Evaluation of Lithium chloride effects as a GSK3-β inhibitor on human Adipose Derived Stem cells differentiation into oligodendrocytes and re-myelination in animal model of multiple sclerosis

Sahar Ghosouri
Isfahan University of Medical Sciences
Mitra Soleimani
Isfahan University of Medical Sciences
Mohammad Bakhtiari
Isfahan University of Medical Sciences
Nazem Ghasemi (n_ghasemi@med.mui.ac.ir)
Isfahan University of Medical Sciences

Research Article

Keywords: Multiple sclerosis, Lithium chloride, Stem cell, Myelination

Posted Date: September 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2083375/v1

Abstract

The application of neuroprotective agents in combination with stem cells, is considered as a potential effective treatment for Multiple sclerosis (MS). So, the effects of lithium chloride as a neuroprotective agent and a GSK3-β inhibitor was evaluated in combination with human adipose derived stem cells on re-myelination, oligodendrocyte differentiation, as well as functional recovery. After inducing mouse model of MS and proving it through hanging wire test, the mice were randomly assigned to five experimental groups including: Cup, Sham, Li, hADSC, and Li + hADSC groups. In addition, a control group with normal feeding was considered. At the end, toluidine blue staining was done to estimate the level of myelination. Moreover, Immunofluorescent staining was used to evaluate the mean of OLIG2 and MOG positive cells. Also, the mRNA levels of β-Catenin, myelin and oligodendrocyte specific genes were determined via Real Time-PCR. The results of hanging wire test and toluidine blue staining showed a significant increase in myelin density and motor function improvement in groups which received lithium and stem cells, especially in Li + hADSC group compared to untreated groups ($P < 0.01$). In addition, immunostaining results indicated that the mean percentage of MOG and OLIG2 positive cells were significantly higher in Li + hADSC group in compared to other groups ($P < 0.01$). Finally, gene expression studies showed that the use of lithium can able to increase the expression of β-Catenin, myelin and oligodendrocyte specific genes. The use of Lithium Chloride can able to increase stem cells differentiation into oligodendrocytes and improve re-myelination in MS.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), which may lead to progressive disabling state. Demyelination is one of the main reasons of neurological disability in MS disease. The penetration of reactive lymphocytes and other inflammatory cells into nervous tissue, oligodendrocytes death and myelin sheath damage, are considered as MS pathology (Mohebi et al. 2019; Lotfi et al. 2021; Templeton et al. 2019). The epidemiologic form of MS is constantly changing around the world due to urbanization, industrialization, and modernization. In spite of this, it is more prevalent in women between the ages of 20 and 40 (Moghadasi 2020; Cheraghmakani et al. 2020; Mahmudi et al. 2019).

The use of some medication which accepted by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) such as dimethyl fumarate (Tecfidera), alemtuzumab (Lemtrada), pegylated interferonβ (Plegridy) and glatiramer acetate (Copaxone) in order to modulate inflammatory and immune responses, is the most common treatment strategy for MS. But these drugs are not able to stop oligodendrocytes death, demyelination and MS progression (Huang et al. 2017; Ganji et al. 2020).

Recently, other therapeutic strategies such as cell-based therapies using either differentiated or undifferentiated cells, has been emerged as promising and effective therapeutic method in neurodegenerative disorders (Mansoor et al. 2019). Previous studies have shown that adipose tissue is an ideal cell source for tissue regeneration, because adipose tissue is abundant and has more stem cells

than other sources such as bone marrow. In addition, adipose derived stem cells (ADSCs) have the ability to differentiate into multi lineage cells such as neural, glial, and neurotrophic factor secreting cells. As a result, these cells can be used in nervous tissue regeneration alone or in combination with other neurotrophic factors such as lithium chloride (Li) (Razavi et al. 2018; Ganji et al. 2020; Ghasemi et al. 2014).

For decades, lithium has been widely used as a long-term mood stabilizer in the treatment of bipolar and depression (Wallin et al. 2019). Moreover, this agent is widely used as an enzymatic inhibitor of GSK3. Thus, plays an important role in Wnt/β-catenin signaling pathway. Recent evidence suggests that lithium has neuroprotective and anti-apoptotic properties. So, it can be used as an ideal substance in treatment of numerous neurodegenerative diseases such as Huntington, Alzheimer's, Parkinson and in experimental autoimmune encephalomyelitis (Chen et al. 2016).

Cuprizone (Cup), as a toxic compound and a copper chelating agent, is able to cause demyelination in different areas of the brain (especially in the corpus callosum) by disrupting the metabolism of oligodendrocytes (Zhen et al. 2017; Kopanitsa et al. 2021). In addition, it has been reported that Cup can reduce the expression of myelin-related genes and proteins, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein in carpus callosum (Han et al. 2020).

Overall, the available data suggest that if lithium can increase the differentiation of ADSCs into oligodendrocyte cells, this factor could be used as a new drug to MS treatment. Thus, the aim of this in-vivo study was to evaluate lithium chloride effects as a GSK3 inhibitor on human ADSCs (hADSCs) differentiation into oligodendrocytes and re-myelination in animal model of multiple sclerosis.

Materials And Methods

hADSCs culture and labeling

Frozen hADSCs from the previous study (Bakhtiari et al. 2021) were thawed and cultured with Dulbecco's modified Eagle's medium DMEM/ F12 (Bioidia, BI1027) containing 10% fetal bovine serum (FBS) (Gibco, 10270106) and 1% penicillin/streptomycin (Bioidia, BI1036) based on a previous study at 37 ◦C in a humidified 5% CO2 incubator . After 80% cell confluency, hADSCs were detached by 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) (Bioidia, BI 1601). 72 hours before cell transplantation, the hADSCs which obtained from passages 3–5, were labeled using PKH26 (a red florescent dye) (Sigma-Aldrich, MINI26) according to our previous study (Ganji et al. 2020).

Behavioral test

During the study, hanging-wire test was performed to assess motor function and muscle stretch. To this end, the mice were hung from a 2-mm-thick metal hanger for 3 min after starting the timer. If the mice fell to the ground before the end of the test period, they were placed on the wire up to two more times, and finally the average maximum hanging time was recorded (Yamazaki et al. 2021).

Animal grouping

Sixty female C57BL/6 mice, 8 weeks old and 18 - 22 g weight, were purchased from the Royan Center, Isfahan, Iran, and maintained on alike light/dark cycles and at stable room temperature in polypropylene cages. In order to induce demyelination, cuprizone dissolved in corn oil according to previous study (Bakhtiari et al. 2021) and the mice were gavaged with 0.5 ml cuprizone 0.2% for 5 weeks (Zhu et al. 2017). Next, in order to prove the destruction of myelin and mice grouping, the results of the behavioral test were examined. Mice with significant or slight motor damage were included in this study. In these mice, the hanging time was reduced by at least 30 seconds compared to the before the study (Yamazaki, Ohno, and Huang 2021). These mice were randomly assigned to five experimental groups including, cuprizone (Cup, n=8) sham (n=8, treated with intra-peritoneal normal saline for 4 weeks), human Adipose Derived Stem Cell (n=8, treated with 1×10^6 labeled hADSC, iv) (Lykhmus et al. 2019), lithium chloride (Li, n=8, treated with 50 mg/kg/day intra-peritoneal lithium) (Makoukji et al. 2012) and Li+ hADSC (n=8, treated simultaneously with 50 mg/kg/day lithium for 4 weeks and 1×10^6 labeled hADSC) groups. In addition, a control group (n=8) with normal feeding was considered for compare the results.

Histological Examination

At the endpoint of experiment, the mice were sacrificed by performing deep anesthesia using ketamine/xylazine and fixation was done by cardiac perfusion method using normal saline and 4% formaldehyde (Merck, 1,040,021,000) (PH 7.4). In the following, the brains were immediately removed and post-fixed with same fixative for 24 h. After tissue processing, 5 µm sections were prepared by rotating microtome (Leitz1512, Germany) and after staining with toluidine blue, the sections were examined with an Olympus Provis light microscope (Hou et al. 2018).

Immunohistochemistry Technique

In order to oligodendrocytes evaluation, 3 µm-thick sections from corpus callosum were prepared and after antigen retrieval using sodium citrate buffer and 1 mM EDTA buffer (Gibco, USA), the slices were incubated with primary antibodies including anti-Olig2 antibody (1:1000; Abcam, Cambridge, MA, USA) and anti-myelin oligodendrocyte glycoprotein (MOG) antibody (1:1000; Abcam, Cambridge, MA, USA) at 4 °C overnight. Afterwards, the slices were washed with PBS, and were treated with FITC - conjugated secondary antibodies (1:500; Abcam, Cambridge, MA, USA) at room temperature for 1 h, and then, with 4',6-diamidino-2-phenylindole (DAPI) (SigmaAldrich, D9542) for 2 min. Finally, the mean percentage of MOG and Olig2 positive cells was calculated in a minimum total of 200 cells per slide using a fluorescent microscope and Image j software (Bakhtiari et al. 2021).

