency. ** $P < 0.01$, *** $P < 0.001$. Abbreviations: CUMS, chronic unpredictable mild stress; Que, Quercetin; Que-L, low dose of Quercetin; Que-H, high dose of Quercetin. ** $P < 0.01$, *** $P < 0.001$.

Abbreviations: CUMS, chronic unpredictable mild stress; Que, Quercetin; Que-L, low dose of Quercetin; Que-H, high dose of Quercetin.

https://doi.org/10.2147/DDDT.S493779

a reduction in both exploration time and frequency within the central area. However, the mice in the high-dose Quercetin group exhibited enhanced exploratory behavior in the open field, with a significant increase observed in both the number of entries into the central area and exploration time (Figure 1B and C). In addition, the immobility time of the model group was prolonged, and the sucrose preference rate was decreased in the FST. However, these effects were ameliorated by Quercetin (Figure 1D and E). Finally, the learning and memory ability of mice was assessed using the MWM test. Mice in the model group exhibited impaired spatial navigation, characterized by difficulty locating the escape platform and prolonged escape latency. Conversely, mice in the Quercetin treatment group demonstrated improved performance with increased frequency of finding the escape platform and reduced escape latency (Figure 1F and G). Collectively, these findings suggest that mice subjected to CUMS exhibit significant depression-like behaviors and cognitive impairment, both of which can be alleviated by treatment with Quercetin.

Quercetin Inhibited Let-7e-5p Expression and Activated Wnt1/β-Catenin Signal Transduction in the Hippocampal DG of CUMS-Induced Mice

Subsequently, we observed a significant increase in microglial activation and a decrease in NSCs population within the DG region of the hippocampus following CUMS exposure compared to the control group (Figure 2A and B). Additionally, both microglia and NSCs exhibited elevated expression levels of let-7e-5p (Figure 2C and D). However, treatment with Quercetin effectively reversed these alterations. Meanwhile, qRT-PCR results revealed a significant upregulation of let-7e-5p expression in the hippocampal DG of the model group compared to the control group, whereas its expression was attenuated in the Quercetin-treated group (Figure 2E). The findings suggest that quercetin exerts an inhibitory effect on the expression of let-7e-5p in microglia and NSCs within the hippocampal DG region of mice induced by CUMS. Notably, Western blotting results revealed a significant reduction in the nuclear expression level of β-catenin within the hippocampal DG in the model group. Additionally, there was a significant decrease observed in the tissue's expression level of Wnt1 protein, while conversely, an evident increase was noted in the expression level of GSK-3β (Figure 2F and G). However, treatment with Quercetin significantly enhanced the expression of Wnt1 and facilitated the nuclear accumulation of β-catenin protein in the DG region of the hippocampus, while also suppressing GSK-3β. Consequently, Quercetin effectively promoted the activation of the Wnt1/β-catenin signaling pathway in the DG of CUMS-induced mice.

Quercetin Reduced the Expression of Let-7e-5p in Peripheral Blood Exosomes of CUMS-Induced Mice

Exosomes serve as a crucial intercellular communication pathway. Hence, we isolated exosomes from the peripheral blood of mice induced with CUMS and identified them through NTA analysis and Western blotting detection of exosome markers CD63 and CD81 (Figure 3A and B). We observed a significant upregulation of let-7e-5p expression in exosomes derived from peripheral blood of mice following CUMS induction, whereas treatment with Quercetin resulted in downregulation of let-7e-5p expression (Figure 3C). The detection of microglia marker proteins in exosomes secreted by microglia has been demonstrated in existing research.[23] In the present study, it was observed that CUMS led to an upregulation of protein expression levels for microglial markers CD11b and TMEM119 in exosomes derived from peripheral blood samples of mice (Figure 3D). This finding suggests an increase in microglia-derived exosomes, which was subsequently attenuated by Quercetin treatment. Therefore, our hypothesis suggests that Quercetin may exert its therapeutic effects in CUMS-induced mice by attenuating the expression of let-7e-5p in microglia-derived exosomes.

Quercetin Promoted NSCs Proliferation and Neurogenesis by Inhibiting the Expression of Let-7e-5p in Microglia Exosomes

To investigate the mechanism of action by which Quercetin improves CUMS-induced mice through modulation of microglial exosome let-7e-5p, we conducted in vitro cell experiments. The optimal concentration of Quercetin for intervention on LPS-induced microglial BV2 cells was determined through a CCK-8 screening method, with 30 μM Quercetin selected for subsequent experiments (Figure 4A). The results of Western blot analysis (Figure 4B), scanning

