Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Synergistic myelin regenerative, immunomodulatory and anti-inflammatory effects of vanillin and adipose derived stem cells in cuprizone animal model of multiple sclerosis

Sara Gazmeh [1], **Mohammad Mardani** [1*], **Nazem Ghasemi** [1*]

[1] Department of Anatomical Sciences and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Feb 19, 2026
Accepted: May 13, 2026

Keywords:
Adipose derived stem cells
Multiple sclerosis
Myelin
Oligodendrocyte
Vanillin

ABSTRACT

Objective(s): Vanillin as a potential therapeutic agent along with transplantation of neurotrophic factors secreting cells, is considered a promising treatment for neurological disorders. In this study, the effects of vanillin in combination with human adipose derived stem cells transplantation were evaluated on oligodendrocyte differentiation, re-myelination, and motor function improvement.

Materials and Methods: Female C57BL/6 mice were fed cuprizone (400 mg/kg/day) for 5 weeks to induce MS. Demyelinated mice were divided into MS, MS/vanillin, MS/stem cells, and MS/vanillin/stem cells groups. Additionally, sixteen mice were divided into control and sham groups for comparison. Motor function was assessed using the hanging wire test. Finally, serum levels of inflammatory (IL-2, IFN-γ) and anti-inflammatory (IL-10, TGF-β) factors, along with IgG, were measured by ELISA. The mean percentage of Olig2 and MOG-positive cells we determined using the immunohistochemical technique, and Luxal Fast Blue staining was performed to assess myelin density of corpus callosum.

Results: The results revealed that the mean percentage of Olig2, Mog positive cells, the serum level of the IL-10 and TGF-β, myelin density, and behavior test score were significantly higher in treated groups specially in MS/vanillin/stem cells group ($P \leq 0.01$), also, the serum levels of the inflammatory factors and IgG were significantly higher in the MS group compare to treated groups ($P \leq 0.01$).

Conclusion: Administration of vanillin with stem cell transplantation could be a suitable approach to accelerate the process of myelin repair in the nervous tissue.

► Please cite this article as:
Gazmeh S, Ghasemi N, Mardani M. Synergistic myelin regenerative, immunomodulatory and anti-inflammatory effects of vanillin and adipose derived stem cells in cuprizone animal model of multiple sclerosis. Iran J Basic Med Sci 2026; 29: 1027-1034. doi: https://dx.doi.org/10.22038/ijbms.2026.94763.20447

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, autoimmune disease of the central nervous system (CNS) (1, 2). It usually occurs in young adults between the ages of 20 and 40, and women are affected up to three times more often than men (3, 4).

Multiple foci of inflammatory reactions, microgliosis, astrocytosis, oligodendrocyte depletion, demyelination, and axonal degeneration are part of the pathogenesis of MS (5). In addition, cytokines released by immune cells are among the other factors that play an important role in the pathogenesis of this pathological condition (6). IL-2 is a key factor in maintaining immunological tolerance, which together with IFN-γ, increases the inflammation of MS lesions (7-9).

In contrast to IL-2, IL-10, as a pivotal anti-inflammatory cytokine, plays a key role in suppressing the immune system (10). In addition, studies have shown that TGF-β, with its function of maintaining immune tolerance by regulating lymphocyte proliferation, differentiation, and survival, is one of the important factors that play a role in protecting against MS (6). Although the exact role of immunoglobulin-producing cells in the pathogenesis of MS is still unclear, studies suggest that some immunoglobulins act as beneficial factors in myelin regeneration (11). In recent years, stem cell therapy has become a very promising scientific research topic (12). Mesenchymal stem cells (MSCs), with their high proliferation and differentiation potential and immunomodulatory properties, are considered a promising source for replacing damaged oligodendrocytes in MS (13). Experimental studies have shown that human adipose-derived stem cells (hADSCs) can differentiate into myelin-producing cells and compensate for myelin loss in MS disease models (14). Compared with other cell types, the use of adipose-derived stem cells can be used for cell-based therapy in MS due to their ease of isolation using a minimally invasive method, high proliferation capacity, and ability to withstand a large number of passages (14-16). However, transplantation of these cells alone has not yielded significant results in treating the disease. Therefore, it is hypothesized that adding a complementary therapy to cell transplantation may enhance therapeutic efficacy.

Natural substances and supplements are commonly used

**Corresponding authors:* Nazem Ghasemi. Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. Email: n-ghasemi@med.mui.ac.ir; Mohammad Mardani. Department of Anatomical Sciences and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, Iran. Email: mardani@med.mui.ac.ir

© 2026. This work is openly licensed via CC BY 4.0.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

for the treatment of various neurological disorders based on their medicinal properties (17, 18). Vanillin (4-hydroxy-3-methoxybenzaldehyde), a phenolic compound and the most popular food flavoring, is extracted from the pods of the orchid Vanilla planifolia (19-21). This phenolic compound has been reported to possess anticancer, antidiabetic, antioxidant, antidepressant, and anti-inflammatory activities, along with neuroprotective and libido-enhancing effects (21-26). Furthermore, its ability to cross the blood-brain barrier has been demonstrated (22). In the present study, it was hypothesized that vanillin could be a promising neuroprotective agent in the management of demyelinating diseases such as MS. There are a wide range of models for inducing MS in laboratory animals. The cuprizone model is widely used in research to study the mechanisms of demyelination and remyelination in MS (27). Cuprizone, a copper chelator, induces demyelination in animal models by inducing metabolic stress and apoptosis in oligodendrocytes, leading to myelin degradation in specific regions of the white matter of the brain, including the corpus callosum (CC). The expression of myelin-related proteins, including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein, is reduced by cuprizone in the CC (28). Since, the *in vivo* effects of vanillin on stem cell differentiation in oligodendrocyte cells and myelination improvement have not been studied, the present study was designed and conducted to evaluate the role of vanillin on the differentiation of hADSCs into oligodendrocytes and remyelination in a cuprizone-induced mouse model (Figure 1).

