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Fatemeh Naghizadeh

Iran University of Medical Sciences

Somayeh Niknazar

Shahid Beheshti University of Medical Sciences

Ali Dadseresht

Iran University of Medical Sciences

Elham Seidkhani

Iran University of Medical Sciences

Rafieh Alizadeh

Iran University of Medical Sciences

Arezo Nahavandi

arezo1999@gmail.com

Iran University of Medical Sciences

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Intranasal Human Olfactory Mucosa-Derived Neural Stem Cells Restore Neuroplasticity and Suppress Neuroinflammation in a Rat Model of Chronic Stress-Induced Depression

Fatemeh Naghizadeh¹, Somayeh Niknazar^{2**}, Ali Dadseresht³, Elham Seidkhani³, Rafieh Alizadeh⁴, Arezo Nahavandi^{*1,5}

1. Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

2. Functional Neurosurgery Research Center, Research Institute of Functional Neurosurgery, Shohada Tajrish Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

3. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

4. ENT and Head and Neck Research Center and Department, The Five Senses Health Institute, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

5. Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran

****Co –corresponding Author**

Somayeh Niknazar, Ph.D. Assistant Professor of Medical Physiology

***Correspondence to:**

Arezo Nahavandi, Ph.D. Professor of Medical Physiology

Tel: (+98) 02122724211 E-mail: arezo1999@gmail.com

[ORCID: 0000-0002-5317-6056](https://orcid.org/0000-0002-5317-6056)

Abstract

Major depressive disorder (MDD) is a leading cause of disability worldwide and is characterized by impaired neurogenesis, neuroinflammation, and limited therapeutic efficacy of currently available treatments in a substantial proportion of patients. Human olfactory mucosa-derived neural stem cells (OM-NSCs) possess regenerative and neurogenic properties that may provide a novel therapeutic strategy for depression. Therefore, this study aimed to investigate the therapeutic effects of intranasally administered human OM-NSCs on behavioral deficits, neurogenesis, and neuroinflammatory responses in a rat model of chronic stress-induced depression. Forty-eight male Wistar rats were randomly assigned to seven groups ($n = 8$ per group): healthy control, untreated depressed, fluoxetine-treated, Neurobasal-treated, fresh culture medium –treated, single-dose human OM-NSC-treated, and double-dose OM-NSC-treated. Depression-like behavior was induced using a six-week chronic unpredictable mild stress (CUMS) protocol. Cultured OM-NSCs were characterized by Nestin and SOX2 expression and administered intranasally. Behavioral assessments included the forced swim test (FST), elevated plus maze (EPM), and sucrose preference test (SPT). Hippocampal cell counts, brain-derived neurotrophic factor (BDNF), serotonin receptor expression, corticosterone, inflammatory cytokines, and cell migration were evaluated. OM-NSC treatment significantly ameliorated depression- and anxiety-like behaviors, restored sucrose preference, reduced inflammatory and oxidative stress markers, increased hippocampal cellularity, and enhanced neuroplasticity-related gene expression. DiI-labeled cells were detected in the hippocampus, confirming successful migration after intranasal administration. Repeated administration produced greater effects than a single dose. **Conclusion:** Intranasal delivery of human OM-NSCs alleviated behavioral and neurobiological alterations induced by chronic stress, supporting their potential as a regenerative therapeutic approach for depression.

Keywords: Chronic unpredictable mild stress; Major depressive disorder; Olfactory Mucosa-Derived Neural Stem Cells; Neurogenesis; Neuroinflammation.

Introduction

Major depressive disorder (MDD) is one of the most prevalent and disabling psychiatric disorders worldwide and represents a major public health challenge. Despite the availability of pharmacological and non-pharmacological interventions, a substantial proportion of patients fail to achieve sustained remission, with approximately 30% exhibiting treatment-resistant depression. Moreover, recurrent episodes and incomplete recovery contribute significantly to functional impairment, reduced quality of life, and considerable socioeconomic burden (Bakhtiarzadeh et al., 2018; Uher, Payne, Pavlova, & Perlis, 2014). Although currently available antidepressants can alleviate symptoms in many patients, their therapeutic efficacy remains limited, and complete restoration of normal emotional and cognitive functioning is often not achieved (Mrozek et al.). Accumulating evidence indicates that neuroinflammation, impaired neuroplasticity, and progressive neurodegenerative changes are central components of the pathophysiology of depression. Chronic stress and depressive states have been associated with neuronal loss, reduced hippocampal neurogenesis, and dysregulation of inflammatory pathways, all of which may contribute to the persistence of depressive symptoms and vulnerability to relapse (Hurley & Tizabi, 2013).

Regenerative medicine has emerged as a promising therapeutic paradigm aimed at restoring damaged tissues and cellular functions through stem cells and their derivatives. Among the various stem cell populations, neural stem cells (NSCs) derived from the olfactory mucosa are of particular interest because of their intrinsic neurogenic potential and accessibility. The olfactory system, together with the hippocampal dentate gyrus and the subventricular zone, is closely associated with

lifelong neurogenesis, suggesting that olfactory mucosa-derived NSCs may possess substantial regenerative capacity within the central nervous system (Velikic et al., 2024). Previous studies have explored the therapeutic potential of olfactory mucosa-derived NSCs in neurodegenerative disorders, including Parkinson's and Alzheimer's diseases. These cells exhibit neuroregenerative, neuroprotective, and immunomodulatory properties and can be delivered through a minimally invasive intranasal route. Following administration, NSCs may migrate toward sites of injury and inflammation, where they contribute to tissue repair, modulation of neuroinflammatory responses, and restoration of neuronal circuitry (Cecerska-Heryć et al., 2023; Jin et al., 2023).

Among the molecular pathways implicated in depression, serotonergic signaling plays a pivotal role. The serotonin receptor family comprises multiple receptor classes and subtypes, among which the 5-hydroxytryptamine 1A receptor (5-HT_{1A} receptor) has received particular attention because of its critical involvement in mood regulation and antidepressant responses. The 5-HT_{1A} receptor functions both as a presynaptic autoreceptor in the raphe nuclei and as a postsynaptic heteroreceptor in limbic and cortical brain regions. Chronic stress has been reported to alter the balance between these receptor populations, resulting in dysregulated serotonergic neurotransmission and increased susceptibility to depressive behavior (Daniali et al., 2013; Kaufman, DeLorenzo, Choudhury, & Parsey, 2016).

Furthermore, genetic and transcriptional variations affecting 5-HT_{1A} receptor expression have been associated with altered serotonergic function and vulnerability to depression. Changes in receptor expression and signaling may interact with neuroinflammatory and neurodegenerative mechanisms, providing a molecular link between environmental stressors and the biological manifestations of depressive disorders (Koller, Bondy, Preuss, Zill, & Soyka, 2006). Given the growing evidence supporting the regenerative and immunomodulatory properties of olfactory

mucosa-derived NSCs, the present study aimed to investigate the effects of intranasal administration of human olfactory mucosa-derived neural stem cells on behavioral outcomes, hippocampal neurogenesis, neuroinflammatory markers, and 5-HT_{1A} receptor expression in a rat model of chronic stress-induced depression.

Materials and Methods

Isolation, Culture, and Characterization of Human Olfactory Mucosa-Derived Stem Cells

Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of Iran University of Medical Sciences. Tissue samples were collected under general anesthesia during routine nasal surgery in the operating room of Hazrat Rasool Akram Hospital. Samples were obtained from either the posterior nasal septum or the medial wall of the superior turbinate, depending on tissue accessibility. The human olfactory mucosal specimens were immediately transferred to Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin. Under sterile conditions, tissue samples were mechanically dissected into small fragments and seeded into 6-well culture plates containing complete culture medium. Cultures were maintained in a humidified incubator at 37°C with 5% CO₂, and the medium was refreshed every three days. Cells migrating from the explants were allowed to proliferate until reaching approximately 80–90% confluence, after which they were detached using trypsin–ethylenediaminetetraacetic acid (EDTA) and expanded in culture flasks for subsequent analyses.

To confirm their cellular phenotype, olfactory mucosa-derived stem cells (OE-MSCs) were characterized by flow cytometry using a panel of mesenchymal stem cell surface markers,

including CD105, CD90, and CD73, as well as the hematopoietic markers CD45 and CD34 (BD Biosciences, San Jose, CA, USA). In addition, immunocytochemical analyses were performed to evaluate the expression of nestin and vimentin, markers associated with neural progenitor and mesenchymal cell populations, respectively. These assessments were conducted to verify the stem cell characteristics and neuroregenerative potential of the cultured OE-MSCs before their use in subsequent experiments.