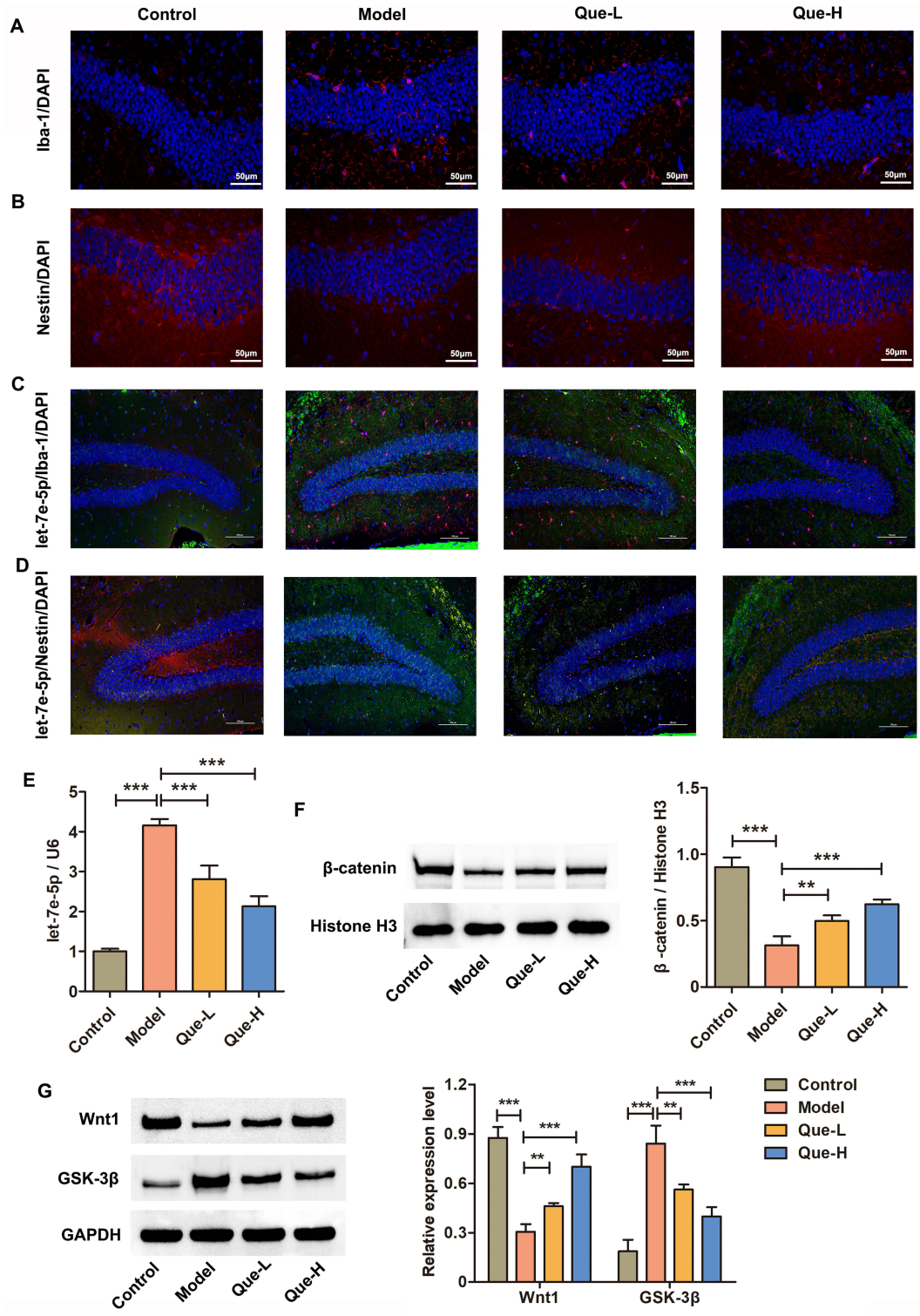


Figure 2 *Effect of Quercetin on let-7e-5p expression and Wnt1/β-catenin signaling pathway activation in the hippocampal DG of in CUMS-induced mice.* (**A** and **B**) Immunofluorescence staining was used to observe the positive expression of microglia and NSCs markers (Iba-1 and Nestin) in the hippocampal DG region. The red fluorescence represents Iba-1 or Nestin, while the blue fluorescence corresponds to DAPI. Bar scale, 50 μm. (**C** and **D**) Fluorescence in situ hybridization/ immunofluorescence staining was used to observe the co-localization of let-7e-5p with Iba-1 or Nestin in the hippocampal DG region. The red fluorescence represents Iba-1 or Nestin, green fluorescence indicates let-7e-5p, while the blue fluorescence corresponds to DAPI. Bar scale, 100 μm. (**E**). qRT-PCR was used to detect the expression of let-7e-5p in the hippocampal DG. (**F** and **G**). Western blotting was used to determine nuclear expression level of β-catenin and protein expression of Wnt1 and GSK-3β within the hippocampal DG. ** $P < 0.01$, *** $P < 0.001$.