Real-Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) Analysis

To determine the expression β-Catenin, human and mouse myelin and oligodendrocyte specific genes in corpus callosum, the samples were evaluated using real-time RT-PCR technique. Total RNA was isolated from the tissue samples using the Total RNA Prep kit (BIOFACT, RP101–050, Korea) according to the

instructions. The RNA was reverse transcribed using the BioFact™ 5X RT Pre-Mix cDNA Synthesis Kit (BIOFACT, 25081) and oligo dT primers. To remove DNA from the samples, the extracted RNA was treated with the RNase-free DNase kit (Qiagen, ID79254 Germany). The real-time PCR was performed using the BioFact™2X Real-Time PCR Master mix kit (BIOFACT, DQ385–40) and the Step One Plus™ quantitative real time PCR detection system (Applied Biosystems). The expression level of each target gene was calculated as $2^{-\Delta\Delta Ct}$. It should be noted that the primers (Table 1) were designed by Allele ID 7.6 and checked against the mice genome using the BLAST site. Also, the β-actin was considered as housekeeping gene to normalize gene expression (Dong et al. 2017).

Table 1. The list of primers used in this study.

Gene	Primer sequences
B-actin-m-F	5'-CGGTTCCGATGCCCTGAGGCTCTT-3'
B-actin-m-R	5'-CGTCACACTTCATGATGGAATTGA-3'
B-ACTIN-H-F	5'-AGCCTCGCCTTTGCCGATCC-3'
B-ACTIN-H-R	5'-ACATGCCGGAGCCGTTGTCG-3'
Mbp-m-F	5'-AACATTGTGACACCTCGAACA-3'
Mbp-m-R	5'-TGTCTCTTCC TCCCCAGCT-3'
MBP-H-F	5'-ACTATCTCTTCCTCCCAGCTTAAAAA-3'
MBP-H-R	5'-TCCGACTATAAATCGGCTCACA-3'
Mog-m-F	5'-TGATTTCCCTCCCTCAACTG-3'
Mog-m-R	5'-CGTATCCTGGTTGGCAGAAT-3'
MOG-H-F	5'-ACCAGGCACCTGAATATCGG-3'
MOG -H-R	5'-CAGGGCTCACCCAGTAGAAAG-3'
Olig2-m-F	5'-TGGAGAGATGCGTTCGTTCC-3'
Olig2-m-R	5'-GTGCTCTGCGTCTCGTCTAA -3'
OLIG2-H-F	5'-AGATCGACGCGACACCAGCG-3'
OLIG2-H-R	5'-TCGGACCCGAAAATCTGGATGCG-3'
β-Catenin-m-F	5'-GTACGCACCATGCAGAATAC-3'
β-Catenin-m-R	5'-TGGAGCAGGAGATTATGCAG-3'

Statistical Analysis

Statistical analysis was done using the software SPSS (IBM, SPSS Statistics Version 25). One-way analysis of variance (ANOVA) and LSD post-hoc test were conducted in order to find significant differences between experimental groups. All data were presented as mean ± standard error of the mean (mean ± SEM).

Result

Characterization of hADSCs before and after labeling

As shown in Fig. 1.A, the cultured hADSCs showed a fibroblast-like morphology that confirms their mesenchymal nature. In addition, after cellular labeling by PKH26 and observation by fluorescent microscopy, the results showed that nearly 80% of the cells were labeled by PKH26 (Fig. 1.B)

Mice weight changes during the study

Mice body weight was calculated and recorded weekly from the beginning to the end of the study. Analysis of the results showed that the use of cuprizone led to weight loss and this reduction increased significantly compared to other groups after the application of Li, hADSC and Li + hADSC ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively) (Figure 2). In addition, the highest weight gain, was observed in the Li + hADSC group in comparison to Li or hADSC ($P < 0.01$, $P < 0.05$, respectively).

hADSC and lithium effects on motor function improvement

During present study, motor function was checked by a hanging wire test (48). Recorded results confirmed that the motor function of the mice was significantly decreases following cuprizone gavage, but its improves with cell transplantation and lithium consumption (Fig. 3).

Overall, in treated groups (Li, hADSC and Li+hADSC), hanging time was significantly higher than Cup and sham groups ($P < 0.05$, $P < 0.05$, $P < 0.01$, respectively). In addition, there was statistically significant differences between Li+hADSC group in comparison to Li and hADSC groups ($P < 0.05$).

Histological analysis

As shown in Fig. 4, toluidine blue staining was performed to evaluate the myelin status in carpus callosum (CC) tissue sections. The obtained images showed clear demyelination in Cup and sham groups (Fig. 4). In this regard, the comparison of myelin density showed that the use of hADSC or Li could improve re-myelination. As a result, the highest increase in myelin density was seen in Li + hADSC group compared to other treated groups (*; $P<0.01$.) (Fig. 5).

Immunohistochemistry analysis

Immunohistochemistry staining with cell type-specific markers was used to identify oligodendrocyte differentiation (Fig. 6). Fluorescence microscopic analysis were showed that the mean percentage of MOG and OLIG2 positive cells were significantly higher in the treated groups compared to the Cup and

sham groups (P<0.05). Meanwhile, there was no significant differences between Li+hADSC group in compared with Li and hADSC groups. Evaluation of cell differentiation in hADSC recipient groups showed that the PKH26/OLIG2+ and PKH26/MOG+ positive cells were significantly higher in Li+hADSC group than hADSC group (P<0.05) (Fig. 7).

β-Catenin, myelin and oligodendrocytes specific genes expression

After RNA extraction from corpus callosum samples, Real Time-PCR assay was done. To this end, β-actin was used as a control marker. The results revealed that the expression of β-Catenin, human and mouse myelin and oligodendrocytes specific genes was significantly reduced in cup group compared to other groups. In addition, there was a significant increase expression of these genes in lithium and cell treated groups, especially in the group that received lithium and cell at the same time (Fig. 8). Finally, the examination of human genes expression showed that there was a significant difference between hADSC group in compared to Li+ hADSC group for MOG and OLIG2 expression although there was no significant difference in MBP expression (Fig. 8.E).

Discussion

The vital role of the myelin sheath in the rapid transmission of nerve impulses is undeniable (Makoukji et al. 2012). During some neurodegenerative diseases such as MS, the destruction of myelin tissue due to immune cells infiltration and oligodendrocytes death is the primary cause of nervous damage. Thus, this abnormal condition lead to CNS plaques formation which can able to impair transmission of nerve impulses and induce severe physical or cognitive disability (Loma et al. 2011; Coles 2009).

Common treatments for MS are based on suppressing inflammation and modulating immunity, but the current immunosuppressive drugs such as mitoxantrone have very serious side effects. Therefore, these compounds not only cannot halt the ongoing progression of neurodegeneration, but also are not effective in treating different types of MS (Bakhtiari et al. 2021).

It has been reported that the use of neurotrophic factors such as lithium as a therapeutic agent and stem cell-based therapies as an alternative source for delivering neurotrophic factors into the CNS, are able to restore and maintain neuronal function during MS (Razavi et al. 2015; Xie et al. 2016). Thus, in present study, the re-myelination of corpus callosum were assessed following the application of hADSCs and littium in cuprizone model of MS. Meanwhile, Oligodendrocyte differentiation and myelin formation were assessed by analyzing the Olig2 (immature oligodendrocytes marker), MOG (mature oligodendrocytes marker) and MBP (myelin marker).

As shown in Fig. 4, the use of cuprizone can be induce obvious demyelination in corpus callosum. In addition, the evaluation of myelin density showed that the use of hADSC or lithium is capable to alleviate of demyelination and trigger re-myelination.

In justifying these results, it can be said that cuprizone through inhibits cytochrome oxidase and monoamine oxidase functions induced megamitochondria induction and so, mitochondrial disturbances in the energy-producing cycle, can lead to oligodendrocytes apoptosis (Mohammadi-Rad et al. 2019). On the other hand, it is possible that lithium, through its neuroprotective effects and hADSC by neurotrophin factors production and oligodendrocyte protection, can reduce cuprizone effects and thus promote re-myelination (Chen et al. 2016; Ghasemi et al. 2014).