Neural Induction and Characterization of Human Olfactory Mucosa-Derived

Neural Stem Cells

Neural Stem Cell Induction

To generate neural stem cells (NSCs), olfactory mucosa-derived mesenchymal stem cells (OE-MSCs) were cultured in Neurobasal medium (Gibco, USA) supplemented with 2% B27 (Gibco, USA), 20 ng/mL basic fibroblast growth factor (bFGF; PeproTech, USA), and 20 ng/mL epidermal growth factor (EGF; Gibco, USA). Culture medium was refreshed every 48 h. Upon reaching 70–80% confluence, cells were passaged and expanded under identical culture conditions. Neural induction was continued through three sequential passages before further characterization and transplantation experiments.

Characterization of Neural Stem Cells

Neurosphere Formation Assay

The self-renewal capacity of induced NSCs was evaluated using a neurosphere formation assay. Dissociated cells were cultured under non-adherent conditions, and neurosphere formation was monitored microscopically. After seven days, the number and diameter of neurospheres were recorded as indicators of stemness and proliferative potential.

Flow Cytometric Analysis

Neural stem cell identity was confirmed by flow cytometry. Neurospheres were enzymatically dissociated into single-cell suspensions, fixed with 4% paraformaldehyde, and incubated with primary antibodies against Nestin, SOX2, CD34, and CD45 (Abcam, Cambridge, UK).

Following incubation with appropriate fluorophore-conjugated secondary antibodies, samples were analyzed using a PartecPAS FacScan flow cytometer (DakoCytomation, Denmark) and FlowJo software. A minimum of 10,000 events were acquired per sample. Cell debris, doublets, and non-viable cells were excluded using forward scatter (FSC), side scatter (SSC), and 7-aminoactinomycin D (7-AAD) gating strategies. Isotype-matched controls were used to define background fluorescence and establish gating thresholds. All analyses were performed in triplicate.

DiI Labeling and Cell Tracking

Prior to transplantation, NSCs were labeled with the lipophilic fluorescent dye DiI to enable in vivo cell tracking. Labeled cells were washed thoroughly with phosphate-buffered saline (PBS) to remove excess dye and incubated under protected light conditions. Following intranasal administration, migration and localization of transplanted cells were evaluated in hippocampal and prefrontal cortical regions using fluorescence microscopy.

Experimental Design and Animal Groups

Forty-eight adult male Wistar rats (150–180 g) were used in this study. Depression-like behavior was induced using a six-week chronic unpredictable mild stress (CUMS) paradigm. The animals were randomly assigned to seven experimental groups (n = 8 per group):: (1) healthy control (no intervention); (2) depression model treated with fluoxetine; (3) depression model treated with fresh culture medium; (4) depression model treated with Neurobasal; (5) depression model treated with a single intranasal administration of OM-NSCs; (6) depression model treated with two intranasal

administrations of OM-NSCs; and (7) untreated depression model (depression only, without any additional intervention). Behavioral, molecular, biochemical, and histological assessments were performed four weeks after treatment to evaluate the therapeutic effects of NSC administration.

Study Design and Behavioral Evaluation in CUMS-Induced Depression Model

Following a six-week experimental period, the control group was maintained under standard laboratory conditions, while all other groups were subjected to the CUMS protocol to induce depression-like phenotypes (Bakhtiarzadeh et al., 2018). After confirmation of depressive behavior, animals received their respective treatments as previously described.

Behavioral assessments were conducted to evaluate depressive- and anxiety-like behaviors, including the Forced Swim Test (FST), Elevated Plus Maze (EPM), and Sucrose Preference Test (SPT). These tests were performed in a controlled environment to minimize external stressors and ensure reproducibility of behavioral outcomes.

Blood Sampling and Biochemical Analysis

At the end of the experimental protocol, animals were deeply anesthetized using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). Whole blood samples (approximately 2 mL) were collected via cardiac puncture under full anesthesia. Blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was subsequently stored at -20°C until biochemical analysis (Niknazar et al., 2013).

Serum corticosterone levels were quantified using an enzyme-linked immunosorbent assay (ELISA). In addition, pro-inflammatory cytokines (IL-6 and TNF- α) and oxidative stress markers (malondialdehyde, MDA) were measured using commercially available ELISA kits following the manufacturer's instructions.

Nissl Staining and Histological Analysis

To evaluate neuronal integrity, Nissl staining was performed on coronal brain sections (5 μ m thickness) prepared according to the Paxinos and Watson rat brain atlas. Cresyl violet staining was used to visualize neuronal cell bodies by binding to Nissl substance. Hippocampal CA1 pyramidal neurons were examined under light microscopy.

Neuronal survival was quantified by counting morphologically intact neurons with clearly visible nuclei using ImageJ software. Cells exhibiting intact morphology were considered viable neurons, and analyses were conducted in a blinded manner to reduce bias.

Intranasal Cell Transplantation

For cell delivery, four million neural stem cells (NSCs) were administered intranasally into each nostril using a fine glass micropipette under mild anesthesia. This minimally invasive route was selected to facilitate direct access to the central nervous system via olfactory pathways.

Groups received either culture medium or conditioned medium following the same administration protocol to control for procedural effects.

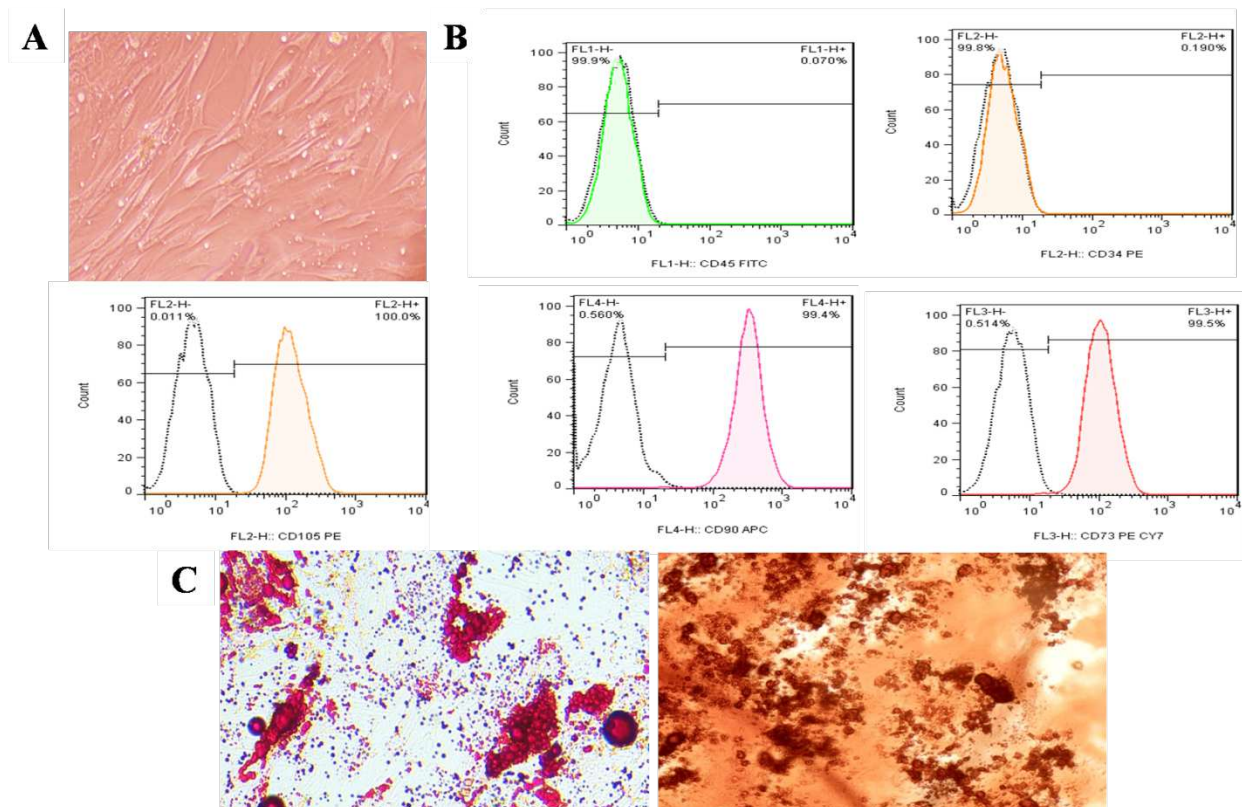
Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test to evaluate differences among experimental groups. Behavioral, biochemical, and molecular data were analyzed using GraphPad Prism software. A p-value of less than 0.05 was considered statistically significant.