Abbreviations: Que-L, low dose of Quercetin; Que-H, high dose of Quercetin; DG, dentate gyrus; NSCs, neural stem cells.

https://doi.org/10.2147/DDDT.S493779

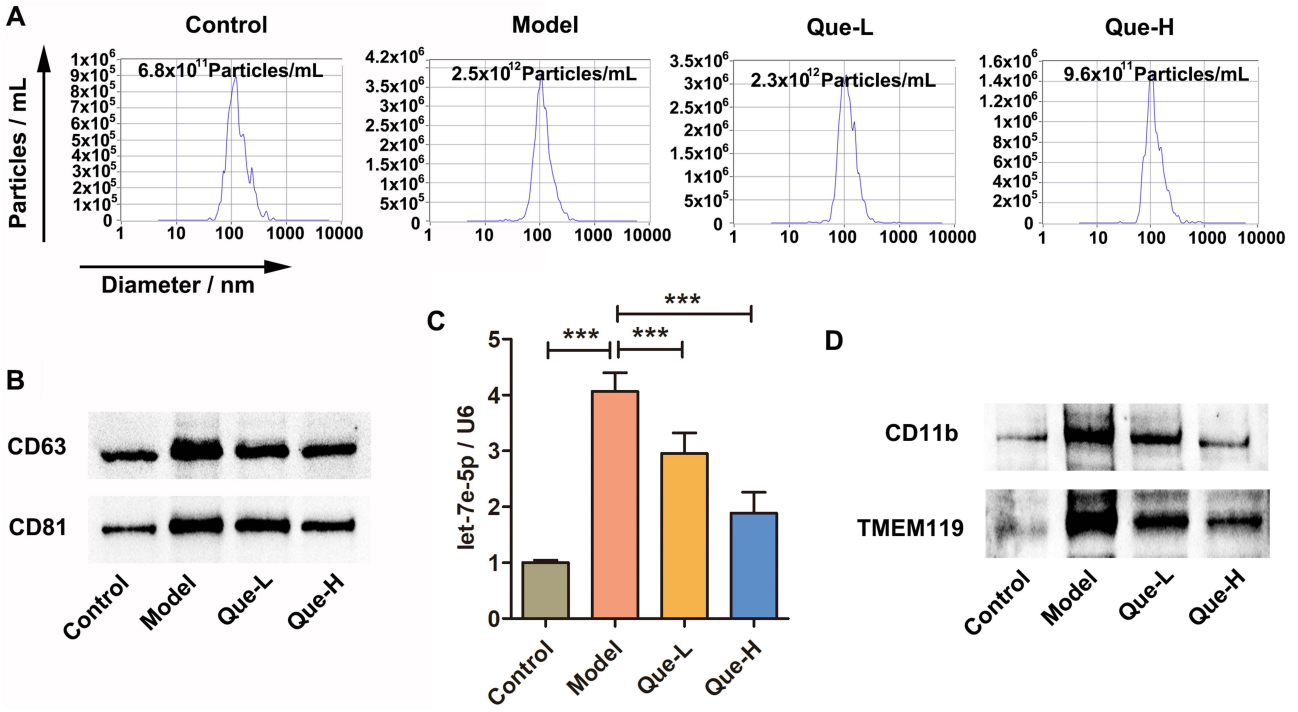


Figure 3 *Effect of Quercetin on the expression level of let-73-5p in exosomes of CUMS-induced mice.* (**A**) Nanoparticle tracking analysis was used to determine the concentration and particle size of exosomes in peripheral blood of mice. (**B**) Western blotting was used to measure the expression of exosome markers CD63 and CD81. (**C**) qRT-PCR was used to detect the expression of let-7e-5p in exosomes. (**D**) Western blotting was used to assess the expression of microglial markers CD11b and TMEM119 in exosomes. *** $P < 0.001$.

Abbreviations: Que-L, low dose of Quercetin; Que-H, high dose of Quercetin.

electron microscopy (Figure 4C), and NTA (Figure 4D) demonstrated successful isolation of exosomes secreted by BV2 cells in each group. Subsequently, we observed a significant upregulation of let-7e-5p expression in exosomes secreted by BV2 cells upon LPS stimulation, whereas Quercetin intervention resulted in a reduction of let-7e-5p expression in exosomes (Figure 4E). To investigate the potential impact of microglial exosomes on the biological characteristics of hippocampal NSCs, we conducted a co-culture experiment wherein microglia-derived exosomes were introduced to hippocampal NSCs, resulting in their engulfment (Figure 4F). Importantly, the exosomes from BV2 cells upon LPS stimulation inhibited the proliferation of NSCs and attenuated the generation of new neurons labeled with DCX positive expression. Conversely, Quercetin effectively promoted the proliferation of NSCs and the generation of new neurons. However, intervention with let-7-5p mimics reverses the beneficial effects exerted by Quercetin (Figure 4G and H). In brief, Quercetin treatment effectively mitigated the inhibitory effects of microglia-derived exosomes on NSCs proliferation and neurogenesis by downregulating let-7e-5p.