Another finding related to our study was that, hADSC transplantation and lithium application can increase the mean percentage of Olig2 and MOG positive cells (Fig. 7). This observation might be explained by this fact that hADSC by either differentiating into oligodendrocyte cells or indirectly by promoting the cell proliferation and cell protection, is capable to increase the population of myelin-producing cells and thus, induce re-myelination as well as motor functional improvement (Fig. 3–5). These results are consistent with others recent studies which have also shown that hADSCs injection in animal model of MS can upregulate the expression of Olig2 and MBP genes and through trigger re-myelination can improve motor functions (Bakhtiari et al. 2021).

It has been reported that lithium can be determining stem cell fate by affect the Wnt /GSK3-β signaling pathway through altering the expression of some genes such as GSK-3β transcription and β-catenin protein (Soleimani et al. 2017). Moreover, according to current published data, it seems that high level of β-catenin can be considered as a novel therapeutic target for MS (Chi-Tso et al. 2011).

β-catenin is a core component of the cadherin protein complex and is essential for the activation of Wnt /GSK3-β signaling in order to regulation of various cellular processes including stem cell proliferation, differentiation, and maturation. Furthermore, β-catenin plays an important role in neuroprotection (Miki et al. 2011; Kühl et al. 2013).

As shown in Fig. 8 in addition to elevation of β-catenin gene expression in lithium-treated groups, the expression of oligodendrocyte and myelin-specific genes also increased. Forasmuch as lithium is capable to inhibit β-catenin phosphorylation through GSK-3 inhibition, it can be argued that lithium probably through increase in cytoplasmic concentration of β-catenin and facilitates β-catenin translocation into the nucleus is capable to enhances the anti-apoptotic genes expression as well as transcription of growth factors) Fig. 9 (. As a result, lithium promotes the re-myelination process and improves motor function by reducing the oligodendrocytes apoptosis, increasing cell viability and proliferation as well as increasing the differentiation of transplanted cells into myelin-producing cells.

In similar experiments, it has been demonstrated that lithium can be increase Schwann cell viability and proliferation. Therefore, it is useful for re-myelination and regeneration of peripheral nerves (Gu et al. 2020). On the other hand, Mingxi li *et al* reported that the nerve transmission and motor function were also improved significantly after intervention with 42.4 mg/kg lithium in intracerebral hemorrhage mice (Li et al. 2020).

Overall, due to the broad biological functions of lithium chloride include: increases neuronal differentiation and neuroprotection, improve the mRNA expression of myelin protein zero (MPZ) and peripheral myelin protein 22 (PMP22), suppress traumatic inflammation, promote axonal regeneration as well as functional recovery, (Zhang et al. 2018; Zhang et al. 2019; Xia et al. 2017) and according to our finding, it is concluded that lithium chloride within the therapeutic range is capable to facilitates differentiation of transplanted stem cells or endogenous precursor cells into oligodendrocytes and promote re-myelination as well as motor function improvement in MS diseases.

Conclusion

Collectively, the results of this study indicate that lithium Chloride via inhibition of GSK-3β activity and β-catenin phosphorylation can able to increase stem cells differentiation into oligodendrocyte cells and improve re-myelination in neurodegenerative diseases such as MS. However, further studies are needed to examine the exact mechanism responsible for these therapeutic effects.

Declarations

Availability of Data and Materials

Data are available from the corresponding authors upon reasonable request.

Acknowledgments

This study was performed as a part of the Ph.D. thesis and we acknowledge the Isfahan University of Medical Sciences, for providing the facility and support.

Funding

This work was supported by Isfahan University of Medical Sciences (grant number: 399831).

Competing Interests

There is not conflict of interest.

Author Contributions

Sahar Ghosouri and Nazem Ghasemi participated in conception, design, statistical analysis, and drafting of the manuscript. Sahar Ghosouri and Mohammad Bakhtiari contributed in data collection and manuscript drafting. Mitra Soleimani revised the manuscript.

Ethics approval

This experimental study was conducted on female C57BL/6 mice model of MS at the Central Laboratory of Isfahan University of Medical Sciences. Meanwhile, all procedures of research were carried out

according to the guidelines of the Iranian Committee of Animal care which approved by the Ethics Committee (Ethics#IR.MUI.MED.REC.1399.925).

Consent to participate

Not applicable.

Consent for Publication

Not applicable.

References

1. Bakhtiari, Mohammad, Nazem Ghasemi, Hossein Salehi, Noushin Amirpour, Mohammad Kazemi, and Mohammad Mardani. 2021. 'Evaluation of Edaravone effects on the differentiation of human adipose derived stem cells into oligodendrocyte cells in multiple sclerosis disease in rats', *Life sciences*, 282: 119812.https://doi.org/10.1016/j.lfs.2021.119812.
2. Chen, Yixun, Jian Weng, Duanyang Han, Bo Chen, Mingtai Ma, Youlai Yu, Ming Li, Zhongdi Liu, Peixun Zhang, and Baoguo Jiang. 2016. 'GSK3β inhibition accelerates axon debris clearance and new axon remyelination', *American journal of translational research*, 8: 5410
3. Cheraghmakani, Hamed, Seyed Mohammad Baghbanian, Reza HabibiSaravi, Arash Azar, and Fariba Ghasemihamedani. 2020. 'Age and sex-adjusted incidence and yearly prevalence of multiple sclerosis (MS) in Mazandaran province, Iran: An 11-years study', *PloS one*, 15: e0235562.https://doi.org/10.1371/journal.pone.0235562.
4. Chi-Tso, CHIU, and De-Maw Chuang. 2011. 'Neuroprotective action of lithium in disorders of the central nervous system', *Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical sciences*, 36: 461.https://doi.org/10.3969/j.issn.1672-7347.2011.06.001.
5. Coles, Alasdair. 2009. 'Multiple sclerosis: the bare essentials', *Practical neurology*, 9: 118-26.http://dx.doi.org/10.1136/jnnp.2008.171132.
6. Dong, Fuxing, Yaping Liu, Zhenzhong Zhang, Rui Guo, Li Ma, Xuebin Qu, Hongli Yu, Hongbin Fan, and Ruiqin Yao. 2017. 'Postnatal alteration of monocarboxylate transporter 1 expression in the rat corpus callosum', *Physiological Research*, 66: 345.https://doi.org/10.33549/physiolres.933365.
7. Ganji, Rasoul, Shahnaz Razavi, Nazem Ghasemi, and Mohammad Mardani. 2020. 'Improvement of Remyelination in Demyelinated Corpus Callosum Using Human Adipose-Derived Stem Cells (hADSCs) and Pregnenolone in the Cuprizone Rat Model of Multiple Sclerosis', *Journal of Molecular Neuroscience*, 70: 1088-99.https://doi.org/10.1007/s12031-020-01515-w.
8. Ghasemi, Nazem, Shahnaz Razavi, Mohammad Mardani, Ebrahim Esfandiari, Hossein Salehi, and Sayyed Hamid Zarkesh Esfahani. 2014. 'Transplantation of human adipose-derived stem cells enhances remyelination in lysolecithin-induced focal demyelination of rat spinal cord', *Molecular biotechnology*, 56: 470-78.https://doi.org/10.22074/cellj.2018.4777.