Results

Characterization of Human Olfactory Mucosa-Derived Stem Cells

Cultured OE-MSCs exhibited a typical spindle-shaped fibroblast-like morphology and strong adherence to the culture surface. Flow cytometric analysis confirmed the mesenchymal phenotype of the isolated cells, demonstrating positive expression of CD73, CD90, and CD105, with negligible expression of the hematopoietic markers CD34 and CD45. Multipotency was verified through successful adipogenic and osteogenic differentiation, as evidenced by lipid droplet accumulation and calcium deposition, respectively. Furthermore, immunocytochemical analyses revealed robust expression of nestin and vimentin, supporting the neuroectodermal characteristics and regenerative potential of the isolated OE-MSCs (Figure 1).



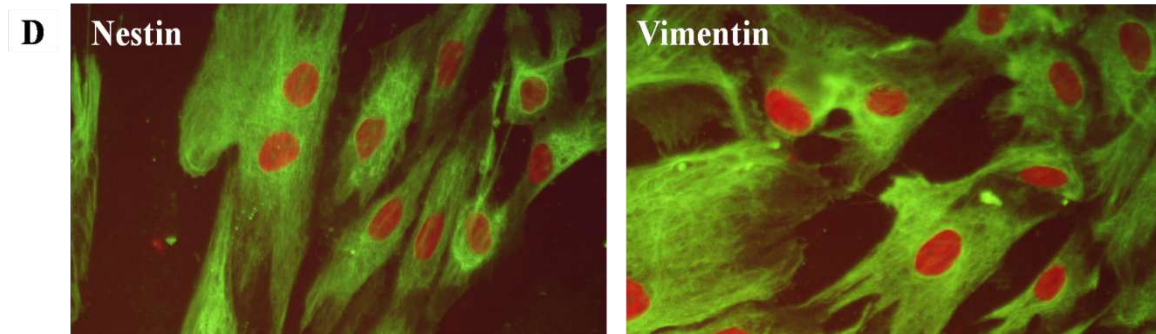


Figure 1. Characterization of human olfactory mucosa-derived mesenchymal stem cells (OE-MSCs). (A) Representative morphology of cultured OE-MSCs displaying a spindle-shaped fibroblast-like appearance. (B) Flow cytometric analysis demonstrating positive expression of mesenchymal stem cell markers (CD73, CD90, and CD105) and minimal expression of hematopoietic markers (CD34 and CD45). (C) Multilineage differentiation capacity of OE-MSCs confirmed by adipogenic differentiation (Oil Red O staining) and osteogenic differentiation (Alizarin Red staining). (D) Immunocytochemical staining showing expression of the neuroectodermal markers nestin and vimentin. Nuclei were counterstained with propidium iodide (PI).

OE-MSC-Derived Neural Stem Cells Induction and Characterization

Neural induction of OE-MSCs resulted in the formation of free-floating neurospheres, indicating successful acquisition of neural stem cell characteristics. The generated neurospheres exhibited robust proliferative capacity and maintained their spherical morphology throughout culture expansion. Flow cytometric analysis further confirmed the neural stem cell phenotype, revealing high expression levels of the neural progenitor markers Nestin (87.24%) and SOX2 (89%), while hematopoietic markers remained negligible. These findings verified the successful generation of a highly enriched neural stem cell population suitable for subsequent transplantation experiments (Figure 2).

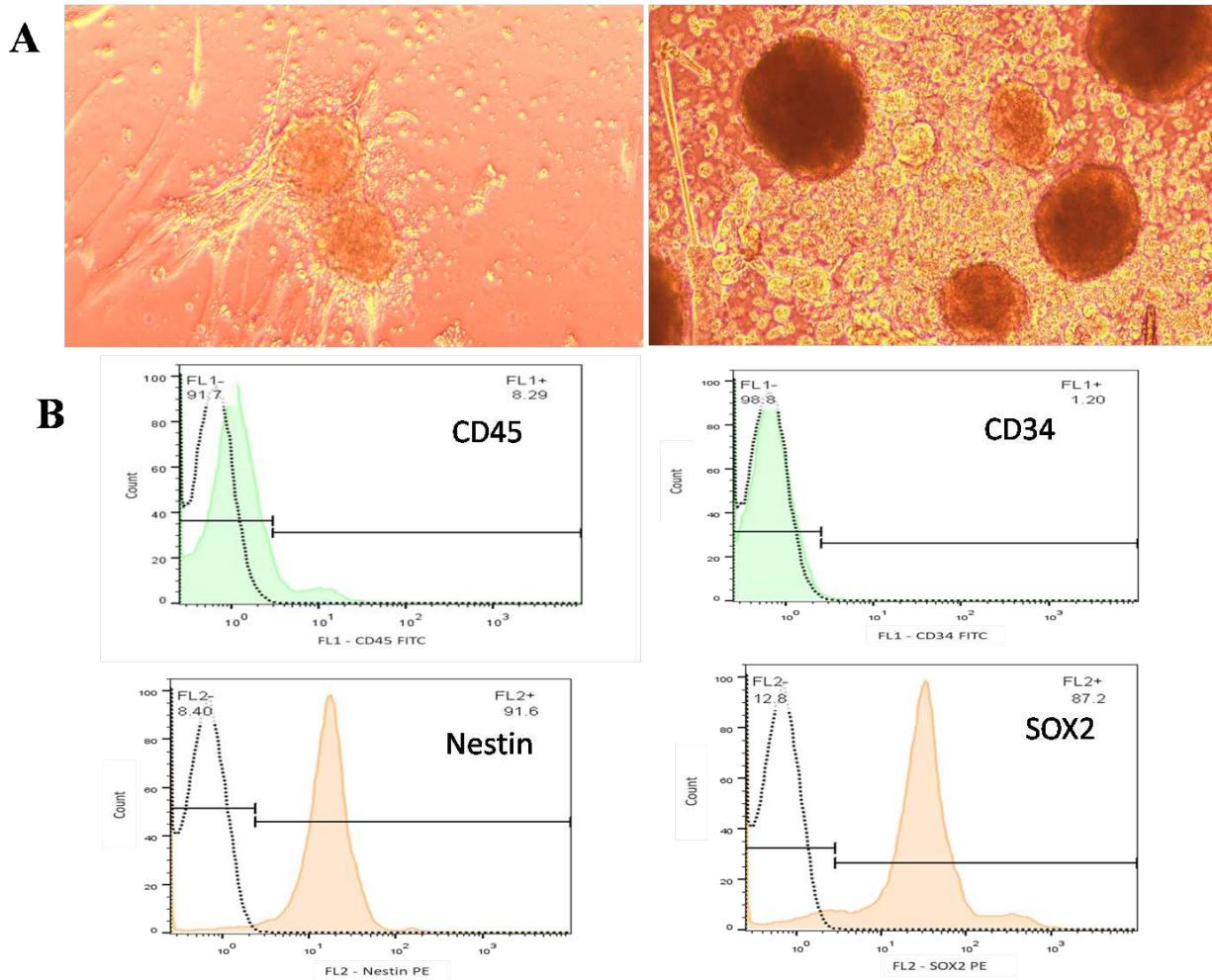


Figure 2. Characterization of olfactory mucosa-derived neural stem cells (NSCs). (A) Representative images of neurosphere formation following neural induction of OE-MSCs in Neurobasal medium supplemented with growth factors. (B) Flow cytometric analysis demonstrating strong expression of the neural stem cell markers Nestin and SOX2 and minimal expression of the hematopoietic markers CD34 and CD45. The high proportion of Nestin- and SOX2-positive cells confirms successful neural lineage commitment and enrichment of the NSC population.

Depression-like behaviors

Forced Swim Test

CUMS significantly increased immobility time in the Forced Swim Test compared with the healthy control group, confirming the establishment of a depression-like phenotype ($P < 0.05$). Treatment with intranasally administered neural stem cells markedly reduced immobility behavior relative to the untreated stress group ($P = 0.0041$). Notably, immobility levels in the neural stem cell-treated animals were comparable to those observed in healthy controls, indicating a substantial reversal of stress-induced behavioral deficits. Similar improvements were observed in the fluoxetine-treated group. These findings suggest that neural stem cell therapy effectively alleviates depressive-like behavior induced by chronic stress (Figure 3).