Materials and Methods

Experimental animals

This experimental study was conducted on 60 C57BL/6 mice, weighing 20–25 g in the animal house of the Medical Research Center, Isfahan University of Medical Sciences, according to the guidelines and regulations established by the Medical Research Ethics Committee (IR.MUI.AEC.1403.033) in 2024.

Cell labeling with PKH26

hADSCs from our previous study was cultured with DMEM/ F12(BIO-IDEA 25131) medium containing 10% fetal bovine serum (FBS) (Invitrogen 10270-106) and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO2 incubator. After 80% cell confluency, hADSCs were detached and cell labeling was done with PKH26 (Sigma) before transplantation according to previous studies (29, 30). To achieve this, after placing cells in a conical tube and washing once with PBS, we centrifuged the cells at 1500 rpm for 5 min. Then PKH26 was added to the cells and washed with PBS. After labeling cessation by 1% BSA (Sigma Aldrich A2153), the cells were washed by DMEM/ F12. Fluorescent microscopy (Olympus BX51, Japan) was employed to assess the mean percentage of the labeled cells.

Induction of demyelination model by cuprizone and animal grouping

In this study, 60 female C57BL/6 mice weighing 20–25 g and aged approximately 8 weeks were used. The mice were provided free access to food and water and maintained under suitable health conditions and temperature control (23 °C ± 2 °C). The MS model lesion was induced using cuprizone (bis-cyclohexanone oxaldihydrazone, Sigma-Aldrich Inc. C9012) in all animals except control and sham groups (n = 8 in each group). To this end, 44 mice were gavaged with 400 mg/kg/day cuprizone solution in 200 µl corn oil for 5 weeks. After confirming of MS induction using the hanging wire test, demyelinated animals (n=32) were randomly divided into 4 groups (n = 8 in each group), including MS, MS/vanillin, MS/stem cells, and MS/ vanillin /stem cells. The control group animals received no intervention. Moreover, the mice in the sham group were gavaged daily with 200 µl corn for five weeks, followed by a single intravenous injection of 200 µl PBS into the tail vein. In vanillin receiving groups, vanillin (40 mg/kg dissolved in water) was injected daily for four weeks and in stem cell receiving groups, a single dose of PBS (200 µl) containing 1×10^6 stem cells were injected intravenously (IV) into the tail.

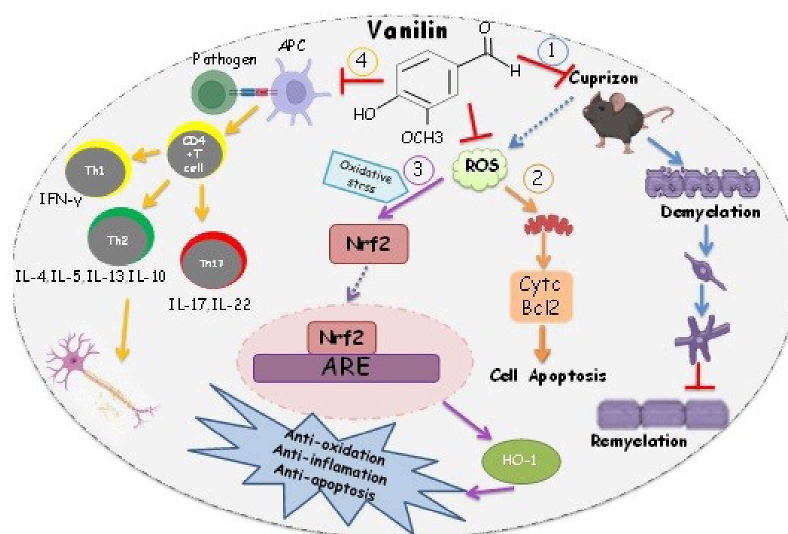

Figure 1. Effects of vanillin on myelin and oligodendrocyte differentiation in mice

Vanillin improves remyelination and OPC differentiation into oligodendrocytes in the central nervous system by inhibiting ROS, following cuprizone gavage (1). In addition, vanillin prevents mitochondrial permeability transition and the release of apoptosis-related proteins such as Bcl-2 and Cyt-c, thus inhibiting apoptosis in oligodendrocytes (2). Vanillin can activate the Nrf2/HO-1 pathway. Under oxidative stress conditions, phosphorylated Nrf2 (p-Nrf2) moves to the nucleus, where it binds to the antioxidant response element (ARE). This leads to up-regulation of HO-1 (a crucial endogenous anti-oxidant) which reduce oxidative stress elements (3). Also, vanillin reduces inflammation by inhibiting immune cell infiltration and the activity of inflammatory cytokines, including IFN-γ, TNF-α, and IL-17, thus preventing oligodendrocyte apoptosis and impaired myelin repair (4).

ROS: Reactive oxygen species, Nrf2: Nuclear factor erythroid 2-related factor 2, ARE: Antioxidant response element, HO-1: Hemooxygenase-1, IFN-γ: Interferon gamma, IL: Interleukin, CytC: Cytochrome C, APC: Antigen presenting cell

Motor function examination by behavioral tests

The hanging wire test was used to compare improvements in balance and motor function in the various experimental groups. Briefly, the animal was hung from a metal wire (90 cm long, 2 mm in diameter, and 50 cm high), and the latency to fall was recorded over a 300 sec period. The maximum hanging time was recorded. This test was performed weekly from one week before induction of MS model to the end of the study (31).

Histological studies

The mice were anesthetized by intraperitoneal injection of combination of ketamine (Sigma) and xylazine (Sigma) and mice brain fixation was performed through the cardiac perfusion method with ice-cold PBS (pH = 7.4) and 4% paraformaldehyde (PFA) (PFA, Sigma-Aldrich, P6148). After removal of the brain from the skull, they were fixed again in 10% formalin for 24 hr. Thereafter, paraffin sections were prepared from the CC in serial sections of 5–7 µm thickness.