9. Gu, Xiao-Kun, Xin-Rui Li, Mei-Ling Lu, and Hui Xu. 2020. 'Lithium promotes proliferation and suppresses migration of Schwann cells', *Neural regeneration research*, 15: 1955.https://doi.org/10.4103/1673-5374.280324.
10. Han, Seung Ro, Yun Hee Kang, Seung-Min Yoo, Myung-Shin Lee, and Seung-Hoon Lee. 2020. 'Diverse gene expressions in the prediction of Cuprizone-induced demyelination', *Neurotoxicity research*, 37: 732-42.https://doi.org/10.1007/s12640-019-00154-3.
11. Hou, Bo, Zhuopeng Ye, Wanqing Ji, Meiqin Cai, Cong Ling, Chuan Chen, and Ying Guo. 2018. 'Comparison of the effects of BMSC-derived schwann cells and autologous schwann cells on remyelination using a rat sciatic nerve defect model', *International journal of biological sciences*, 14: 1910.https://doi.org/10.7150/ijbs.26765.
12. Huang, Wen-Juan, Wei-Wei Chen, and Xia Zhang. 2017. 'Multiple sclerosis: Pathology, diagnosis and treatments', *Experimental and therapeutic medicine*, 13: 3163-66.https://doi.org/10.3892/etm.2017.4410.
13. Kopanitsa, Maksym V, Kimmo K Lehtimäki, Markku Forsman, Ari Suhonen, Juho Koponen, Tuukka O Piiponniemi, Anna-Mari Kärkkäinen, Pavlina Pavlidi, Artem Shatillo, and Patrick J Sweeney. 2021. 'Cognitive disturbances in the cuprizone model of multiple sclerosis', *Genes, Brain and Behavior*, 20: e12663.https://doi.org/10.1111/gbb.12663.
14. Kühl, Susanne J, and Michael Kühl. 2013. 'On the role of Wnt/β-catenin signaling in stem cells', *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830: 2297-306.https://doi.org/10.1016/j.bbagen.2012.08.010.
15. Li, Mingxi, Min Xia, Weixiang Chen, Jie Wang, Yi Yin, Chao Guo, Chengcheng Li, Xiaoqin Tang, Hengli Zhao, and Qiang Tan. 2020. 'Lithium treatment mitigates white matter injury after intracerebral hemorrhage through brain-derived neurotrophic factor signaling in mice', *Translational Research*, 217: 61-74.https://doi.org/10.1016/j.trsl.2019.12.006.
16. Loma, Ingrid, and Rock Heyman. 2011. 'Multiple sclerosis: pathogenesis and treatment', *Current neuropharmacology*, 9: 409-16.https://doi.org/10.2174/157015911796557911.
17. Lotfi, Alireza, Mitra Soleimani, and Nazem Ghasemi. 2021. 'Astaxanthin reduces demyelination and oligodendrocytes death in a rat model of multiple sclerosis', *Cell Journal (Yakhteh)*, 22: 565.https://doi.org/10.22074/cellj.2021.6999.
18. Lykhmus, Olena, Lyudmyla Koval, Larysa Voytenko, Kateryna Uspenska, Serhiy Komisarenko, Olena Deryabina, Nadia Shuvalova, Vitalii Kordium, Alina Ustymenko, and Vitalii Kyryk. 2019. 'Intravenously injected mesenchymal stem cells penetrate the brain and treat inflammation-induced brain damage and memory impairment in mice', *Frontiers in Pharmacology*, 10: 355.https://doi.org/10.3389/fphar.2019.00355.
19. Mahmudi, Leily, Moslem Moslemirad, Bafrin Dabestani, Masoumeh Shohani, and Milad Azami. 2019. 'Clinical and demographic features in Iranian multiple sclerosis patients: a systematic review and meta-analysis', *Future Neurology*, 14: FNL19.https://doi.org/10.2217/fnl-2018-0025.

20. Makoukji, Joelle, Martin Belle, Delphine Meffre, Ruth Stassart, Julien Grenier, Ghjuvan'Ghjacumu Shackleford, Robert Fledrich, Cosima Fonte, Julien Branchu, and Marie Goulard. 2012. 'Lithium enhances remyelination of peripheral nerves', *Proceedings of the National Academy of Sciences*, 109: 3973-78.https://doi.org/10.1073/pnas.1121367109.
21. Mansoor, Sahar Rostami, Ebrahim Zabihi, and Maryam Ghasemi-Kasman. 2019. 'The potential use of mesenchymal stem cells for the treatment of multiple sclerosis', *Life sciences*, 235: 116830.https://doi.org/10.1016/j.lfs.2019.116830.
22. Miki, Toshio, Shin-ya Yasuda, and Michael Kahn. 2011. 'Wnt/β-catenin signaling in embryonic stem cell self-renewal and somatic cell reprogramming', *Stem Cell Reviews and Reports*, 7: 836-46.https://doi.org/10.1007/s12015-011-9275-1.
23. Moghadasi, Abdorreza Naser. 2020. 'History of Multiple Sclerosis in Iran', *Archives of Iranian Medicine (AIM)*, 23
24. Mohammadi-Rad, Maedeh, Nazem Ghasemi, and Mehdi Aliomrani. 2019. 'Evaluation of apamin effects on myelination process in C57BL/6 mice model of multiple sclerosis', *Research in pharmaceutical sciences*, 14: 424.https://doi.org/10.4103/1735-5362.268203.
25. Mohebi, Farnam, Sharareh Eskandarieh, Mohammad Ali Mansournia, Bahram Mohajer, and Mohammad Ali Sahraian. 2019. 'Multiple sclerosis in Tehran: rising prevalence alongside stabilizing incidence–true increase or enhanced diagnosis?', *Archives of Iranian medicine*, 22: 429-34
26. Razavi, Shahnaz, Ghasemi Nazem, Mohammad Mardani, Ebrahim Esfandiari, Hossein Salehi, and Sayyed Hamid Zarkesh Esfahani. 2015. 'Neurotrophic factors and their effects in the treatment of multiple sclerosis', *Advanced biomedical research*, 4.https://doi.org/10.4103/2277-9175.151570.
27. Razavi, Shahnaz Razavi, Nazem Ghasemi, Mohammad Mardani, and Hossein Salehi. 2018. 'Co-transplantation of human neurotrophic factor secreting cells and adipose-derived stem cells in rat model of multiple sclerosis', *Cell Journal (Yakhteh)*, 20: 46.https://doi.org/10.22074/cellj.2018.4777.
28. Soleimani, Mitra, and Nazem Ghasemi. 2017. 'Lithium chloride can induce differentiation of human immortalized RenVm cells into dopaminergic neurons', *Avicenna Journal of Medical Biotechnology*, 9: 176
29. Templeton, Nikki, Bronwyn Kivell, Amy McCaughey-Chapman, Bronwen Connor, and Anne Camille La Flamme. 2019. 'Clozapine administration enhanced functional recovery after cuprizone demyelination', *PloS one*, 14: e0216113.https://doi.org/10.1371/journal.pone.0216113.
30. Wallin, Mitchell T, William J Culpepper, Emma Nichols, Zulfiqar A Bhutta, Tsegaye Tewelde Gebrehiwot, Simon I Hay, Ibrahim A Khalil, Kristopher J Krohn, Xiaofeng Liang, and Mohsen Naghavi. 2019. 'Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016', *The Lancet Neurology*, 18: 269-85.https://doi.org/10.1016/S1474-4422(18)30443-5.
31. Xia, Ming-Yu, Xue-Yan Zhao, Qi-Lin Huang, Hai-Ying Sun, Chen Sun, Jie Yuan, Chang He, Yu Sun, Xiang Huang, and Wen Kong. 2017. 'Activation of Wnt/β-catenin signaling by lithium chloride

attenuates d-galactose-induced neurodegeneration in the auditory cortex of a rat model of aging', *FEBS open bio*, 7: 759-76.https://doi.org/10.1002/2211-5463.12220.

32. Xie, Chong, Yan-Qun Liu, Yang-Tai Guan, and Guang-Xian Zhang. 2016. 'Induced stem cells as a novel multiple sclerosis therapy', *Current stem cell research & therapy*, 11: 313-20.https://doi.org/10.2174/1574888x10666150302110013.
33. Yamazaki, Reiji, Nobuhiko Ohno, and Jeffrey K Huang. 2021. 'Acute motor deficit and subsequent remyelination-associated recovery following internal capsule demyelination in mice', *Journal of neurochemistry*, 156: 917-28.https://doi.org/10.1111/jnc.15142.
34. Zhang, Jian, Lu He, Zhong Yang, Lihong Li, and Wenqin Cai. 2019. 'Lithium chloride promotes proliferation of neural stem cells in vitro, possibly by triggering the Wnt signaling pathway', *Animal cells and systems*, 23: 32-41.https://doi.org/10.1080/19768354.2018.1487334.
35. Zhang, Li-Qun, Wen-Ming Zhang, Lingxiao Deng, Zi-Xing Xu, Wen-Bin Lan, and Jian-Hua Lin. 2018. 'Transplantation of a peripheral nerve with neural stem cells plus lithium chloride injection promote the recovery of rat spinal cord injury', *Cell Transplantation*, 27: 471-84.https://doi.org/10.1177/0963689717752945.
36. Zhen, Wei, Ailian Liu, Jiaqi Lu, Wandong Zhang, David Tattersall, and Jianfei Wang. 2017. 'An alternative cuprizone-induced demyelination and remyelination mouse model', *ASN neuro*, 9: 1759091417725174.https://doi.org/10.1177/1759091417725174.
37. Zhu, Keying, Jingxian Sun, Zheng Kang, Zaofeng Zou, Gencheng Wu, and Jun Wang. 2017. 'Electroacupuncture promotes remyelination after cuprizone treatment by enhancing myelin debris clearance', *Frontiers in neuroscience*, 10: 613.https://doi.org/10.3389/fnins.2016.00613.