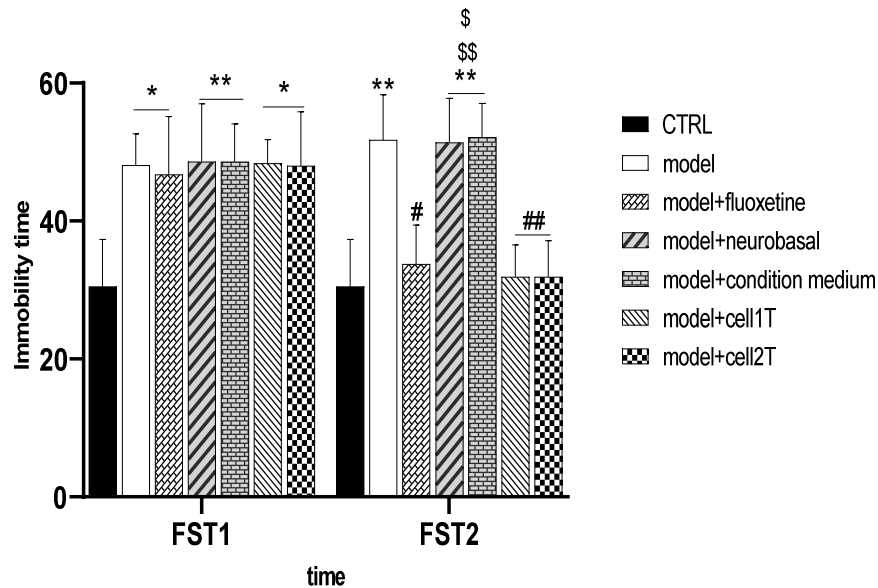


Figure 3. Effects of neural stem cell treatment on immobility time in the Forced Swim Test. Chronic stress significantly increased immobility behavior, whereas neural stem cell and fluoxetine treatments reduced immobility compared with the untreated stress group. Data are presented as mean $\pm$ SEM ($n = 8$ per group). * $P < 0.05$, ** $P < 0.01$ vs. healthy control, # $P < 0.05$, ## $P < 0.01$ vs model group. $^sP < 0.05$, $^{ss}P < 0.01$ vs. fluoxetine

Anxiety-Like Behavior in the Elevated Plus Maze Test

The Elevated Plus Maze (EPM) test revealed significant differences among the experimental groups in both the percentage of time spent in the open arms and the number of open-arm entries ($P < 0.001$). Exposure to CUMS induced a marked anxiety-like phenotype, characterized by reduced exploration of the open arms compared with healthy controls. Treatment with human olfactory mucosa-derived neural stem cells significantly increased both open-arm time and open-arm entries relative to the untreated stress group ($P < 0.05$). These effects were observed following both single and repeated administrations of neural stem cells, indicating a substantial reduction in anxiety-like behavior. Moreover, the behavioral improvement reached levels comparable to those of the healthy control group, suggesting effective reversal of stress-induced anxiety-related deficits (Figure 4).

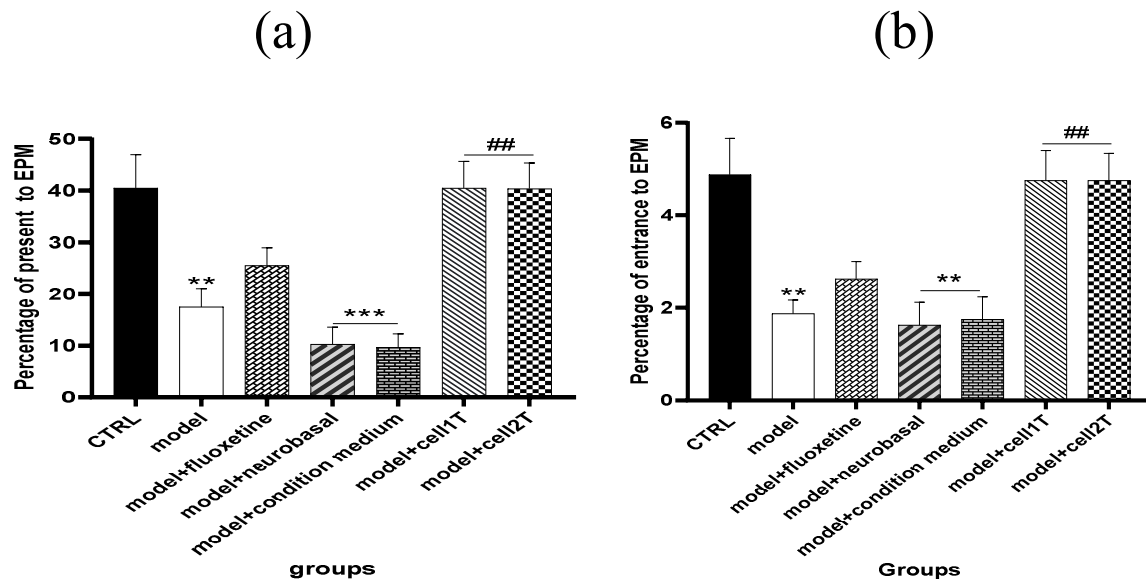


Figure 4. Effects of neural stem cell treatment on anxiety-like behavior in the Elevated Plus Maze (EPM) test. (A) Percentage of time spent in the open arms. (B) Percentage of open-arm entries. Chronic stress reduced open-arm exploration, whereas neural stem cell treatment significantly increased both parameters. Data are presented as mean $\pm$ SEM ($n = 8$ per group). ** $P < 0.01$, *** $P < 0.001$ vs. healthy control, ## $P < 0.01$ vs model group.

Anhedonia Assessment Using the Sucrose Preference Test

CUMS significantly reduced sucrose preference compared with the healthy control group, indicating the development of anhedonia, a core feature of depressive-like behavior ($P < 0.0001$). Animals treated with human olfactory mucosa-derived neural stem cells exhibited a marked increase in sucrose consumption at both one and six hours after testing compared with the untreated stress group ($P < 0.0001$). The therapeutic effect was observed following both treatment regimens and restored sucrose preference to levels comparable with those of healthy controls. Fluoxetine treatment also improved sucrose preference, although the effect was less pronounced than that observed in the neural stem cell-treated groups. These findings demonstrate that neural stem cell therapy effectively attenuates stress-induced anhedonic behavior (Figure 5).

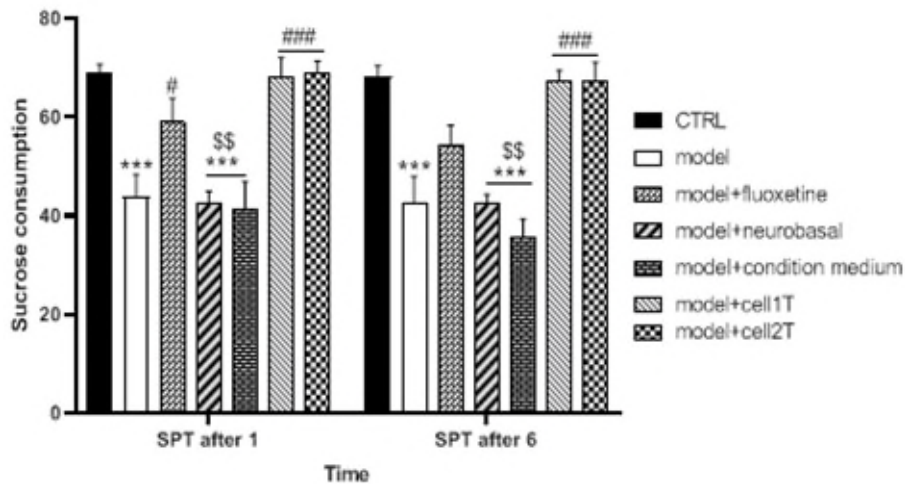


Figure 5. Effects of neural stem cell treatment on anhedonia-like behavior in the Sucrose Preference Test (SPT). Sucrose consumption was assessed at 1 h and 6 h following sucrose exposure. Chronic stress significantly reduced sucrose preference, whereas neural stem cell treatment restored sucrose consumption to near-control levels. Data are presented as mean $\pm$ SEM ($n = 8$ per group). *** $P < 0.0001$ vs. healthy control group; # $P < 0.05$, ### $P < 0.001$ vs. model group. \$\$ $P < 0.01$ vs. fluoxetine???

Histological Analysis

Neuronal Cell Counts in the Hippocampal CA1 Region

Nissl staining was performed to evaluate neuronal integrity in the hippocampal CA1 region following chronic stress exposure and subsequent treatments. Quantitative analysis using ImageJ software revealed a significant reduction in the number of viable neurons in the CA1 region of rats subjected to chronic unpredictable mild stress compared with healthy controls ($P < 0.0001$). In addition, animals receiving vehicle treatments (Neurobasal medium and conditioned medium) also exhibited a marked decrease in neuronal counts relative to the control group ($P < 0.0001$).

In contrast, intranasal administration of human olfactory mucosa-derived neural stem cells significantly preserved neuronal density in the CA1 region compared with the untreated stress group ($P < 0.0001$). Notably, repeated NSC administration demonstrated a greater neuroprotective effect than a single dose ($P = 0.0001$), suggesting a dose- and frequency-dependent enhancement of neuronal survival and/or neurogenic processes in the hippocampus (Figure 6).