Quercetin Activated Wnt1/β-Catenin Signaling Pathway by Down-Regulating Let-7e-5p in NSCs

In addition, we observed that NSCs which engulfed exosomes derived from BV2 cells upon LPS stimulation exhibited upregulated expression of let-7e-5p (Figure 5A), reduced nuclear β-catenin and cellular Wnt1 protein expression, and enhanced GSK-3β expression (Figure 5B and C). The expression of let-7e-5p was downregulated and the Wnt1/β-catenin signaling pathway was activated in NSCs treated with Quercetin-treated exosomes, however, these regulatory effects were reversed upon introduction of let-7e-5p mimics. We also found that the online prediction website TargetScan (https://www.targetscan.org/vert_72/) predicted the presence of binding sites between let-7e-5p and Wnt1 3' UTR (Figure 5D). Therefore, we verified that let-7e-5p could directly target Wnt1 by dual-luciferase reporter assay. Therefore, Quercetin may enhance the activation of the Wnt1/β-catenin signaling pathway in NSCs through let-7e-5p down-regulation.

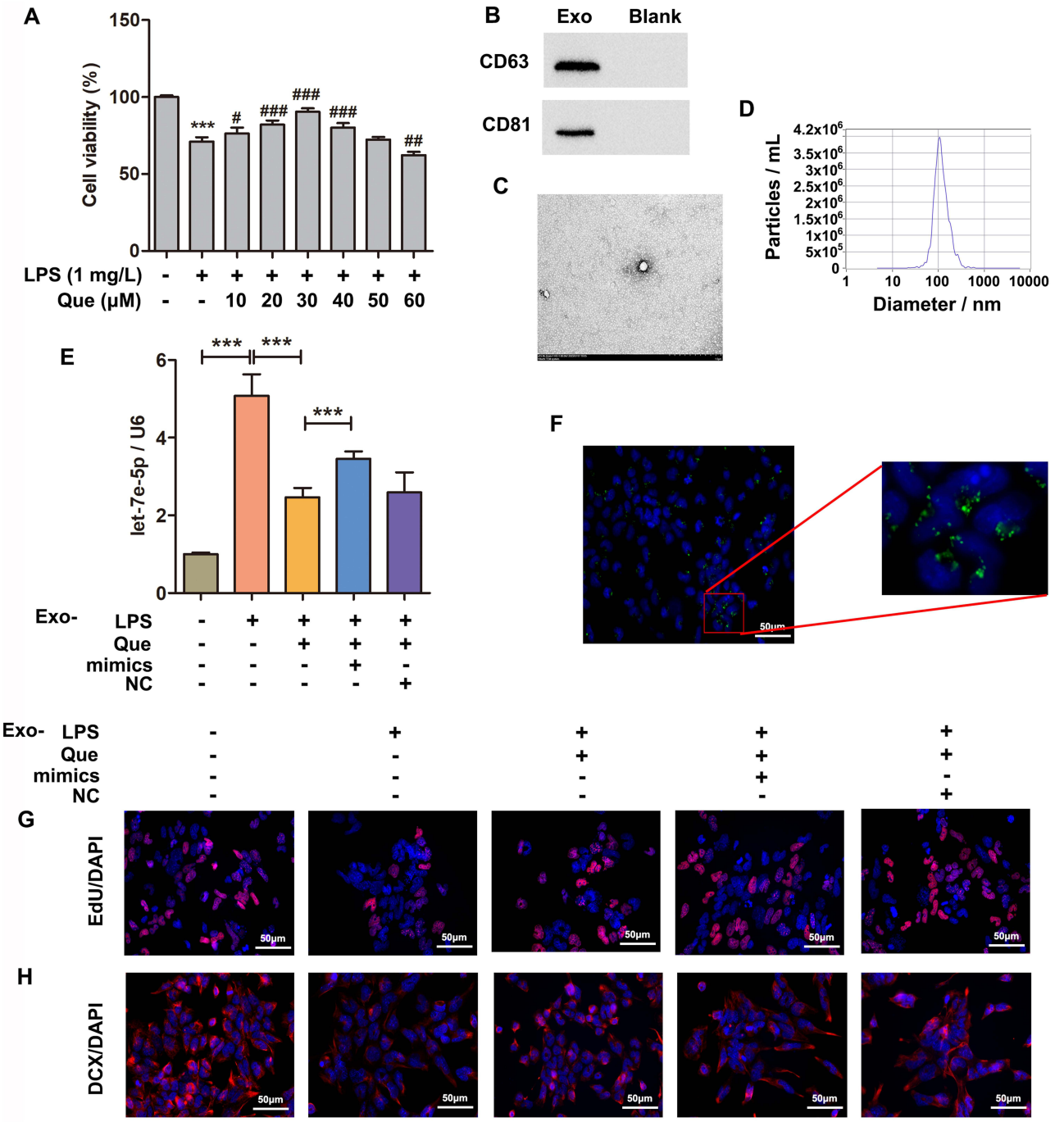


Figure 4 *Effects of Quercetin on proliferation of NSCs and neurogenesis.* (**A**) CCK-8 assay was used to assess the cell proliferation activity. (**B**) Western blotting was used to determine the protein expression of exosome markers CD63 and CD81. (**C**) Scanning electron microscopy was used to observe the morphology of exosomes. (**D**) Nanoparticle tracking analysis was used to determine the concentration and particle size of exosomes. (**E**) qRT-PCR was used to detect the expression of let-7e-5p in exosomes. (**F**) The uptake of PKH-67-labeled exosomes by NSCs was observed. The green fluorescence represents PKH-67-labeled exosomes, while the blue fluorescence corresponds to nucleus. Bar scale, 50 μm. (**G**) EdU incorporation assay was used to observe the proliferation ability of NSCs. The red fluorescence indicates the newly proliferating cells labeled with EdU, while the blue fluorescence corresponds to DAPI. Bar scale, 50 μm. (**H**) Immunofluorescence staining was used to observe the generation of new neurons. The red fluorescence indicates the newly generated neurons labeled with DCX, while the blue fluorescence corresponds to DAPI. Bar scale, 50 μm. *** $P < 0.001$.

Abbreviations: Exo, exosome; Que, Quercetin; NSCs, neural stem cells.