Figures

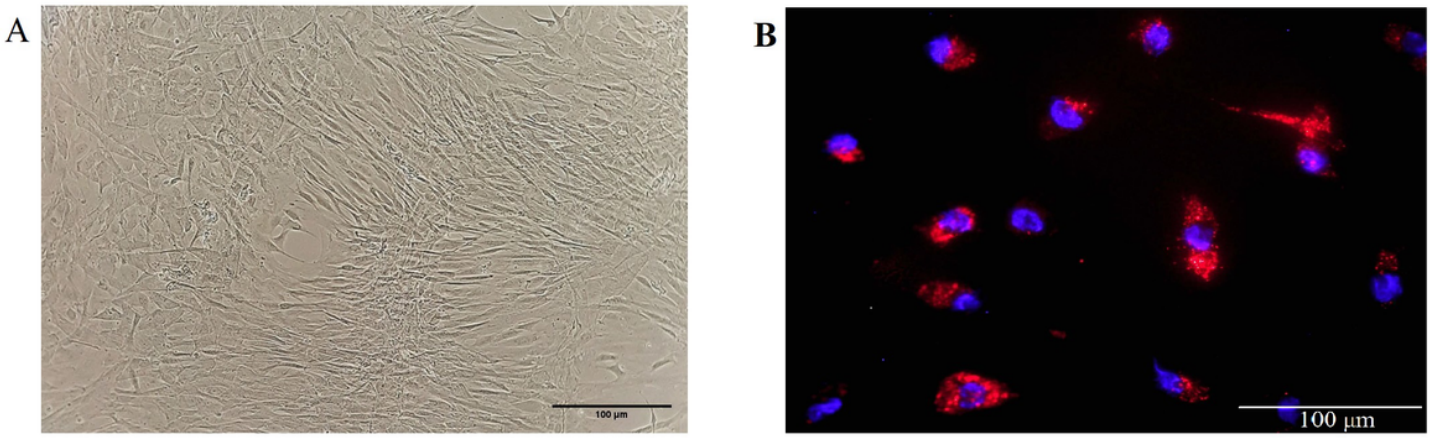

Figure 1

A. Cultured human adipose derived stem cells (hADSCs) with the appearance of like fibroblast on the bottom of the flask. B. The cytoplasm of PKH26-labeled hADSC, was red and their nucleus was blue

(DAPI –stained) Scale bar = 100μm

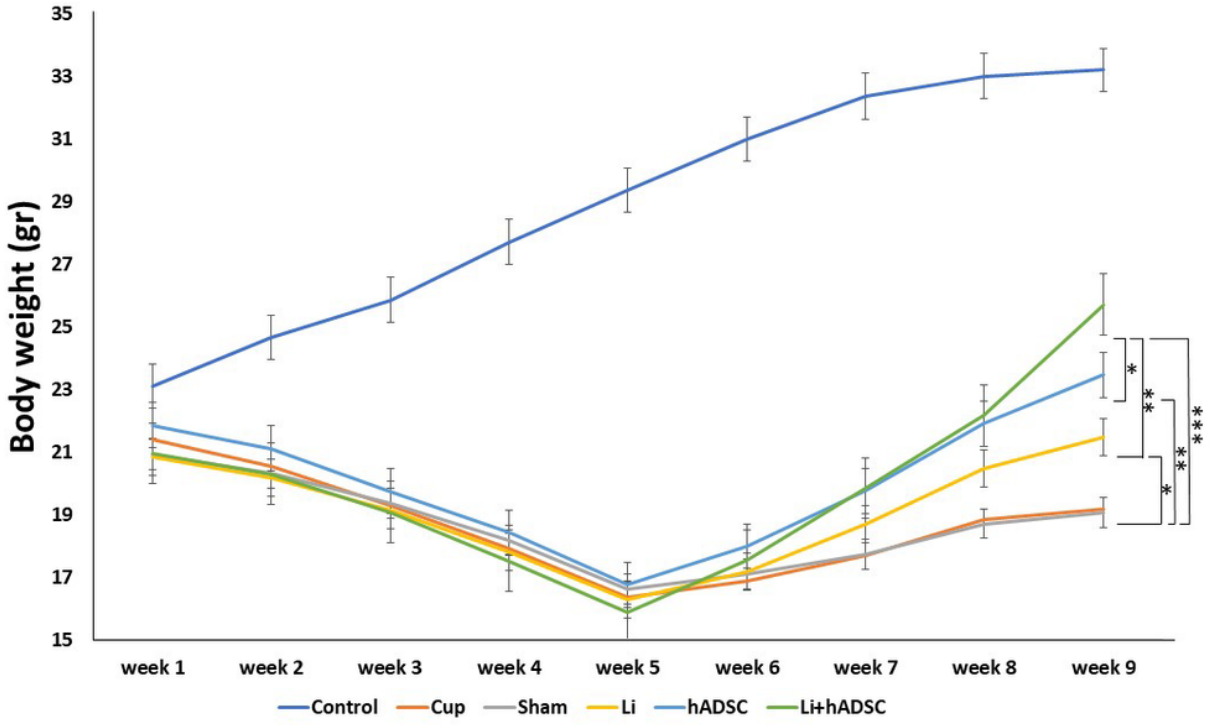

Figure 2

Effects of Li, hADSC and Li+hADSC on bodyweight after cuprizone gavage. The use of lithium and hADSC led to weight gain in mice. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC), and Li+hADSC groups. *P < 0.05, **P < 0.01, ***P < 0.001.

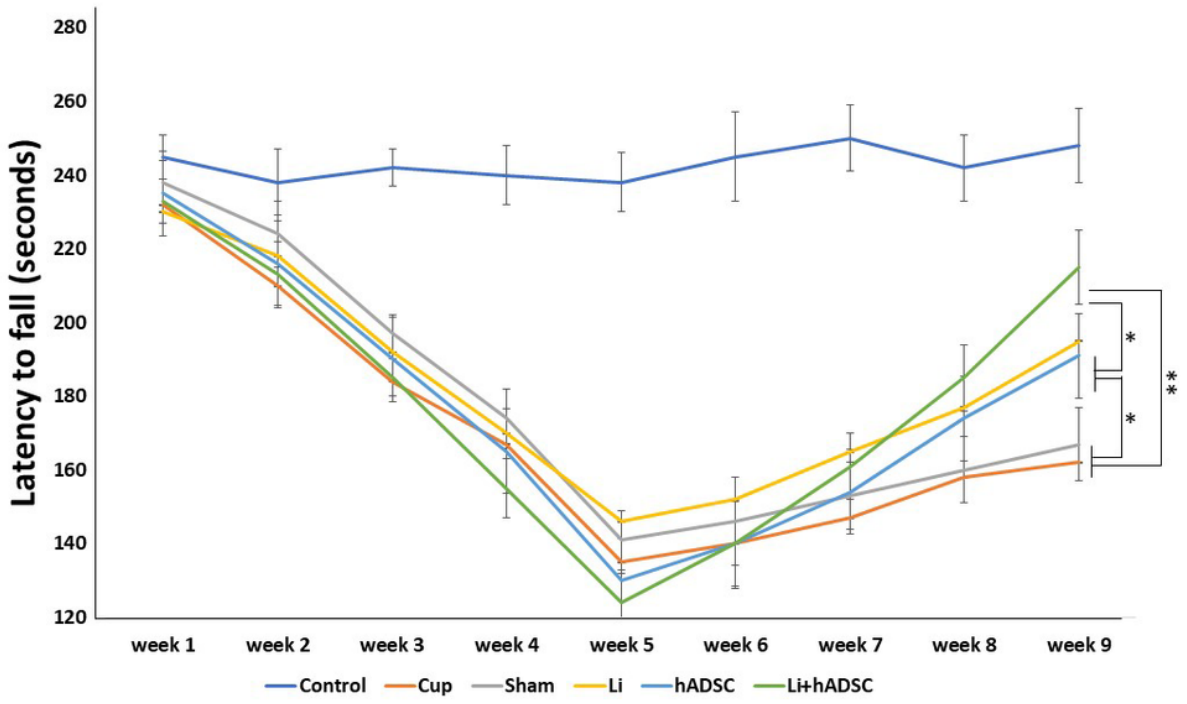

Figure 3

Hanging wire test. Effects of Li, hADSC and Li+hADSC on mice motor function. Simultaneous administration of Li and hADSC had the best effect on motor function improvement. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC), and Li+hADSC groups. *P < 0.05, **P < 0.01.