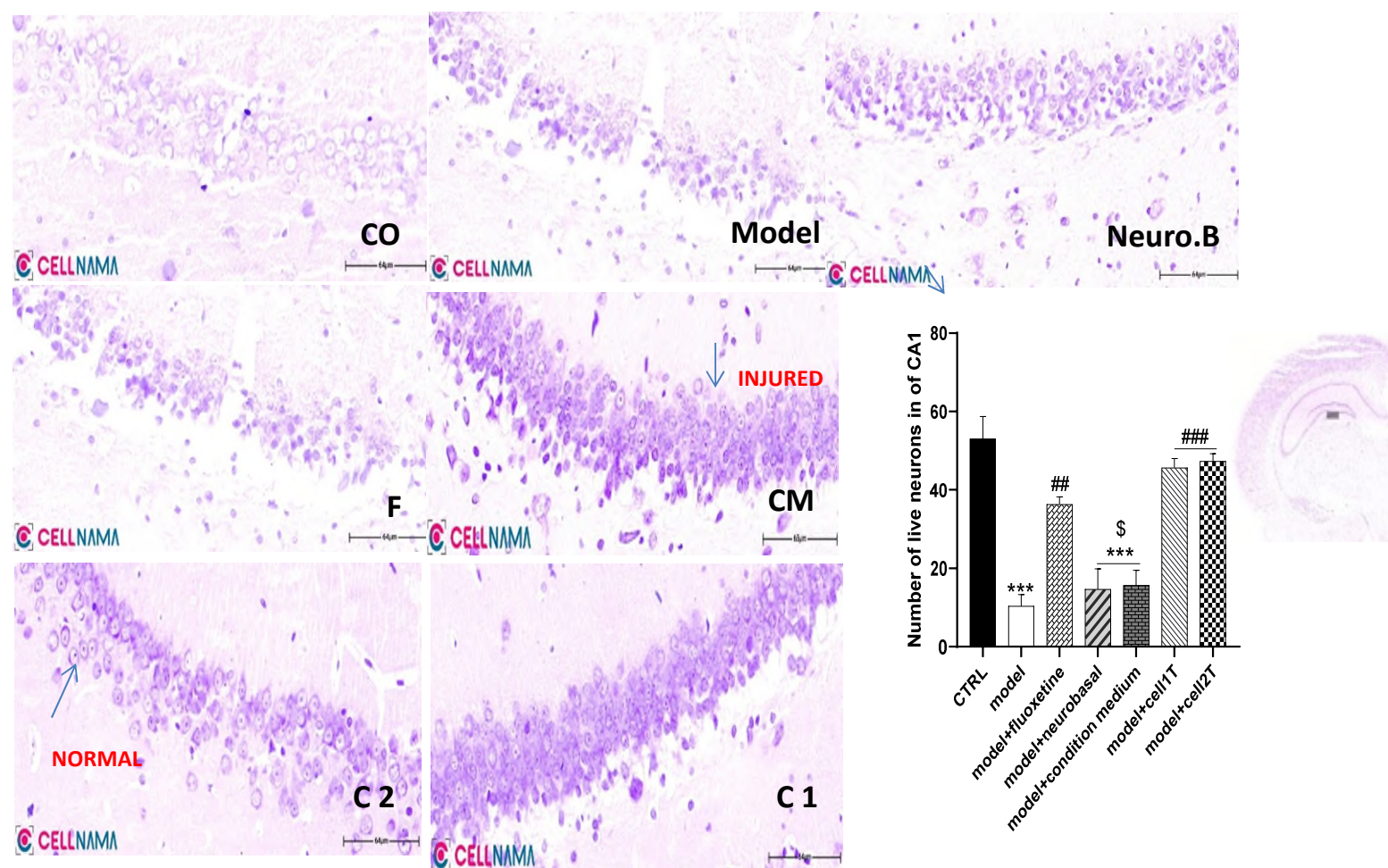


Figure 6. Neuronal cell counts in the hippocampal CA1 region following chronic stress and treatment. Nissl staining was used to quantify viable neurons in the CA1 area. Chronic stress significantly reduced neuronal density, whereas neural stem cell treatment preserved CA1 neuronal counts, particularly in the repeated administration group. Data are presented as mean $\pm$ SEM ($n = 3$ per group). *** $P < 0.001$ vs. healthy control group; ## $P < 0.01$, ### $P < 0.001$ vs. model group. \$ $P < 0.05$ vs. fluoxetine.

Biochemical Analyses

Tissue Interleukin-6 (IL-6) Levels

CUMS significantly increased hippocampal IL-6 levels compared with the healthy control group ($P < 0.0001$). Intranasal administration of human olfactory mucosa-derived neural stem cells significantly reduced IL-6 concentrations in both single- and double-dose groups, restoring levels close to those observed in healthy animals. In contrast, fluoxetine treatment did not produce a significant reduction in IL-6 compared with the stress group, indicating a limited anti-inflammatory effect in this model (Figure 7).

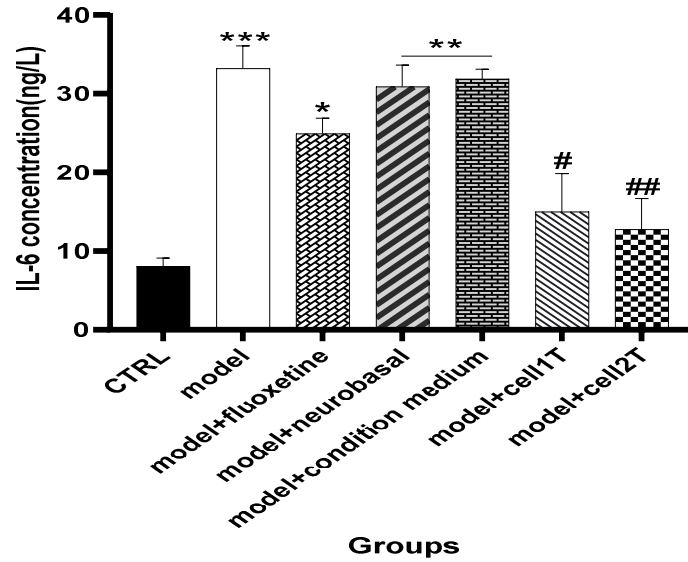


Figure 7. Effects of neural stem cell treatment on hippocampal IL-6 levels. Chronic stress increased IL-6 concentrations, whereas intranasal neural stem cell administration significantly reduced IL-6 in both treatment regimens. Fluoxetine showed no significant effect compared with the stress group. Data are presented as mean $\pm$ SEM (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs. healthy control group; #P < 0.05, ##P < 0.01 vs. model group.

Tissue Tumor Necrosis Factor- α (TNF- α) Levels

TNF- α levels were markedly elevated following CUMS exposure ($P < 0.0001$). Neural stem cell treatment significantly attenuated TNF- α expression in a dose-dependent manner, with the double-dose regimen showing greater efficacy. Fluoxetine also reduced TNF- α levels; however, its effect was less pronounced than that of stem cell therapy. Notably, TNF- α levels in the high-dose NSC group were comparable to those of the control group (Figure 8).

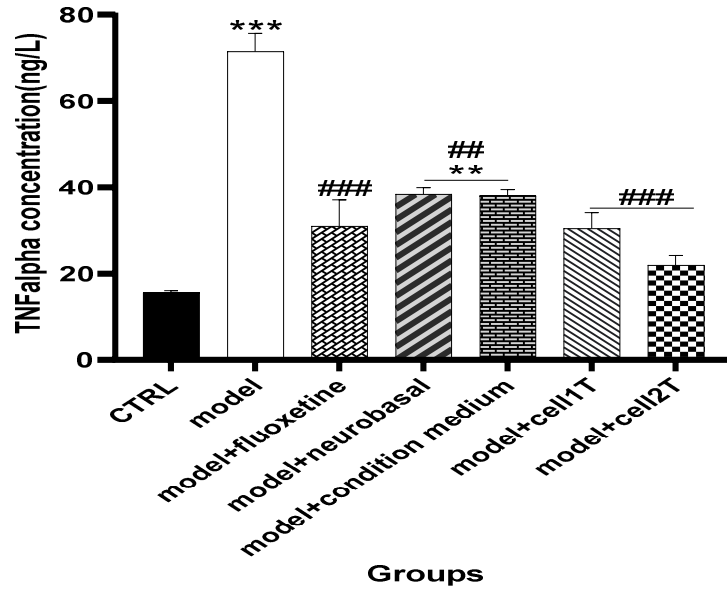


Figure 8. Hippocampal TNF- α levels across experimental groups. Chronic stress significantly elevated TNF- α expression. Neural stem cell treatment markedly reduced TNF- α levels in a dose-dependent manner, with the highest reduction observed in the double-dose group. Fluoxetine partially reduced TNF- α levels but was less effective than stem cell therapy. Data are presented as mean $\pm$ SEM (n = 3). **P < 0.01, ***P < 0.001 vs. healthy control group; #P < 0.05, ###P < 0.01 vs. model group.