https://doi.org/10.2147/DDDT.S493779

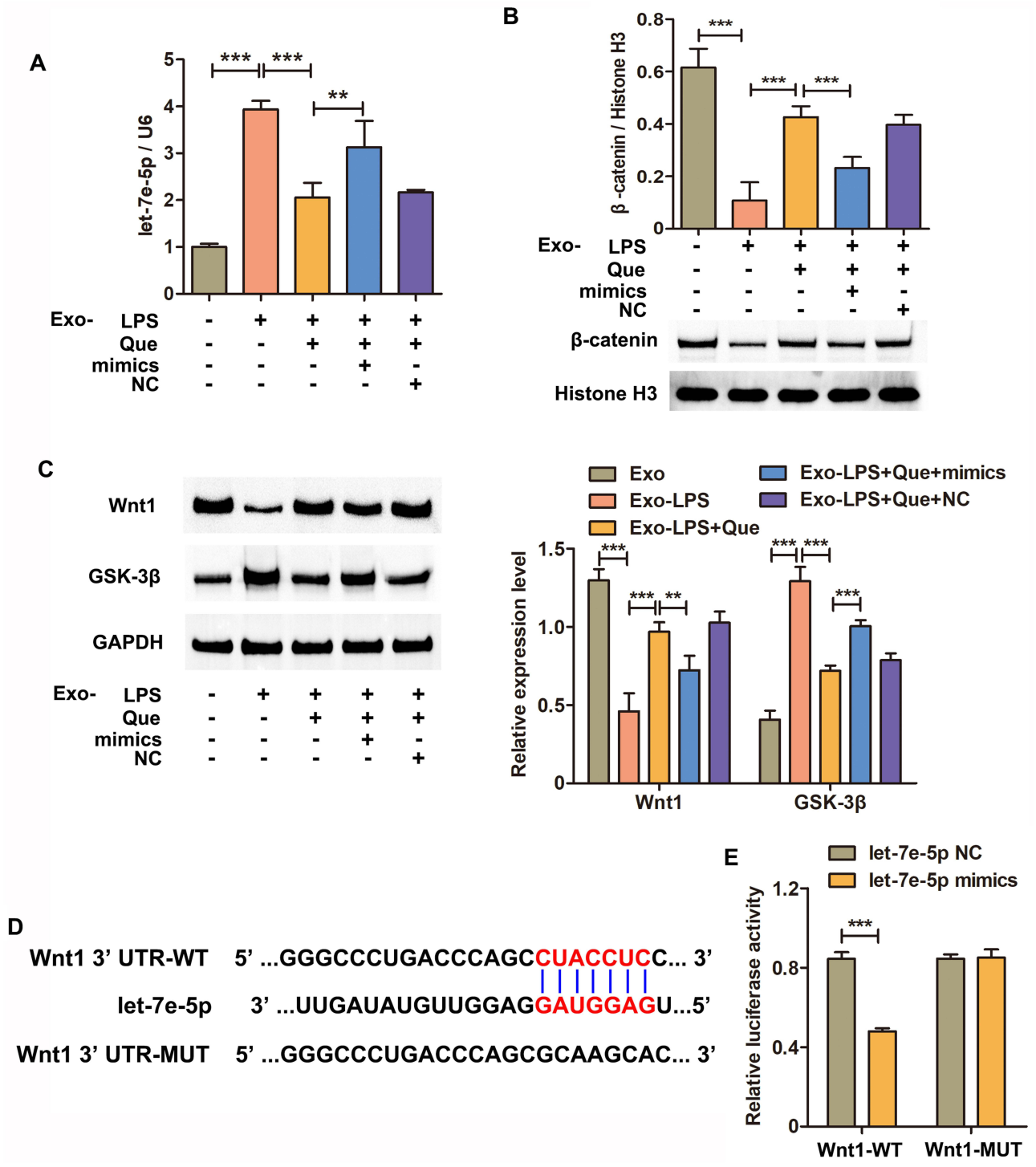


Figure 5 *Quercetin down-regulated let-7e-5p to promote the activation of Wnt1/β-catenin signaling pathways in NSCs.* (**A**) qRT-PCR was used to detect the expression of let-7e-5p in NSCs. (**B** and **C**) Western blotting was used to determine nuclear expression level of β-catenin and protein expression of Wnt1 and GSK-3β in NSCs. (**D**) TargetScan website predicted the target binding sites between let-7e-5p and Wnt1 3' UTR. (**E**) Dual-luciferase reporter assay was used to verify the targeted regulatory relationship between let-7e-5p and Wnt1. ** $P < 0.01$, *** $P < 0.001$.

Abbreviations: Exo, exosome; Que, Quercetin; NSCs, neural stem cells.

Discussion

In this study, we found that Quercetin may improve depression-like behavior and cognitive impairment in mice by regulating hippocampal DG neurogenesis, and preliminarily revealed its mechanism of action. Our data present a novel hypothesis suggesting that let-7e-5p in microglial exosomes inhibits neurogenesis associated with depression by blocking the Wnt1/β-catenin signaling pathway, which can be effectively reversed through Quercetin treatment. Therefore, Quercetin holds promising potential for the future treatment of depression.

The process of adult neurogenesis holds significant implications for the functional regulation of the hippocampus, which is a crucial brain structure responsible for learning and memory formation.[24] Adult neurogenesis is also a crucial foundation for stress recovery. Research has demonstrated that impaired adult hippocampal neurogenesis can result in emotional alterations following social failure in mice, whereas enhanced neurogenesis can facilitate the restoration of normal behavior after social failure, suggesting a direct involvement of diminished adult hippocampal neurogenesis in the etiology of depression.[25] The DG region of the hippocampus in adult mammals serves as one of the predominant site of neurogenesis, where NSC continuously generate new neurons.[26] The findings of a study demonstrated that enhancing the proliferation of NSCs can exert a protective effect on hippocampal neurons, thereby ameliorating depression-like behavior in rats.[27] The number of microglia in the DG of the hippocampus is negatively correlated with the number of NSCs under physiological conditions of voluntary running and aging, as discovered by several scholars.[28] It is suggested that microglia inhibit NSCs proliferation and reduce neurogenesis even without inflammatory stimulation. Our study also focused on the DG of the hippocampus, where CUMS induced a decrease in nestin-labeled NSCs and an increase in Iba-1 labeled microglia. Furthermore, exosomes from LPS-stimulated microglial cells significantly suppressed NSCs proliferation and neurogenesis. However, treatment with quercetin reversed these alterations. Previous studies have demonstrated that treatment with quercetin can enhance the proliferation of NSCs in the DG of rats with an Alzheimer's disease model, thereby promoting adult neurogenesis and subsequently improving learning and memory formation.[29] By integrating our findings with previous reports, it is plausible to hypothesize that quercetin may ameliorate depression-like behaviors and cognitive impairment in mice induced by CUMS through the promotion of neurogenesis in the hippocampal DG.