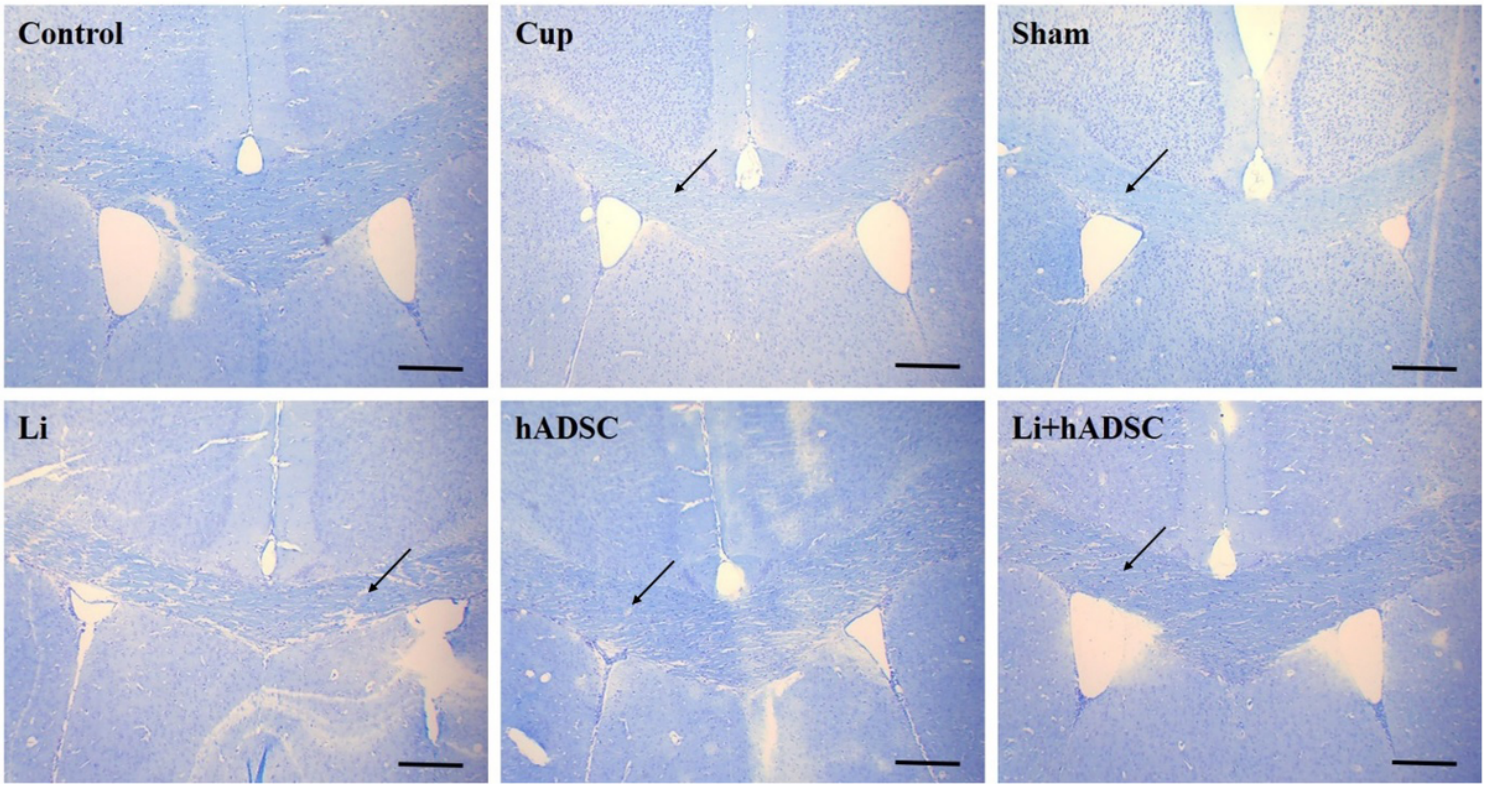

Figure 4

Toluidine blue staining in mouse brain sections. Arrows indicate clear demyelination in the corpus callosum of the Cuprizone and Sham groups as well as re-myelination in treatment groups especially in Li+hADSC group. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC) and Li+hADSC groups. Scale bar = 300μm

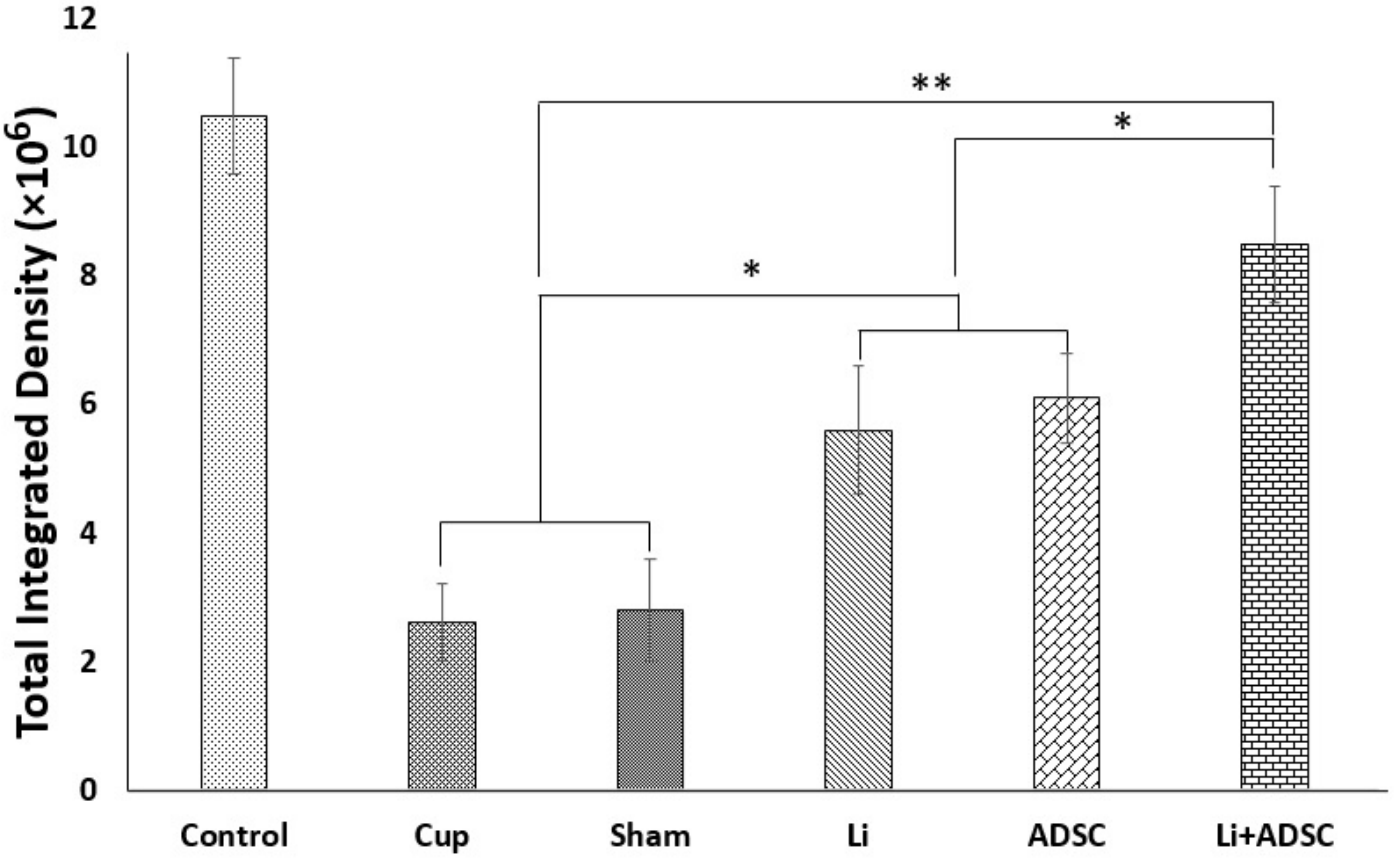

Figure 5

The total integrated density of myelin calculated by ImageJ software. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC) and Li+hADSC groups. *; P<0.05.