Tissue Malondialdehyde (MDA) Levels

Oxidative stress marker MDA was significantly increased in stressed animals (P < 0.0001). NSC administration markedly decreased MDA levels, with the most robust reduction observed in the double-dose group, restoring values near baseline. Fluoxetine partially reduced MDA levels but remained significantly higher than both control and NSC-treated groups, indicating a weaker antioxidative effect (Figure 9).

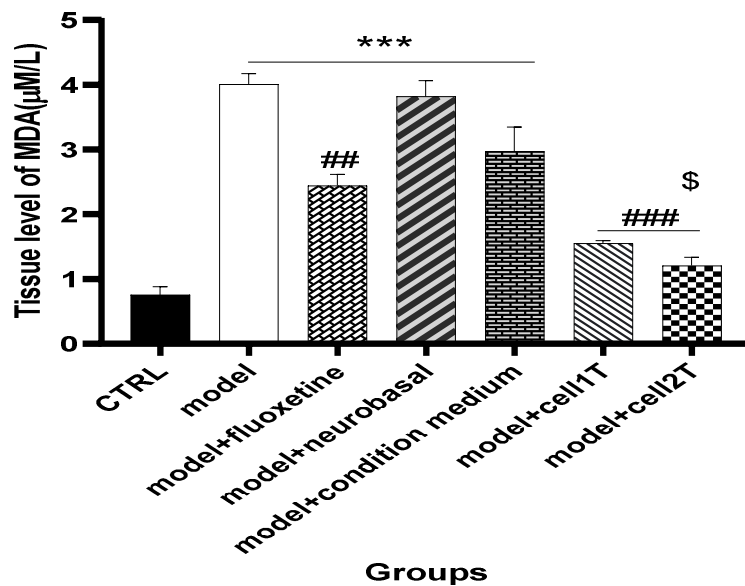


Figure 9. Comparison of MDA tissue level in healthy, stress and treatment groups (SEM $\pm$ Mean). Data are presented as mean $\pm$ SEM (n = 3). ***P < 0.001 vs. healthy control group; ##P < 0.01, \$\$\$P < 0.001 vs. model group. \$P < 0.05 vs. fluoxetine

Plasma Corticosterone Levels

Serum corticosterone levels were significantly elevated in the CUMS group compared with controls (P < 0.0001). Neural stem cell treatment significantly reduced corticosterone levels in both treatment regimens, with greater improvement observed in the double-dose group, approaching control values. Fluoxetine showed only partial normalization and remained

significantly different from healthy controls, suggesting limited efficacy in restoring hypothalamic–pituitary–adrenal axis activity (Figure 10).

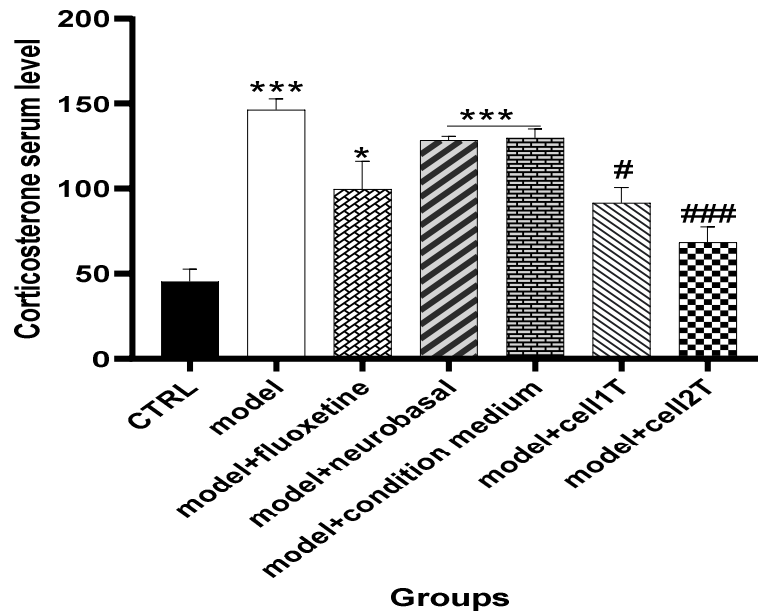


Figure 10. Serum corticosterone levels following chronic stress and treatment. Chronic stress significantly increased corticosterone levels. Neural stem cell treatment significantly reduced corticosterone, with greater normalization observed in the double-dose group. Fluoxetine showed partial improvement but remained significantly different from control levels. Data are presented as mean $\pm$ SEM ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. healthy control group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. model group.

Molecular Analyses

Hippocampal Brain-Derived Neurotrophic Factor (BDNF) Expression

One-way ANOVA demonstrated a significant difference in hippocampal BDNF mRNA expression among the experimental groups (Figure 11). Post hoc analysis revealed that chronic unpredictable mild stress significantly reduced BDNF expression compared with the healthy

control group ($P < 0.05$). Intranasal administration of human OM-NSCs significantly increased hippocampal BDNF expression in both the single-dose ($P < 0.05$) and double-dose ($P < 0.01$) treatment groups relative to the untreated stress group. No significant difference was observed between either OM-NSC-treated group and the healthy controls, indicating restoration of BDNF expression to control levels. In contrast, fluoxetine treatment did not significantly alter BDNF expression compared with either the untreated stress group or the healthy controls ($P > 0.999$). Analysis of ΔCT values showed a similar pattern, with OM-NSC-treated animals exhibiting ΔCT values comparable to those of the healthy control group. (Figure 11).

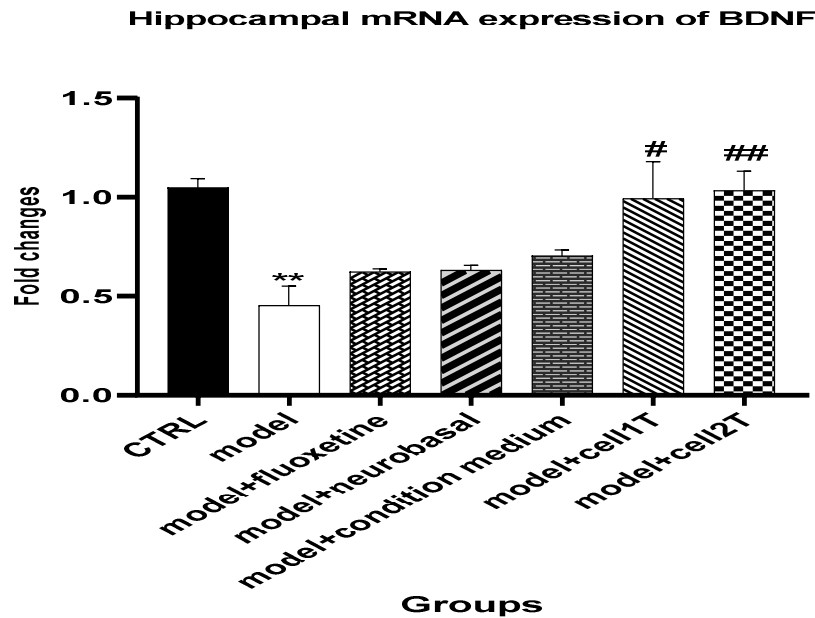


Figure 11. Hippocampal BDNF mRNA expression across experimental groups. Chronic stress reduced BDNF expression, whereas neural stem cell treatment significantly restored BDNF levels in both single- and double-dose groups. Fluoxetine showed no significant effect. Data are presented as mean $\pm$ SEM ($n = 3$). ** $P < 0.01$ vs. healthy control group; # $P < 0.05$, ## $P < 0.001$ vs. model group.

Hippocampal 5-HT1A Receptor Expression

Post hoc analysis showed that CUMS significantly increased 5-HT1A mRNA expression compared with the healthy control group ($P < 0.001$). Animals receiving Neurobasal medium also exhibited significantly higher 5-HT1A expression than healthy controls ($P < 0.05$). In contrast, intranasal administration of human OM-NSCs significantly reduced 5-HT1A mRNA expression in both the single-dose and double-dose treatment groups compared with the untreated stress group (both $P < 0.05$). No significant differences were observed between either OM-NSC-treated group and the healthy controls ($P > 0.05$). No significant difference was detected between the fluoxetine and untreated stress groups ($P > 0.999$). Analysis of ΔCT values demonstrated the same expression pattern, with ΔCT values in both OM-NSC-treated groups approximating those of the healthy control group. (Figure 12).