The release of exosomes is a common occurrence among various types of nerve cells, including neurons, astrocytes, and microglia, both in normal physiological states and during pathological conditions.[30] Serving as crucial mediators of intercellular communication, exosomes transport mRNAs and miRNAs to modulate the function of recipient cells, thereby actively participating in the onset and progression of depression. The regulation of hippocampal neurogenesis in depression by microglia involves a complex mechanism, with exosome-mediated delivery of miRNAs being one of the contributing factors.[31] Fan et al discovered that in rats with CUMS, microglia in the hippocampal DG secreted exosomes abundant in miR-146a-5p, which effectively suppressed neurogenesis and spontaneous firing of DG neurons by down-regulating the target protein.[15] In the present study, we observed an up-regulation of let-7e-5p in the hippocampal DG tissues of mice induced with CUMS, as well as in microglia-derived exosomes upon LPS stimulation. Furthermore, our findings suggest that quercetin treatment may enhance neurogenesis in the hippocampal DG region during depression by modulating the secretion of let-7e-5p-containing exosomes from microglia. However, further investigations are required to elucidate the precise molecular mechanisms through which let-7e-5p regulates neurogenesis in the DG.

The Wnt protein family is widely recognized as a pivotal regulator of neural stem cell behavior. When the Wnt signaling pathway is obstructed, it exerts inhibitory effects on the neurogenesis process of hippocampal NSCs.[32] Wnt1 is one of the representative members of the Wnt protein family. Research has demonstrated the significance of the Wnt1 signaling pathway in maintaining the functionality and development of hippocampal neural stem cells.[33] The activation of Wnt/β-catenin signaling has been demonstrated to stimulate the proliferation of neural stem cells, while also promoting their differentiation into neurons and inhibiting their differentiation into astrocytes.[34] Sun et al also demonstrated that the activity-dependent neuroprotective protein exerts its neurogenesis-promoting effects through inhibition of GSK-3β-mediated phosphorylation and degradation of β-catenin, leading to the accumulation and nuclear translocation of stable β-catenin for downstream target regulation.[35] Based on the presence of a targeted binding site between Wnt1 and let-7e-5p, our luciferase assay results have confirmed that Wnt1 is indeed a direct target gene of let-7e-5p. Our

findings strongly suggest that let-7e-5p plays a crucial role in regulating neurogenesis associated with depression by specifically targeting the Wnt1/β-catenin signaling pathway, which can be activated through Quercetin treatment.