Luxol fast blue staining

According to a previous study (32), after deparaffinization and clarification of the prepared tissue sections, the samples were placed in Luxol Fast Blue (LFB) solution (0.1% w/v, Sigma-Aldrich, USA) at 56 °C overnight. After that, the samples were washed in 70% ethanol, 0.05% lithium chloride solution for 30 sec, and then in 70% ethanol. After washing in distilled water, they were immersed in cresyl violet acetate solution for 60 sec. In order to assess the degree of demyelination, the stained sections were photographed under a microscope and myelin density was measured using ImageJ.

Immunohistochemical technique for identifying Mbp, Olig2, and Mog markers

Based on our previous studies (31-33), oligodendrocyte transcription factor 2 (Olig2), myelin oligodendrocyte glycoprotein (Mog), and MBP (myelin basic protein or myelin marker) were used to identify oligodendrocytes and myelin. After antigen retrieval using sodium citrate buffer, blocking of nonspecific antigens was performed using 10% goat serum albumin, diluted in PBS for 30 min at room temperature. Next, primary antibodies, including rabbit anti-Mog antibody (1:1000; Abcam, ab32760, RRID: AB -2142685); Rat anti-Mbp antibodies (1:1000; Abcam, ab7349 Cambridge, MA, USA, RRID: AB - 306726) and rabbit anti-Olig2 (1:1000; Abcam, ab109186, RRID: AB -10861310) were used overnight at 4 °C in a humidified environment. After washing with PBS, the slides were incubated with FITC-conjugated secondary antibodies (1:500; Abcam, Cambridge, MA, USA) for 1 hr at room temperature. Subsequently, the sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, D9542) for 2 min to reveal the cell nuclei, and the cells were examined using a fluorescence microscope (Olympus BX51, Japan). Finally, the average percentage of cells expressing Mog and Olig2 markers was determined from 200 cells per slide using ImageJ. In addition, the average of myelin density was calculated using ImageJ.

ELISA test for interleukin evaluation

This method was used to assess the serum levels of inflammatory factors (IL-2, interferon gamma (IFN-γ)), anti-inflammatory factors (IL-10 and transforming growth factor beta (TGF-β)) and immunoglobulin G factor. First of all, washing buffer (KPG-WB, karmaniaparsgen, Iran) and avidin-HRP solution (KPG-HA, karmaniaparsgen, Iran) were prepared according to the company's protocol. In the first step, 50 µl of standard solutions 1 to 4 (KPG- MIL2 NS1-4, karmaniaparsgen, Iran) were added to the first to fourth wells, and the fifth well was considered a control. In the next step, 50 µl of the sample was added to the remaining wells and incubated for 50 min on a shaker (speed 200 rpm). After that, the plates were washed 3 times using washing solution (step 3). Next, 50 µl of conjugated antibody (Detection ab, KPG- MIL2D, karmaniaparsgen, Iran) was added to all wells except the control and incubated again for 50 min under the previous conditions (step 4). After incubation, the plates were washed 3 times using washing solution (step 5). Next, 50 µl of HRP-avidin solution (HAA, karmaniaparsgen, Iran) was added to all wells except the control well and placed on a shaker for 50 min (step 6). After incubation, the plates were washed 5 times (step 7) and then 50 µl of substrate was added to all wells and incubated for 15 min (step 8). Finally, 2 µl of stopping solution (KPG-ST, karmaniaparsgen, Iran) was added to all wells (step 9) and the absorbance of the samples was measured in an ELISA reader at a wavelength of 4.0 nm. It should be noted that all steps except steps 4 and 6 were performed for the control well.

Statistical analysis

Statistical differences between groups were determined using one-way analysis of variance (ANOVA) using SPSS version 25 (IBM, Armonk, NY, USA). Results were presented as mean ± standard error, and *P*-values less than 0.05 were considered significant.

Results

Effects of stem cells and vanillin on mouse motor function improvement

Data evaluation showed that the motor function of the mice significantly decreased following the administration of cuprizone; however, it improved with cell transplantation and vanillin consumption. As shown in Figure 2, In the fourth week a significant increase of hanging time in mice was seen in MS/Vanillin group (133±4.4) ($P≤0.05$), Ms/Stem group (130±4.2) ($P≤0.05$) and in Ms/stem/Vanillin group (153±3) ($P≤0.01$) in compared to the Ms group (105±3), Additionally Ms/Stem/Vanillin group had a statistically significant increase compared to MS/Vanillin and Ms/Stem groups ($P≤0.05$).

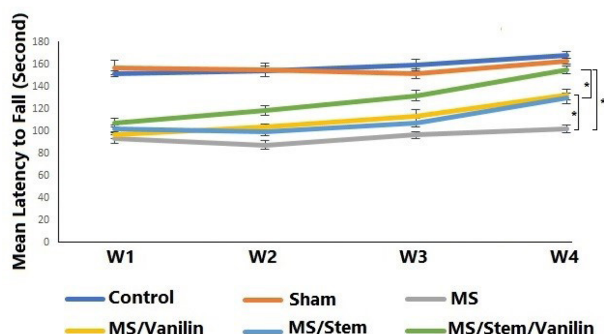

Figure 2. Mouse hanging wire test results

A significant increase in the hanging time was observed in the groups receiving stem cells. Additionally, Ms/Stem/Vanillin group had a statistically significant increase compared to MS group ($P<0.01$) and MS/Vanillin and Ms/Stem groups ($P<0.05$). (*$P≤0.05$, **$P≤0.01$).