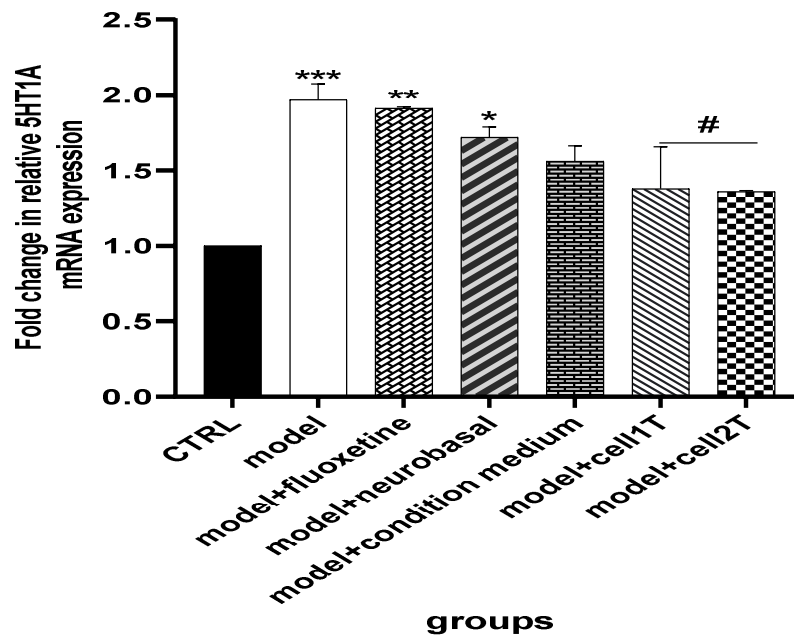
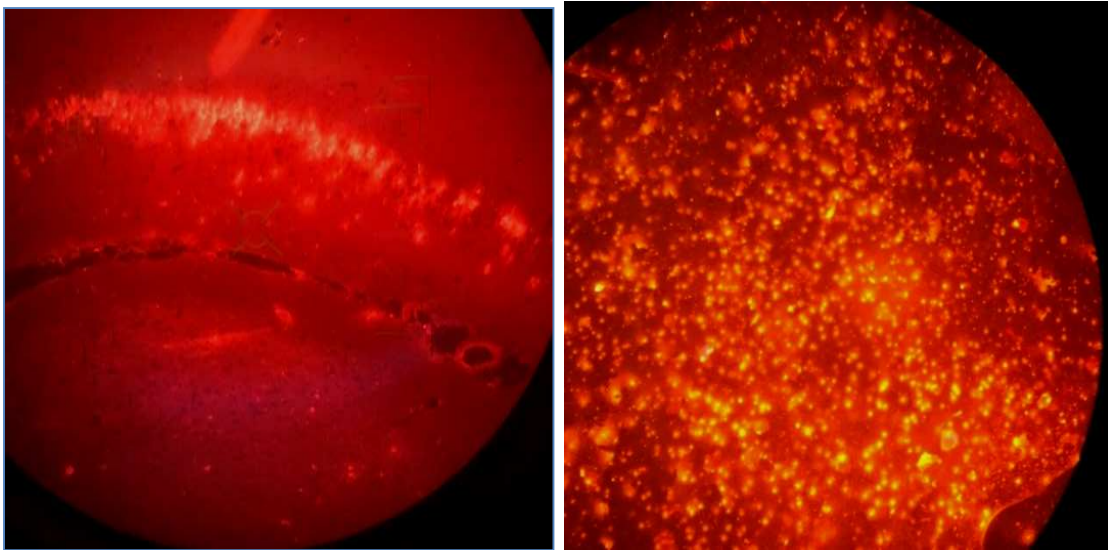


Figure 12. Hippocampal 5-HT1A receptor mRNA expression in experimental groups. Chronic stress increased 5-HT1A expression, whereas neural stem cell treatment significantly reduced its expression to control-like levels. Fluoxetine failed to normalize 5-HT1A expression. Data are presented as mean $\pm$ SEM ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. healthy control group; # $P < 0.05$ vs. model group.

In Vivo Tracking of Intranasally Delivered Neural Stem Cells

Fluorescence microscopy was used to evaluate the migration and localization of DiI-labeled neural stem cells following intranasal administration. Bright fluorescent signals corresponding to labeled cells were detected in both the prefrontal cortex and hippocampal regions at different time points. Specifically, transplanted cells were observed in the prefrontal cortex after two weeks, while hippocampal localization was confirmed after one month, indicating progressive migration along neuroanatomical pathways. These findings demonstrate that intranasally delivered neural stem cells are capable of reaching key brain regions implicated in mood regulation and stress-related pathology (Figure 13).



Hippocampus area (after one month) Prefrontal area (after two weeks)

Figure 13. In vivo tracking of DiI-labeled neural stem cells following intranasal administration.

Fluorescent microscopy revealed migration of labeled cells to the prefrontal cortex at two weeks and to the hippocampus at one-month post-transplantation, confirming successful CNS homing of transplanted cells.

Discussion

The present study demonstrates that intranasal administration of human olfactory mucosa-derived neural stem cells (OM-NSCs) markedly attenuated behavioral, histological, biochemical, and molecular abnormalities induced by chronic unpredictable mild stress (CUMS). Compared with fluoxetine, OM-NSC therapy produced a more comprehensive therapeutic response, restoring depressive-like behaviors, reducing neuroinflammation and oxidative stress, promoting hippocampal neurogenesis, normalizing corticosterone concentrations, increasing BDNF expression, and reversing the pathological alteration of 5-HT_{1A} receptor expression. Collectively, these findings suggest that OM-NSCs target multiple interconnected pathological pathways underlying depression rather than acting through a single neurotransmitter system.

4.1. Behavioral Restoration and Depression-Related Phenotypes

Chronic stress significantly induced depressive-like behavior, including increased immobility in the forced swim test, elevated anxiety in the elevated plus maze, and anhedonia in the sucrose preference test. These findings are consistent with established CUMS models of depression, which reliably reproduce core affective symptoms and neurobiological changes of major depressive disorder (Nollet, Guisquet, & Belzung, 2013; Strekalova et al., 2022; Willner, 2017). OM-NSC treatment significantly reversed all behavioral deficits, outperforming fluoxetine in reducing immobility and restoring reward sensitivity. Importantly, fluoxetine only partially improved behavioral outcomes, consistent with previous reports indicating limited efficacy of SSRIs in reversing stress-induced neuroplastic deficits (Rosas-Sanchez et al., 2024; Zavvari, Nahavandi, & Goudarzi, 2020). The superior efficacy of OM-NSCs suggests engagement of broader regenerative mechanisms beyond serotonergic modulation. Recent studies have demonstrated that neural stem cell-derived extracellular vesicles exert antidepressant-like effects via microRNA-mediated

neuroprotection and HPA-axis regulation (Li et al., 2024; Wu et al., 2025). These mechanisms likely contribute to the behavioral normalization observed in the present study.

Histology, Neuroinflammation, and Oxidative Stress

Beyond behavioral alterations, CUMS induced pronounced structural and biochemical abnormalities in the hippocampus, particularly within the CA1 subregion. The hippocampus is highly vulnerable to stress-induced damage due to its dense expression of glucocorticoid receptors and its central role in regulating emotional and cognitive processes (Höschl & Hajek, 2001; Podgorny & Gulyaeva, 2021). Prolonged exposure to elevated glucocorticoids disrupts neuronal integrity, suppresses neurogenesis, and enhances excitotoxic vulnerability, particularly in CA1 pyramidal neurons (Saaltink & Vreugdenhil, 2014).

Histological analysis demonstrated significant neuronal loss in the CA1 hippocampal region following chronic stress, consistent with stress-induced neurodegeneration and impaired synaptic plasticity (Gao et al., 2025; Kim & Diamond, 2002). Importantly, intranasal administration of OM-NSCs significantly restored CA1 neuronal density. This effect was more pronounced in the repeated-dose group, suggesting a dose-dependent neuroregenerative response. OM-NSCs possess strong neurogenic potential and can integrate into hippocampal circuits, supporting neuronal replacement and synaptic remodeling (Alvites et al., 2022; Liu et al., 2023). Additionally, olfactory-derived stem cells are known to respond to injury-associated chemotactic signals, enhancing migration toward inflamed brain regions (Kronner et al., 2022).