In conclusion, this study is the first to demonstrate the involvement of let-7e-5p in the regulatory mechanism between microglial exosomes and DG neurogenesis in depression. Let-7e-5p-rich microglia-derived exosomes are transferred to DG neurons, where they inhibit neurogenesis by blocking Wnt1/β-catenin signaling. More importantly, we also discovered that quercetin treatment effectively alleviated depressive-like behavior and cognitive impairment in mice stimulated by CUMS. Furthermore, the underlying mechanism was found to be associated with the down-regulation of let-7e-5p in the DG. Consequently, our experimental findings suggest that let-7e-5p derived from microglia could serve as a promising therapeutic target for depression, while Quercetin could offer a novel avenue for the development of innovative treatment strategies for this disorder.

Abbreviations

CUMS, chronic unpredictable mild stress; DG, dentate gyrus; NSCs, neural stem cells; OFT, Open field test.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the Natural Science Foundation of Hubei Province [grant number 2023AFD114, 2023AFD134].

Disclosure

The authors declare that they have no conflict of interest.

References

1. Zhang M. Major depressive disorder treatment guidelines in China. *J Clin Psychiatry*. 2010;71 Suppl E1:e06. doi:10.4088/JCP.9058se1c.06gry
2. Kovich H, Kim W, Quaste AM. Pharmacologic Treatment of Depression. *Am Fam Physician*. 2023;107(2):173–181.
3. Kim HJ, Kim W, Kong SY. Antidepressants for neuro-regeneration: from depression to Alzheimer's disease. *Arch Pharm Res*. 2013;36 (11):1279–1290. doi:10.1007/s12272-013-0238-8
4. Eisch AJ, Petrik D. Depression and hippocampal neurogenesis: a road to remission? *Science*. 2012;338(6103):72–75. doi:10.1126/science.1222941
5. Kronmüller KT, Pantel J, Köhler S, et al. Hippocampal volume and 2-year outcome in depression. *Br J Psychiatry*. 2008;192(6):472–473. doi:10.1192/bjp.bp.107.040378
6. Kim IB, Park SC. Neural circuitry-neurogenesis coupling model of depression. *Int J Mol Sci*. 2021;22(5):2468.
7. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci*. 2007;10(9):1110–1115. doi:10.1038/nn1969
8. Planchez B, Lagunas N, Le Guisquet A-M. Increasing adult hippocampal neurogenesis promotes resilience in a mouse model of depression. *Cells*. 2021;10(5):972. doi:10.3390/cells10050972
9. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication. *Int J Biochem Cell Biol*. 2012;44(1):11–15. doi:10.1016/j.biocel.2011.10.005
10. Sokolova V, Ludwig AK, Hornung S, et al. Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. *Colloids Surf B Biointerfaces*. 2011;87(1):146–150. doi:10.1016/j.colsurfb.2011.05.013
11. Frühbeis C, Fröhlich D, Krämer-Albers EM. Emerging roles of exosomes in neuron-glia communication. *Front Physiol*. 2012;3:119. doi:10.3389/fphys.2012.00119
12. Ji XY, Guo YX, Wang LB, et al. Microglia-derived exosomes modulate myelin regeneration via miR-615-5p/MYRF axis. *J Neuroinflammation*. 2024;21(1):29. doi:10.1186/s12974-024-03019-5
13. Zhu Y, Zhao X, Liu R, Yang D, Ge G. Effect of oxygen-glucose deprivation of microglia-derived exosomes on hippocampal neurons: a study on miR-124 and inflammatory cytokines. *J Mol Histol*. 2024;55:349–357. doi:10.1007/s10735-024-10193-6
14. Jiang W, Wu Y, Pang A, Li P, Mei S. M1-type microglia-derived exosomes contribute to blood-brain barrier damage. *Brain Res*. 2024;1835:148919. doi:10.1016/j.brainres.2024.148919
15. Fan C, Li Y, Lan T, Wang W, Long Y, Yu SY. Microglia secrete miR-146a-5p-containing exosomes to regulate neurogenesis in depression. *Mol Ther*. 2022;30(3):1300–1314. doi:10.1016/j.ymthe.2021.11.006
16. Davis-Dusenbery BN, Hata A. Mechanisms of control of microRNA biogenesis. *J Biochem*. 2010;148(4):381–392. doi:10.1093/jb/mvq096