MS: Multiple sclerosis

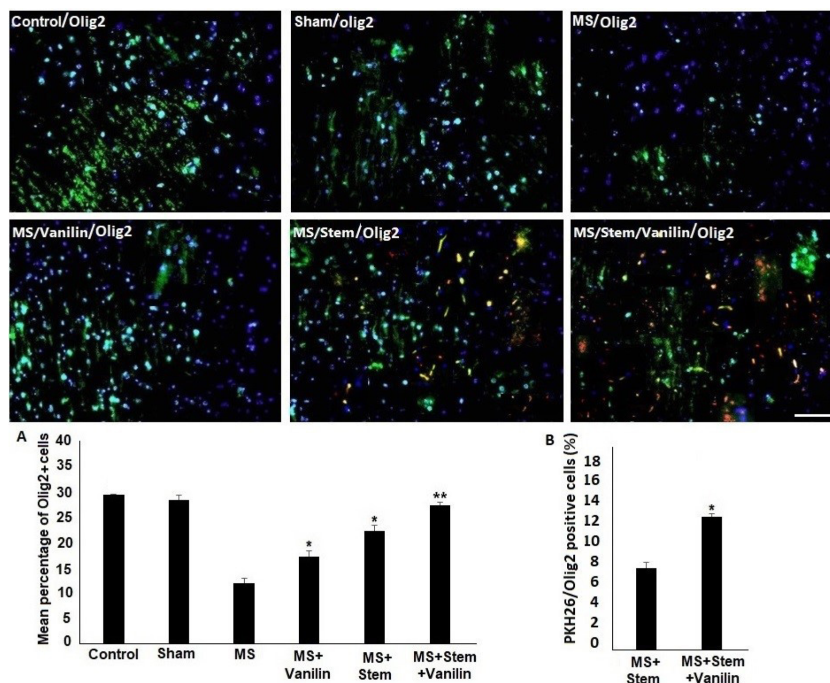

Figure 3. Immunohistochemistry staining for Olig2 marker in mice
Percentage of OLIG2-positive cells (green) increased in all treated groups specially in Ms/stem/Vanilin group (A). Moreover, the PKH26/Olig 2 positive cells were significantly higher in the Ms/stem/Vanilin groups than in Ms/stem group (B). DAPI (blue) was used for nuclear counterstaining. The PKH26 labeled cells (hADSCs) are shown red, (Scale bars: 200 μm) (*P≤0.05, **P≤0.01)
Olig2: Oligodendrocyte transcription factor; DAPI: 4,6-diamidino-2-phenylindole; MS: Multiple sclerosis

Vanillin and hADSCs reduced demyelination and increased oligodenderogenesis

Analysis of the results showed the average percentage of Olig2 and Mog positive cells increased significantly in treated groups, especially in the Ms/stem/Vanillin group (27.5 ±.6 for Olig 2 and 24.5± .7 for Mog) compared to the Ms group (12±.8 for Olig2 and 7.5±.8 for Mog) (P≤0.01) (Figures 3A and 4A). Furthermore, quantification of the data showed that PKH26/Olig 2 and PKH26/Mog positive cells were significantly higher in Ms/stem/Vanillin groups (12.5±.4 for PKH26/Olig2 and 10.5±.7 for PKH26/Mog) (P≤0.05) than in the Ms/stem group (7.5±.3 for PKH26/Olig2 and 6.3±.1 for

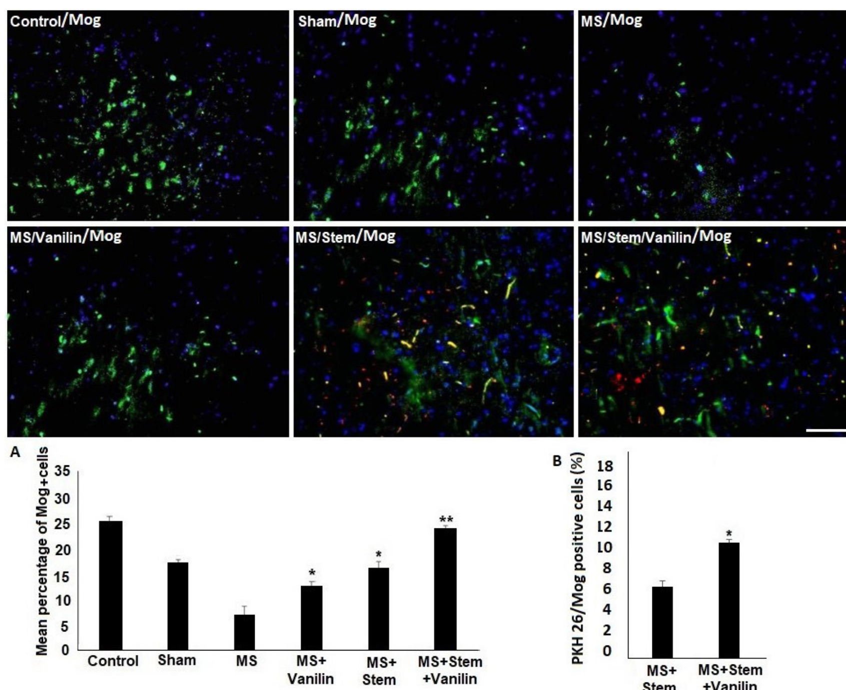

Figure 4. Immunohistochemistry staining for Mog marker in mice
Percentage of MOG-positive cells (green) increased in all treated groups specially in Ms/stem/Vanilin group (A) compared with the Ms group. Moreover, the PKH26/Mog positive cells (oranged cells) were significantly higher in the Ms/stem/Vanilin groups than in the Ms/stem group (B). DAPI (blue) was used for nuclear counterstaining. The PKH26 labeled cells (hADSCs) are shown red, (Scale bars: 200 μm) (*P≤0.05, **P≤0.01).
Mog: Myelin oligodendrocyte glycoprotein; DAPI: 4,6-diamidino-2-phenylindole; MS: Multiple sclerosis