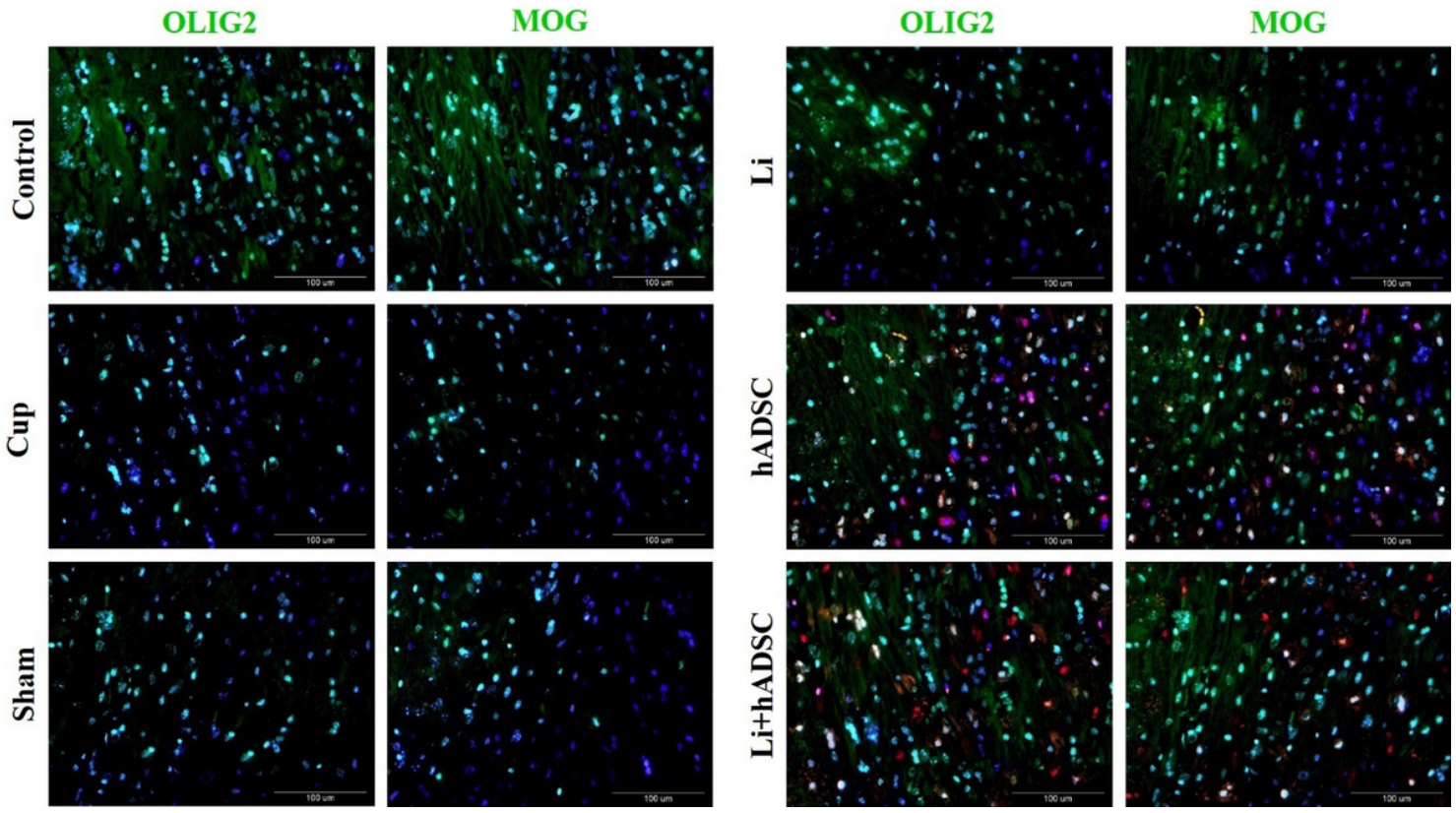

Figure 6

Immunofluorescent images of oligodendrocyte markers. hADSCs pre-labeled with PKH26 (red) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). The PKH26 positive transplanted cells, presented Olig2 and MOG markers. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC), and Li+hADSC groups. Scale bar = 100μm

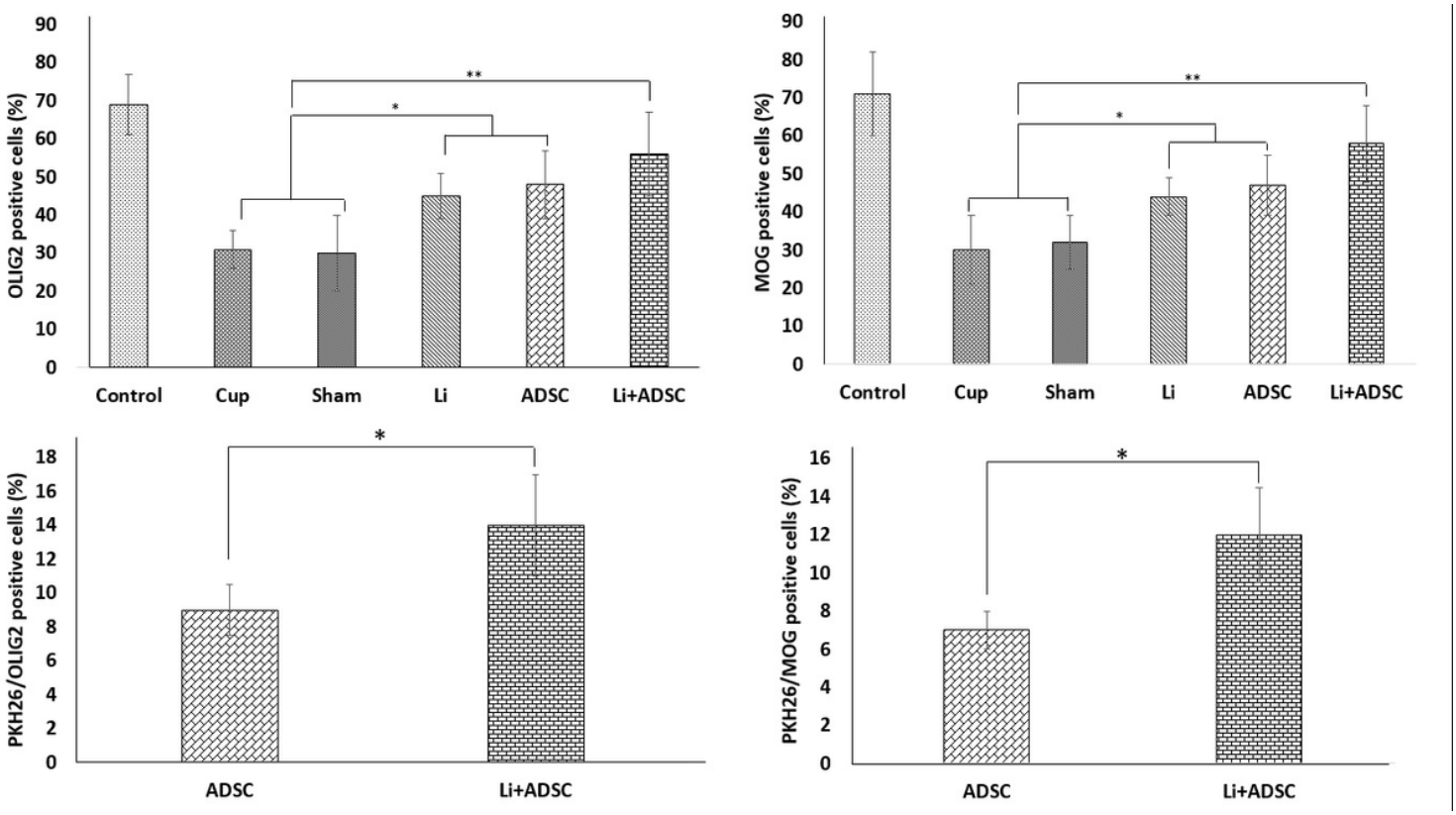

Figure 7

The comparison of OLIG2 and MOG expression in all experimental groups. In the treated groups, the mean percentage of cells which express OLIG2 and MBP markers was statistically significant in comparison with Cuprizone and sham groups (*P<0.05, ** P<0.01).