17. Gecys D, Dambrauskiene K, Simonyte S. Circulating hsa-let-7e-5p and hsa-miR-125a-5p as possible biomarkers in the diagnosis of major depression and bipolar disorders. *Dis Markers*. 2022;2022:3004338. doi:10.1155/2022/3004338
18. Kale A, Piskin Ö, Bas Y, et al. Neuroprotective effects of quercetin on radiation-induced brain injury in rats. *J Radiat Res*. 2018;59(4):404–410. doi:10.1093/jrr/rry032
19. Kaur S, Singla N, Dhawan DK. Neuro-protective potential of quercetin during chlorpyrifos induced neurotoxicity in rats. *Drug Chem Toxicol*. 2019;42(2):220–230. doi:10.1080/01480545.2019.1569022
20. Pakrashi S, Chakraborty J, Bandyopadhyay J. Neuroprotective role of quercetin on rotenone-induced toxicity in SH-SY5Y cell line through modulation of apoptotic and autophagic pathways. *Neurochem Res*. 2020;45(8):1962–1973. doi:10.1007/s11064-020-03061-8
21. Guan Y, Wang J, Wu X, et al. Quercetin reverses chronic unpredictable mild stress-induced depression-like behavior in vivo by involving nuclear factor-E2-related factor 2. *Brain Res*. 2021;1772:147661. doi:10.1016/j.brainres.2021.147661
22. Du L, Fan X, Yang Y, Wu S, Liu Y. Quercetin ameliorates cognitive impairment in depression by targeting HSP90 to inhibit NLRP3 inflammasome activation. *Mol Neurobiol*. 2024;61(9):6628–6641. doi:10.1007/s12035-024-03926-x
23. Luong N, Olson JK. Exosomes secreted by microglia during virus infection in the central nervous system activate an inflammatory response in bystander cells. *Front Cell Dev Biol*. 2021;9:661935. doi:10.3389/fcell.2021.661935
24. Pérez-Rodríguez DR, Blanco-Luquin I, Mendioroz M. The participation of microglia in neurogenesis: a review. *Brain Sci*. 2021;11(5):658. doi:10.3390/brainsci11050658
25. Lehmann ML, Brachman RA, Martinowich K, Schloesser RJ, Herkenham M. Glucocorticoids orchestrate divergent effects on mood through adult neurogenesis. *J Neurosci*. 2013;33(7):2961–2972. doi:10.1523/JNEUROSCI.3878-12.2013
26. Cameron HA, Woolley CS, McEwen BS, Gould E. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience*. 1993;56(2):337–344. doi:10.1016/0306-4522(93)90335-D
27. Jiang C, Zheng S, Chen T, et al. Repetitive transcranial magnetic stimulation improves depression-like behavior in rats by promoting neural stem cell proliferation and differentiation. *Neuroscience*. 2023;524:1–10. doi:10.1016/j.neuroscience.2022.09.013
28. Gebara E, Sultan S, Kocher-Braissant J, Toni N. Adult hippocampal neurogenesis inversely correlates with microglia in conditions of voluntary running and aging. *Front Neurosci*. 2013;7:145. doi:10.3389/fnins.2013.00145
29. Karimipour M, Rahbarghazi R, Tayefi H, et al. Quercetin promotes learning and memory performance concomitantly with neural stem/progenitor cell proliferation and neurogenesis in the adult rat dentate gyrus. *Int J Dev Neurosci*. 2019;74:18–26. doi:10.1016/j.ijdevneu.2019.02.005
30. Gupta A, Pulliam L. Exosomes as mediators of neuroinflammation. *J Neuroinflammation*. 2014;11:68. doi:10.1186/1742-2094-11-68
31. Fang S, Wu Z, Guo Y, et al. Roles of microglia in adult hippocampal neurogenesis in depression and their therapeutics. *Front Immunol*. 2023;14:1193053. doi:10.3389/fimmu.2023.1193053
32. Lie DC, Colamarino SA, Song HJ, et al. Wnt signalling regulates adult hippocampal neurogenesis. *Nature*. 2005;437(7063):1370–1375. doi:10.1038/nature04108
33. Wexler EM, Paucer A, Kornblum HI, Palmer TD, Geschwind DH. Endogenous Wnt signaling maintains neural progenitor cell potency. *Stem Cells*. 2009;27(5):1130–1141. doi:10.1002/stem.36
34. Zhang WM, Zhang ZR, Yang XT, Zhang YG, Gao YS. Overexpression of miR-21 promotes neural stem cell proliferation and neural differentiation via the Wnt/β-catenin signaling pathway in vitro. *Mol Med Rep*. 2018;17(1):330–335. doi:10.3892/mmr.2017.7856
35. Sun X, Peng X, Cao Y, Zhou Y, Sun Y. ADNP promotes neural differentiation by modulating Wnt/β-catenin signaling. *Nat Commun*. 2020;11(1):2984. doi:10.1038/s41467-020-16799-0