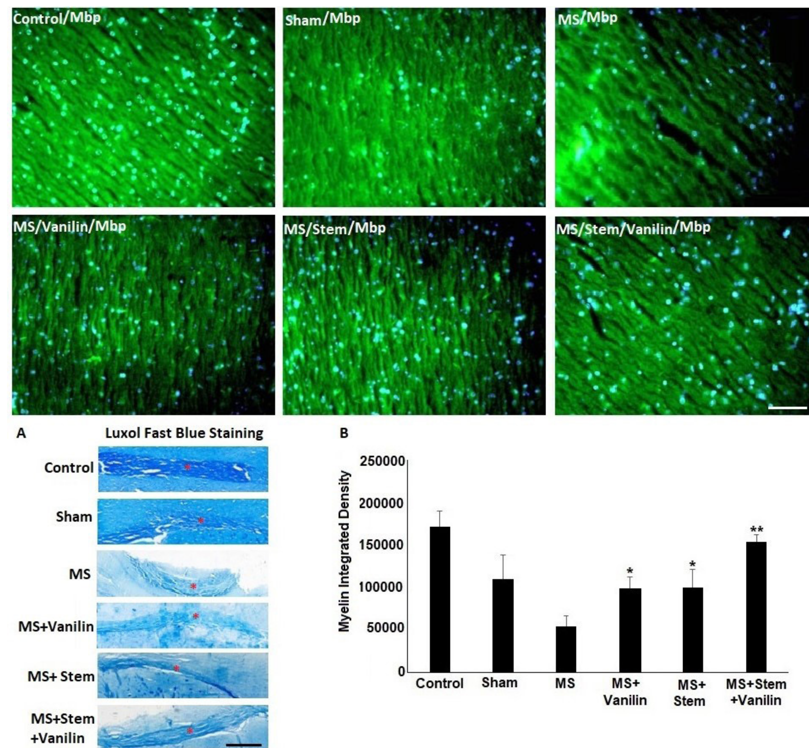

Figure 5. Immunohistochemistry staining for myelin marker and Luxol fast blue staining:
Mean of myelin density (green) increased significantly in all treated groups compared to the Ms group (A). In Luxal fast blue staining, stars indicate demyelination in the corpus callosum of the MS group as well as re-myelination in the treatment groups, especially in the MS + Stem+Vanillin group. Also, the mean of myelin integrated density increased in all treated groups, espesiaally in MS + Stem+Vanillin group (B). DAPI (blue) is used for nuclear counterstaining, Scale bar = 200 μm.
DAPI: 4,6-diamidino-2-phenylindole; MS: Multiple sclerosis

PKH26/Mog) ($P \le 0.05$) (Figures 3B and 4B).

In order to assess myelin density and the extent of demyelination/remyelination in mice, the average myelin density was measured in sections stained with LFB and in sections stained using the myelin-specific marker (Figure 5). The results showed that the density of myelin in CC sections significantly increased in treated groups, especially in the Ms/stem/Vanillin group (153000±98) compared to the Ms group (52000±120) ($P \le 0.01$) (Figure 5A, B).

ELISA test results

The findings showed that the serum level of proinflammatory cytokine (IL-2 and IFN- γ) in the MS group were considerably higher (76.98±.9 for IL-2 and 77.89±1.1 for IFN- γ) compared to other groups ($P \le 0.01$) (Figure 6 A, B). Moreover, the serum levels of the anti-inflammatory factors (IL-10 and TGF-β) in the treated groups showed a significant increase especially in the Ms/stem/Vanillin group (78.52±.88 for IL-10 and 73.82±.9 for

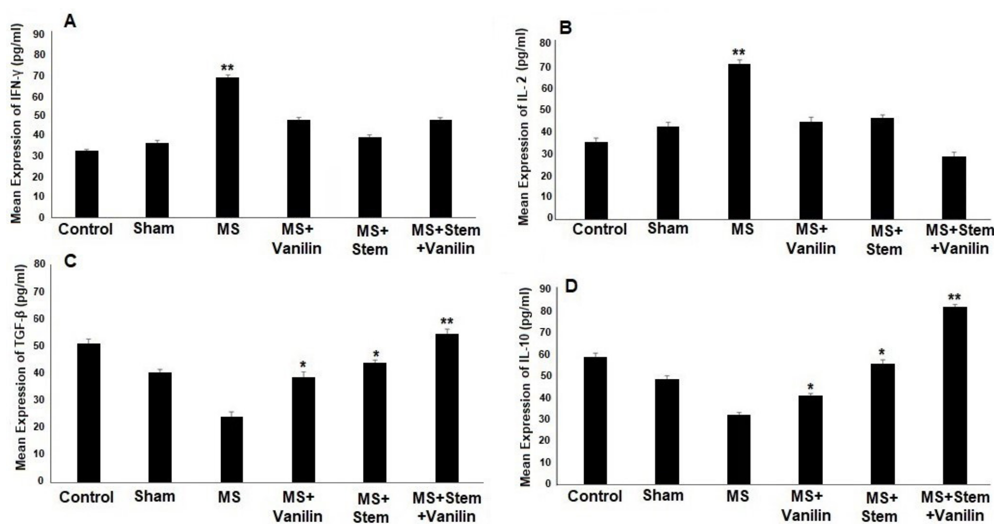

Figure 6. ELISA results for mouse pro and anti-inflammatory factors
Mean serum levels of IFN-γ and IL-2 were significantly higher in the MS group compared to the other groups (A, B). In addition, the mean serum level of TGF-β and IL-10 increased significantly in treated groups compared to the Ms group, especially in the MS + Stem+Vanillin group (C, D). (*$P \le 0.05$, **$P \le 0.01$).
IFN-γ: Interferon gamma; IL-2: Interleukin-2; MS: Multiple sclerosis; TGF-β: Transforming growth factor beta

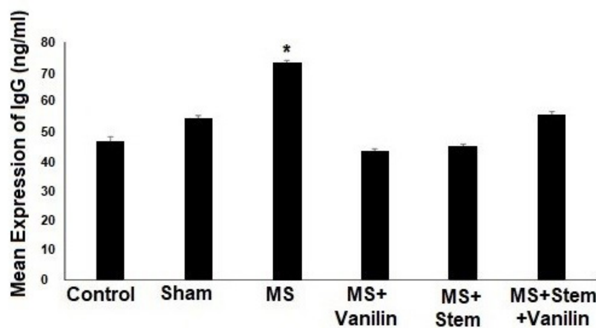

Figure 7. ELISA results for mouse IgG factor
The mean serum level of IgG is significantly higher in MS group compared to other groups. (**P*≤0.05).
IgG: Immunoglobulin G; MS: Multiple sclerosis

TGF-β) compared to the MS group (30.91±.58 for IL-10 and 30.77±.79 for TGF-β) (*P*≤0.01) (Figure 6 C, D). Also, a significant difference was seen in the serum levels of IgG in the MS group (70.91±.67) compared to the other groups (57.34±.9 in Ms/Vanillin group, 32.81±.77 in Ms/stem group, and 34.83±.55 in Ms/stem/Vanillin group) (Figure 7).