A key pathological hallmark of depression is neuroinflammation, characterized by elevated pro-inflammatory cytokines such as IL-6 and TNF- α . These cytokines contribute to synaptic dysfunction, reduced neurogenesis, and altered neurotransmitter metabolism (Miller & Raison,

2016; Sălcudean et al., 2025) (Hassamal, 2023; Schramm & Waisman, 2022). Chronic stress is known to induce persistent neuroinflammation mediated by microglial activation and pro-inflammatory cytokine release. NSC-based therapies have been shown to regulate microglial polarization toward an anti-inflammatory M2 phenotype, thereby reducing neurotoxicity and enhancing tissue repair (de Almeida, Goodkey, & Voronova, 2023; Zhou et al., 2022). OM-NSC treatment markedly attenuated these inflammatory markers, restoring them toward baseline levels. A key finding of this study is the significant reduction of IL-6 and TNF- α following OM-NSC treatment. Furthermore, NSC-derived exosomes carry bioactive microRNAs that suppress inflammatory signaling pathways such as NF- κ B and MAPK (Marei & Cenciarelli, 2026). Oxidative stress marker MDA was also significantly reduced following OM-NSC treatment. Oxidative stress is increasingly recognized as a key contributor to depression, promoting mitochondrial dysfunction, impairing synaptic plasticity, and accelerating neuronal damage, particularly within the hippocampus (Lucca et al., 2009) (Bessa et al., 2009; Rezin et al., 2008). NSC-derived factors have been shown to enhance antioxidant defenses and reduce lipid peroxidation in neurodegenerative disorders (Díaz Reyes, Gatti, Delgado Ocaña, Ortega, & Banchio, 2025; Madhu et al., 2026). In addition, chronic stress-induced elevation of corticosterone confirmed sustained activation of the hypothalamic–pituitary–adrenal (HPA) axis (Du & Pang, 2015). Dysregulation of this system is a core feature of depression and is strongly associated with hippocampal dysfunction. Corticosterone normalization further indicates restoration of hypothalamic–pituitary–adrenal (HPA) axis dysregulation, a hallmark of chronic stress and depression (Pannu & Goyal, 2026).

Molecular Mechanisms: BDNF and 5HT1A Signaling

At the molecular level, one of the most important findings of the present study was the reciprocal regulation of BDNF and 5-hydroxytryptamine receptor 1A (5-HT1A) following chronic stress and their normalization after treatment with OM-NSCs. These molecular alterations provide mechanistic insight into how OM-NSCs exert antidepressant effects beyond symptomatic behavioral improvement and further support the concept that regenerative cell therapy targets the underlying pathophysiology of depression rather than simply correcting neurotransmitter imbalance. Consistent with previous reports, CUMS significantly reduced hippocampal BDNF expression. BDNF is widely recognized as one of the principal regulators of neuronal survival, synaptic plasticity, dendritic remodeling, and adult hippocampal neurogenesis (De Vincenti, Ríos, Paratcha, & Ledda, 2019; Kowiański et al., 2018). Reduced BDNF signaling has been consistently reported in both patients with major depressive disorder and experimental models of chronic stress and is considered a hallmark of impaired neuroplasticity (Ayatollahi et al., 2020; Correia, Cardoso, & Vale, 2023; L Neto, Borges, Torres-Sanchez, A Mico, & Berrocoso, 2011). Activation of the BDNF–TrkB pathway promotes neuronal differentiation, enhances long-term potentiation, and supports the structural remodeling required for recovery from depressive disorders. Therefore, restoration of BDNF represents one of the major therapeutic targets in modern antidepressant research (Mohandas et al., 2025; Tejeda & Díaz-Guerra, 2017).

In the present study, OM-NSC administration restored hippocampal BDNF expression to levels comparable with healthy controls. These findings are highly consistent with the histological evidence demonstrating preservation of CA1 neurons and the marked improvement in depressive-like behaviors. Rather than acting solely through neurotransmitter modulation, OM-NSCs appear to recreate a neuroregenerative microenvironment that favors neuronal survival and endogenous repair. Previous investigations have demonstrated that transplanted neural stem cells secrete

multiple neurotrophic molecules, including BDNF, glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF), all of which collectively enhance neuronal survival and synaptic remodeling(De Gioia et al., 2020; Trigo, 2020). In addition, neural stem cell-derived extracellular vesicles transfer regulatory microRNAs that stimulate endogenous neurogenesis while simultaneously inhibiting apoptosis, thereby amplifying regenerative signaling throughout the injured brain (Bonetto & Grilli, 2023).

Fluoxetine failed to significantly restore BDNF expression, consistent with clinical evidence showing delayed or partial neurotrophic effects of selective serotonin reuptake inhibitors (SSRIs) (Talaee et al., 2024).

Interestingly, 5HT1A receptor expression was increased following stress exposure(Briones-Aranda, Rocha, & Picazo, 2005). Although traditionally depression has been associated with reduced serotonergic signaling, recent evidence suggests that stress can induce compensatory upregulation of 5HT1A autoreceptors and altered receptor trafficking in hippocampal and prefrontal regions(Kaufman et al., 2016; Turcotte-Cardin et al., 2019). OM-NSC treatment normalized 5HT1A expression, indicating restoration of serotonergic homeostasis at the transcriptional level. This may occur indirectly via anti-inflammatory and neurotrophic pathways, as cytokines are known to regulate serotonin receptor expression (Torrise et al., 2025).

Taken together, the behavioral, histological, biochemical, and molecular findings strongly suggest that OM-NSCs exert therapeutic effects through multiple complementary mechanisms rather than a single pharmacological target. In contrast to fluoxetine, which primarily enhances serotonin availability (Stahl, 1998), OM-NSCs simultaneously suppress neuroinflammation, reduce oxidative stress, normalize HPA-axis activity, preserve hippocampal neurons, stimulate neurotrophic signaling, and restore molecular pathways governing neuronal plasticity. Such

multimodal activity is particularly relevant because major depressive disorder is increasingly recognized as a complex neuroimmune disease involving reciprocal interactions among inflammation, oxidative stress, endocrine dysfunction, impaired neurogenesis, and neurotransmitter imbalance rather than an isolated deficiency of monoamines (Duman & Aghajanian, 2012; Miller & Raison, 2016; Wohleb, Franklin, Iwata, & Duman, 2016).

Another important observation was the superior efficacy of repeated OM-NSC administration compared with a single dose across nearly all behavioral and biological outcomes. These findings suggest that sustained regenerative stimulation may be required to maintain an optimal therapeutic microenvironment within chronically injured brain tissue. Repeated administration may prolong paracrine signaling, increase cumulative secretion of neurotrophic and immunomodulatory factors, and improve survival of transplanted cells (Habiba et al., 2026) (Santos, Moreira, Santos, & Solá, 2025). Future studies should therefore investigate optimal dosing schedules, treatment intervals, and long-term persistence of transplanted OM-NSCs to maximize therapeutic efficacy.

The intranasal delivery strategy employed in this study also deserves particular attention. Unlike intracerebral transplantation, intranasal administration represents a minimally invasive approach capable of bypassing the blood–brain barrier through the olfactory and trigeminal pathways (Pardeshi & Belgamwar, 2013). The successful migration of DiI-labeled OM-NSCs into both the hippocampus and prefrontal cortex confirms efficient brain targeting and supports the growing interest in intranasal cell therapy as a clinically feasible strategy for neurological disorders (Danielyan et al., 2011). Because the olfactory mucosa is relatively accessible and can be harvested with minimal morbidity, autologous OM-NSCs may eventually provide a practical source of patient-specific regenerative therapy while minimizing immunological complications (Lindsay, Riddell, & Barnett, 2010).

Conclusion

In conclusion, the present study demonstrates that OM-NSCs provide substantially greater therapeutic benefit than conventional fluoxetine therapy in a rat model of chronic stress-induced depression. By simultaneously restoring hippocampal integrity, suppressing neuroinflammation and oxidative stress, normalizing HPA-axis activity, enhancing BDNF signaling, and correcting stress-induced serotonergic dysregulation, OM-NSCs address several core mechanisms underlying depression. These findings support the concept that OM-NSC therapy represents a promising disease-modifying regenerative strategy, rather than merely a symptomatic antidepressant intervention, and warrants further preclinical and translational investigation for the treatment of major depressive disorder.

Limitations and Future Directions

This study has some limitations that warrant further investigation. Although our findings demonstrate promising neuroprotective effects of intranasally administered human olfactory mucosa-derived neural stem cells, the underlying molecular mechanisms and protein-level changes were not comprehensively evaluated. Long-term behavioral outcomes and optimal dosing frequency also require further investigation. Future studies addressing these aspects may further clarify the mechanisms and therapeutic potential of this approach.

Ethical Approval

The present study was approved by the Ethical Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1401.145).

Consent for publication

Not applicable.

Availability of supporting data

Data are available from the corresponding author.

Competing interests

There are no competing interests to declare.

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Author Contributions

AN designed, conceived, and planned the study. F. N performed the experiments, S. N, A. D, E.S and RA drafted the manuscript, analyzed the data, calculated statistics and revised intellectual content of the manuscript. F.N, A.N and S.N played a revised intellectual content of the manuscript, respectively. All authors reviewed the manuscript.

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