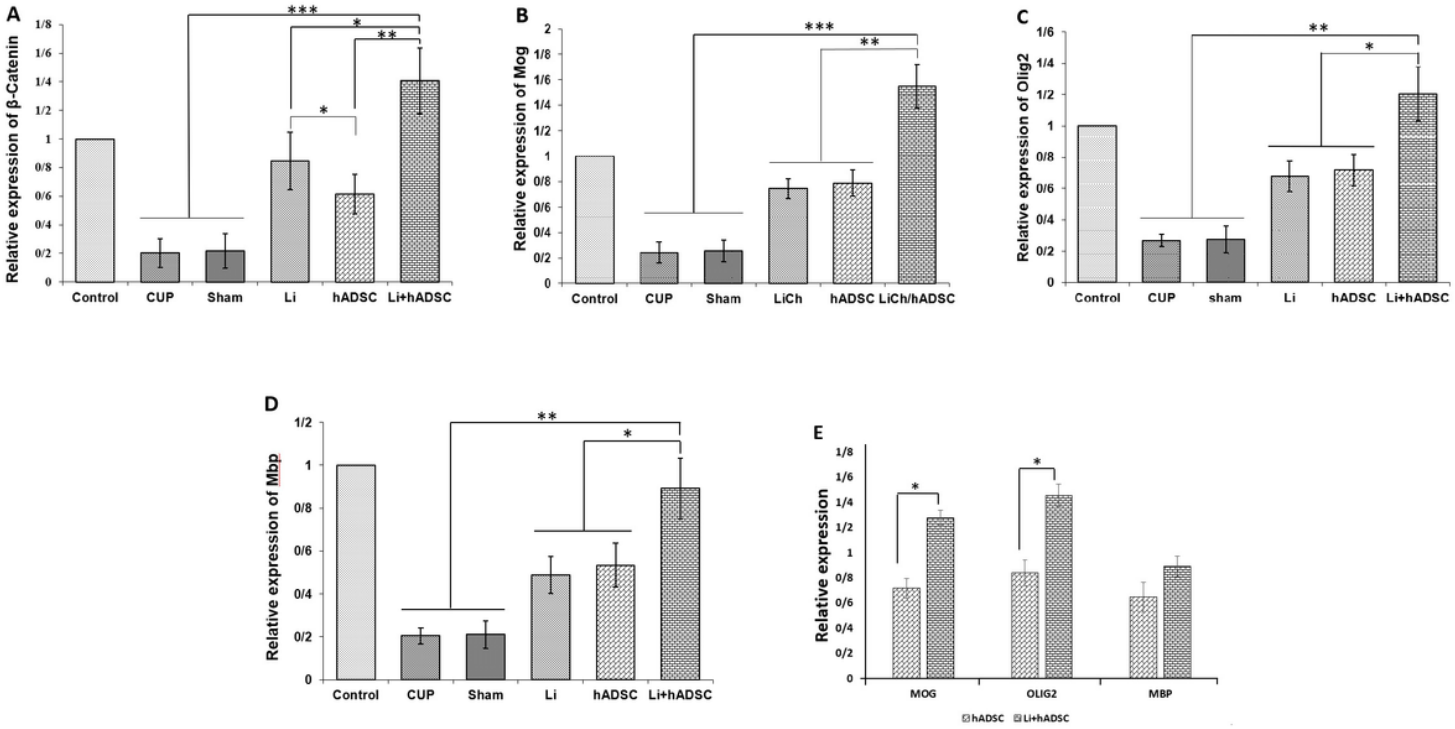

Figure 8

Expression of β-Catenin, myelin and oligodendrocytes specific genes in corpus callosum. Quantitative RT-PCR data revealed a significant upregulation of Mbp, Olig2 and Mog in treated groups compared with Cup and sham groups. E shows the expression of human MBP, OLIG2 and MOG in hADSC and Li+hADSC treated groups. Cuprizone (Cup), Lithium Chloride (Li), human Adipose Derived Stem Cell (hADSC), and Li+hADSC groups. (*P < 0.05, **P < 0.01, ***P < 0.001).

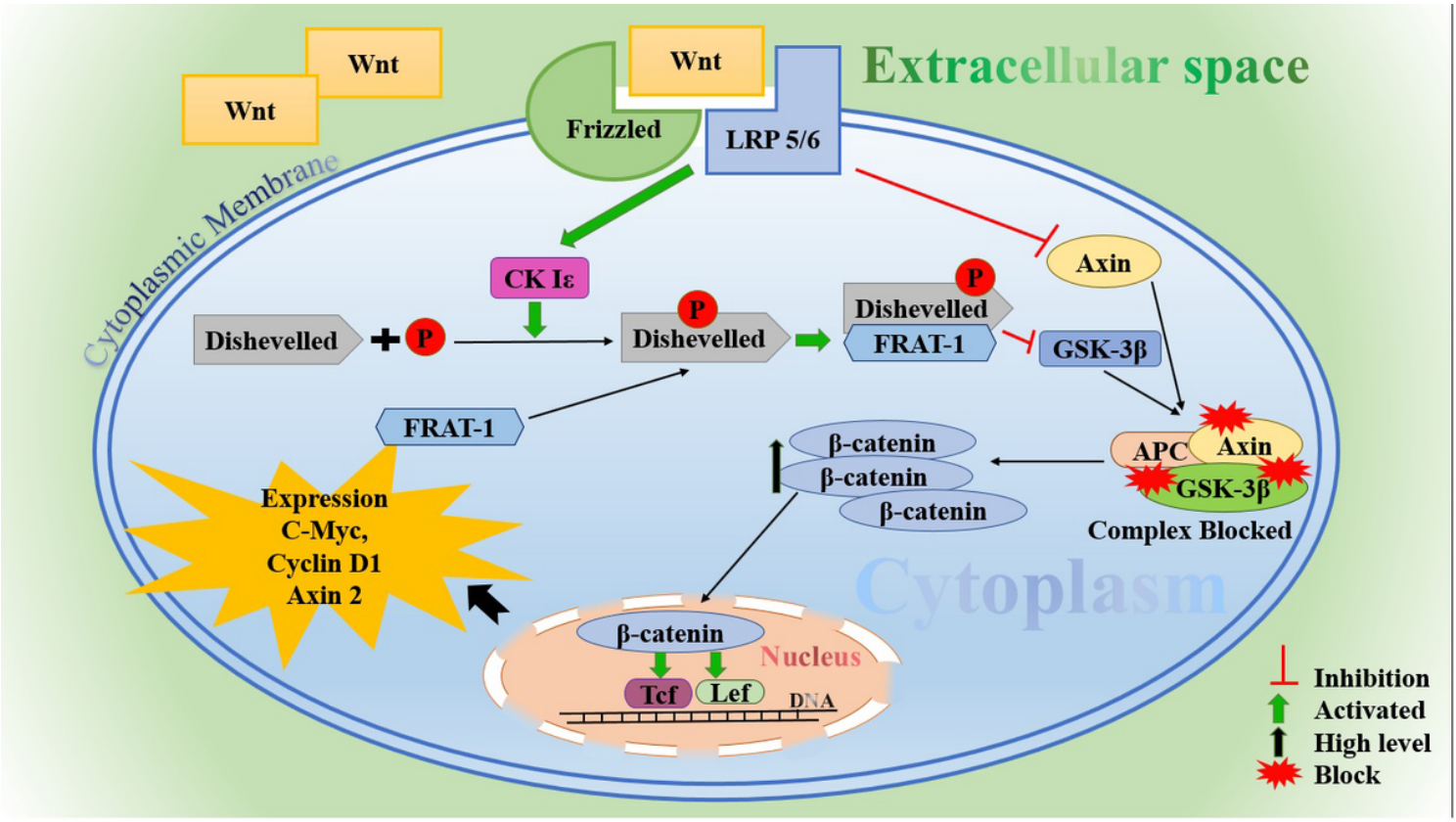

Figure 9

The Wnt signaling pathway, be started with the binding of the Wnt ligand to the Frizzled transmembrane receptor and the co-receptor LRP-5/6. In the following, the Wnt-Frizzled-LRP5/6 complex formation causes recruitment of Dishevelled (DVL). Thus, casein kinase Iε (Ck Iε) will phosphorylates DVL and inhibits GSK-3β activity due to complexation with Frat1. In addition, Axin degradation is performed by LRP5/6 in the Wnt-Frizzled-LRP5/6 complex. As a result, the formation of the GSK-3β, Axin, and adenomatous polyposis coli (APC) complex blocked and GSK-3β inhibition occurs. The inhibition of this complex activity leads to inhibition of β-catenin phosphorylation and increase cytoplasmic concentration of β-catenin. Once β-catenin enters the nucleus, as a transcription factor, it activates nuclear complexes consisting of the Tcf / Lef transcription factor. These events lead to the initiation of transcription and expression of target genes such as c-Myc, cyclin D1 and Axin 2. In the absence of Wnt ligand, β-catenin phosphorylation occurs due to the formation of GSK-3β, Axin and APC complexes and gene expression will be stopped.