Discussion

Neurological diseases are among the major threats to human health. During neurodegenerative diseases, lymphocyte cell infiltration into the nervous tissue and specific interleukins secretion lead to the initiation of oligodendrocyte cell death (34). This process leads to myelin tissue destruction and the development of functional disorders in the CNS. Among these abnormal conditions, MS can be mentioned. MS is an autoimmune disease in which the immune system reacts against oligodendrocyte cells and the myelin sheath (35). The role of pro-inflammatory cytokines is very important in the development of this abnormal condition. Pro-inflammatory cytokines such as TNF-α, IL-2, IL-6, IL-17, and IFN-γ play an important role in myelin destruction by activating immune cells and increasing inflammation, followed by disrupting the transmission of nerve signals, leading to the development of sensory-motor problems. The role of anti-inflammatory cytokines such as IL-10 in reducing inflammation and myelin damage has also received special attention (35). Although the role of immunoglobulin-producing cells is still unclear, some studies suggest that some of the immunoglobulins produced may be beneficial in myelin regeneration (11). Therefore, there may be a relationship between the onset or prevention of MS and immunoglobulins. Current published data suggest that cell-based and drug-based therapies could be a new paradigm for the treatment of MS.

For example, transplantation of human adipose stem cells (hADSCs) into a cuprizone model of MS has been shown to improve motor function and reduce pathological symptoms such as demyelination (32). This finding is consistent with a previous study which demonstrated that intravenous injection of hADSCs can reverse the clinical course of EAE (36). Taken together, ADSCs, with their wide range of valuable properties, can replace degenerated neurons, provide a suitable environment for the maintenance of remaining neurons, and promote tissue regeneration. Therefore, ADSCs are a promising cell source for cell-based therapies in MS, although further studies are needed to confirm their efficacy and safety in humans. In line with the results of the present study, Razavi *et al.* in 2018 showed a significant increase in remyelination in the groups receiving adipose-derived stem cells (37). Also, another study showed that intravenous administration of adipose tissue-derived MSCs increased remyelination and improved motor and cognitive function (38).

In addition, the use of specific drugs or agents can enhance the differentiation of stem cells into target cells. Therefore, the present study aimed to evaluate the role of vanillin as a neuroprotective, anti-inflammatory, and immunomodulatory agent in the differentiation of hADSCs into oligodendrocytes and remyelination in a cuprizone-induced mouse model.

Vanillin is an aromatic organic compound with various therapeutic effects, including anticancer (24), antidiabetic (26), antioxidant (23), antidepressant (39), antibacterial (40), and neuroprotective properties (41). Several studies suggest that vanillin has promising potential in the prevention and treatment of various metabolic and nonmetabolic diseases (42). It has been reported that this natural compound significantly protects the brain by reducing the level of lipid peroxidation and increasing the activity of antioxidant enzymes. Furthermore, it improves motor and non-motor deficits by reducing apoptosis, reducing stress, and reducing mitochondrial dysfunction in the brain (19).

The results of the present study showed that co-treatment of stem cells with vanillin increased oligodendrocyte differentiation and enhanced remyelination. As shown in immunohistochemical images (Figure 2), the mean percentage of cells which expressing Olig2 and Mog markers was significantly reduced in the cuprizone-treated groups. In justification of this, cuprizone may cause mitochondrial enzyme dysfunction associated with oxidative stress, leading to metabolic stress in oligodendrocytes and myelin loss due to selective oligodendrocyte death in several brain regions (43).

Our findings further revealed that cuprizone has an important function in increasing serum IgG levels which play an important role in demyelination by forming immune complexes and causing cytotoxicity. As seen in figure 7, serum levels of IgG significantly decreased in the groups receiving vanillin. Furthermore, the duration of hanging in the groups that received vanillin significantly increased compared to the cuprizone group. In justification of these results, it can be said that vanillin has significant immunomodulatory and antioxidative effects, thus, with inhibitory effects on immune cell activity (44) can lead to decreased immunoglobulins secretion. On the other hand, vanillin, due to its neuroprotective effects, is able to prevent cell death by inhibiting ERK1/2, P38 and the NF-κB Signaling Pathway (45). Therefore, by preserving neuroglia cells and the myelin sheath, muscle contractile strength will increase and the mice's motor balance will be maintained.

Inflammation plays a main role in the MS pathogenesis (46). As seen in Figure 6, proinflammatory factors, including IL-2 and IFN-gamma were significantly increased following the use of cuprizone. Another relevant result of our study was that, serum levels of anti-inflammatory factors were significantly increased in the groups that used vanillin and stem cells. In justification of these findings, it can be concluded that vanillin reduced neuroinflammation with reducing proinflammatory factors and neutralizing the effects of cuprizone. Also, vanillin improves mitochondrial function in neuroglia cells by neutralizing free radicals and increasing endogenous antioxidant enzymes. So, vanillin

probably suppresses apoptosis of oligodendrocytes and myelin tissue through its antioxidant and anti-inflammatory activities. The results of the present study showed that hADSCs are able to reduce proinflammatory cytokines and IgG factor. In addition, they increase serum levels of anti-inflammatory factors. In justification of these results, it can be said that hADSCs probably modulate the function of monocytes/macrophages with paracrine functions and therefore play an important role in the immune response and are able to suppress inflammation (47).

Conclusion

The results of this study showed that vanillin, with its anti-inflammatory and neuronal modulatory properties, can induce differentiation of hADSCs into oligodendrocytes and improve remyelination in the brain tissue. Also, hADSCs are able to facilitate the reduction of inflammation by reducing inflammatory cytokines and immunoglobulin G. Therefore, the use of vanillin alone and simultaneously with hADSCs transplantation will be an effective step towards improving the health of MS patients.

Acknowledgment

The results presented in this paper were part of a student thesis, and we acknowledge the facility and funding provided by Isfahan University of Medical Sciences, Iran.

Ethical Approval and Consent to Participate

This research project, titled “The Synergistic myelin regenerative, immunomodulatory and Anti-inflammatory Effects of vanillin and adipose derived stem cells in Cuprizone Animal Model of Multiple Sclerosis” was carried out in strict accordance with the “Guideline for the Care and Use of Laboratory Animals in Iran”. This experimental study was conducted on the female C57BL/6 mice model of MS at the Central Laboratory of Isfahan University of Medical Sciences, Iran. All procedures of research were conducted according to the guidelines of the Iranian Committee of Animal Care approved by the Ethics Committee (Ethics# IR.MUI.AEC.1403.033).

Funding

Isfahan university of Medical Sciences, Iran, supported this work (grant number: 3403223).

Authors’ Contributions

All authors contributed to the study conception, design, statistical analysis, and drafting of the manuscript. S G and N G contributed to data collection and manuscript drafting. M M revised the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

All authors confirm that they have reviewed and understood the journal’s conflicts of interest and authorship policies. They declare no competing interests related to this work. Each author has made significant contributions to the research and the manuscript, and has reviewed and approved the final version for publication.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

References

1. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and treatment of multiple sclerosis: A review. JAMA 2021; 325: 765-779.
2. Xiao J, Yang R, Biswas S, Qin X, Zhang M, Deng W. Mesenchymal stem cells and induced pluripotent stem cells as therapies for multiple sclerosis. Int J Mol Sci 2015; 16: 9283-9302.
3. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, *et al.* Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS. Mult Scler 2020; 26: 1816-1821.
4. Ghasemi N, Razavi S, Nikzad E. Multiple sclerosis: Pathogenesis, symptoms, diagnoses and cell-based therapy. Cell J 2017; 19:1-10.
5. Stadelmann C, Wegner C, Brück W. Inflammation, demyelination, and degeneration—recent insights from MS pathology. Biochim Biophys Acta 2011; 1812: 275-282.
6. Mirshafiey A, Mohsenzadegan M. TGF-β as a promising option in the treatment of multiple sclerosis. Neuropharmacology 2009; 56: 929-936.
7. Damoiseaux J. The IL-2–IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor. Clin Immunol 2020; 218: 108515.
8. Sivieri S, Ferrarini A, Gallo P. Multiple sclerosis: IL-2 and sIL-2R levels in cerebrospinal fluid and serum. Review of literature and critical analysis of ELISA pitfalls. Mult Scler 1998; 4: 7-11.
9. Ali Shokrgozar M, Sarial S, Amirzargar A, Shokri F, Rezaei N, Arjang Z, *et al.* IL-2, IFN-γ, and IL-12 gene polymorphisms and susceptibility to multiple sclerosis. J Clin Immunol 2009; 29: 747-751.
10. Grunwald C, Krętowska-Grunwald A, Adamska-Patruno E, Kochanowicz J, Kułakowska A, Chorąży M. The role of selected interleukins in the development and progression of multiple sclerosis—A systematic review. Int J Mol Sci 2024; 25: 2589.
11. Li Y, Noto D, Hoshino Y, Mizuno M, Yoshikawa S, Miyake S. Immunoglobulin directly enhances differentiation of oligodendrocyte-precursor cells and remyelination. Sci Rep 2023; 13: 9394.
12. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther 2019; 10:1-22.
13. Jiang H, Zhang Y, Tian K, Wang B, Han S. Amelioration of experimental autoimmune encephalomyelitis through transplantation of placental derived mesenchymal stem cells. Sci Rep 2017; 7: 41837.
14. Klinker MW, Wei C-H. Mesenchymal stem cells in the treatment of inflammatory and autoimmune diseases in experimental animal models. World J Stem Cells 2015; 7: 556.
15. Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, *et al.* Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. Biomed Pharmacother 2019; 114: 108765.
16. Planchon SM, Lingas KT, Reese Koç J, Hooper BM, Maitra B, Fox RM, *et al.* Feasibility of mesenchymal stem cell culture expansion for a phase I clinical trial in multiple sclerosis. Mult Scler J Exp Transl Clin 2018; 4: 2055217318765288.
17. Kim HN, Shin JY, Kim DY, Lee JE, Lee PH. Priming mesenchymal stem cells with uric acid enhances neuroprotective properties in parkinsonian models. J Tissue Eng 2021; 12: 20417314211004816.
18. Brockmueller A, Mahmoudi N, Movaeni AK, Mueller A-L, Kajbafzadeh A-M, Shakibaei M, *et al.* Stem cells and natural agents in the management of neurodegenerative diseases: A new approach. Neurochem Res 2023; 48: 39-53.
19. Cho J-H, Park JH, Ahn JH, Lee J-C, Hwang IK, Park SM, *et al.* Vanillin and 4-hydroxybenzyl alcohol promotes cell proliferation and neuroblast differentiation in the dentate gyrus of mice via the increase of brain-derived neurotrophic factor and tropomyosin-related kinase B. Mol Med Rep 2016; 13: 2949-2956.
20. Dhanalakshmi C, Janakiraman U, Manivasagam T, Justin Thenmozhi A, Essa MM, Kalandar A, *et al.* Vanillin attenuated behavioural impairments, neurochemical deficts, oxidative stress and apoptosis against rotenone induced rat model of Parkinson’s disease. Neurochem Res 2016; 41: 1899-1910.
21. Arya SS, Rookes JE, Cahill DM, Lenka SK. Vanillin: A review on the therapeutic prospects of a popular flavouring molecule. Adv

Tradit Med (ADTM) 2021; 21: 1-17.
22. Lan X-B, Wang Q, Yang J-M, Ma L, Zhang W-J, Zheng P, *et al*. Neuroprotective effect of Vanillin on hypoxic-ischemic brain damage in neonatal rats. Biomed Pharmacother 2019; 118: 109196.
23. Bezerra-Filho CS, Barboza JN, Souza MT, Sabry P, Ismail NS, de Sousa DP. Therapeutic potential of vanillin and its main metabolites to regulate the inflammatory response and oxidative stress. Mini Rev Med Chem 2019; 19: 1681-1693.
24. Karathanos VT, Mourtzinos I, Yannakopoulou K, Andrikopoulos NK. Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with β-cyclodextrin. Food Chem 2007; 101: 652-658.
25. Kanedi M, Nurhidayah S, Nurcahyani E, Widiastuti EL. Fruit extract of vanilla (Vanilla planifolia Andrews) lowers total blood glucose in alloxan-induced hyperglycemic mice. Eur J Pharm Med Res 2019; 6: 314-316.
26. Kumar R, Sharma P, Mishra PS. Vanillin derivatives showing various biological activities. Int J Pharmtech Res 2012; 43.
27. Praet J, Guglielmetti C, Berneman Z, Van der Linden A, Ponsaerts P. Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. Neurosci Biobehav Rev 2014; 47: 485-505.
28. Morgan ML, Teo W, Hernandez Y, Brideau C, Cummins K, Kuipers HF, *et al*. Cuprizone-induced demyelination in mouse brain is not due to depletion of copper. ASN Neuro 2022; 14: 17590914221126367.
29. Ganji R, Razavi S, Ghasemi N, Mardani M. Improvement of remyelination in demyelinated corpus callosum using human adipose-derived stem cells (hADSCs) and pregnenolone in the cuprizone rat model of multiple sclerosis. J Mol Neurosci 2020; 70: 1088-1099.
30. Bakhtiari M, Ghasemi N, Salehi H, Amirpour N, Kazemi M, Mardani M. Evaluation of Edaravone effects on the differentiation of human adipose derived stem cells into oligodendrocyte cells in multiple sclerosis disease in rats. Life Sci 2021; 282: 119812.
31. Ghosouri S, Soleimani M, Bakhtiari M, Ghasemi N. Evaluation of in vivo lithium chloride effects as a GSK3-β inhibitor on human adipose derived stem cells differentiation into oligodendrocytes and re-myelination in an animal model of multiple sclerosis. Mol Biol Rep 2023; 50: 1617-1625.
32. Ghasemi N, Razavi S, Mardani M, Esfandiari E, Salehi H, Zarkesh Esfahani SH. Transplantation of human adipose-derived stem cells enhances remyelination in lysolecithin-induced focal demyelination of rat spinal cord. Mol Biotechnol 2014; 56: 470-478.
33. Ghosouri S, Bakhtiari M, Mitra S, Ghasemi N. Valproic acid effects on human adipose-derived stem cell differentiation into oligodendrocytes and improved remyelination in a mouse model of Multiple Sclerosis. Int J Dev Biol 2023; 67: 101-108.
34. Gitler AD, Dhillon P, Shorter J. Neurodegenerative disease: Models, mechanisms, and a new hope. Dis Model Mech 2017; 10: 499-502.
35. Steinman L. Multiple sclerosis: A coordinated immunological attack against myelin in the central nervous system. Cell 1996; 85: 299-302.
36. Constantin G, Marconi S, Rossi B, Angiari S, Calderan L, Anghileri E, *et al*. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. Stem Cells 2009; 27: 2624-35.
37. Razavi SR, Ghasemi N, Mardani M, Salehi H. Co-transplantation of human neurotrophic factor secreting cells and adipose-derived stem cells in rat model of multiple sclerosis. Cell J 2018; 20:46-52.
38. Barkat MA, El-Agwany AM, Khanfor AA, Kelada MNB, Nabil I, Abdel-monsif DA, *et al*. The potential therapeutic effect of adipose tissue-derived mesenchymal stem cell transplantation on cuprizone model of multiple sclerosis in the mice. Egy J Histol 2020; 43: 122-143.
39. Dhingra D, Sharma A. Evaluation of antidepressant-like activity of glycyrrhizin in mice. Indian J Physiol Pharmacol 2005; 37: 390-394.
40. Fuqua C, Greenberg EP. Listening in on bacteria: Acyl-homoserine lactone signalling. Nat Rev Mol Cell Biol 2002; 3: 685-695.
41. Salau VF, Islam MS. Vanillin: A natural phenolic compound with neuroprotective benefits. Natural Molecules in Neuroprotection and Neurotoxicity: Elsevier 2024. p. 1857-1879.
42. Olatunde A, Mohammed A, Ibrahim MA, Tajuddeen N, Shuaibu MN. Vanillin: A food additive with multiple biological activities. Eur J Med Chem Rep 2022; 5:100055.
43. Safwat SM, El Tohamy M, Aboonq MS, Alrehaili A, Assinnari AA, Bahashwan AS, *et al*. Vanillic acid ameliorates demyelination in a cuprizone-induced multiple sclerosis rat model: Possible underlying mechanisms. Brain Sci 2023; 14:12.
44. Sobhy S, Elmetwalli A, Baraka DM, Hassan J, Hassan MG. Immunomodulatory and antioxidative effects of vanillin on human acute monocytic leukemia cells: A potential therapeutic approach for AMoL. J Basic Environ Sci 2024; 11: 755-768.
45. Yan X, Liu D-F, Zhang X-Y, Liu D, Xu S-Y, Chen G-X, *et al*. Vanillin protects dopaminergic neurons against inflammation-mediated cell death by inhibiting ERK1/2, P38 and the NF-κB signaling pathway. Int J Mol Sci 2017; 18: 389.
46. Haase S, Linker RA. Inflammation in multiple sclerosis. Ther Adv Neurol Disord 2021; 14:17562864211007687.
47. Guillén MI, Platas J, Perez del Caz MD, Mirabet V, Alcaraz MJ. Paracrine anti-inflammatory effects of adipose tissue-derived mesenchymal stem cells in human monocytes. Front Physiol 2018; 9:661.