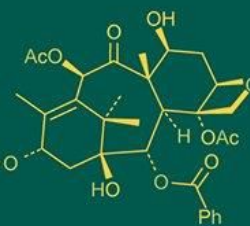


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
NAAS Rating(2026): 5.29  
IJABR 2026; 10(3): 415-424  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 15-01-2026  
Accepted: 17-02-2026

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## Evaluation of the neuro-modulatory potential of odontogenic derived mesenchymal stem cells - conditioned medium against cuprizone-induced multiple sclerosis in mice

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3f.7885>

### Abstract

**Background:** Neurodegenerative diseases are a major cause of global morbidity and mortality and are characterized by demyelination, axonal damage, and neuronal death mainly caused by neuroinflammation and oxidative stress. In multiple sclerosis (MS), immune cell infiltration and chronic inflammatory responses disrupt the blood-brain barrier and exacerbate neurodegeneration. Odontogenic mesenchymal stem cell-conditioned medium(OMSC-CM) is a cell-free therapeutic approach that supports neuronal survival and tissue regeneration, making it a promising strategy for treating neuroinflammatory conditions such as MS.

**Aim:** To evaluate the neuromodulatory and immunoregulatory potential of odontogenic-derived mesenchymal stem cells-conditioned medium in a cuprizone-induced MS model in C57BL/6J mice by estimating catalase(CAT) activity, interleukin-6(IL-6), and glial fibrillary acidic protein(GFAP).

**Materials and Methods:** Thirty male C57BL/6J mice were divided into 3 equal groups control, MS model, and odontogenic-derived mesenchymal stem cells-conditioned medium. MS induction was done by cuprizone in diet and confirmed by motor clinical assessment, body weight measurement, and histopathological examination. GFAP and IL-6 were measured using ELISA, while catalase activity was determined by a colorimetric method.

**Results:** The group treated by odontogenic-derived mesenchymal stem cells-conditioned medium showed significantly reduced GFAP and IL-6 levels and increased catalase activity compared with the MS model group.

**Keywords:** Odontogenic derived- mesenchymal Stem cells- CM exerts a therapeutic effect in MS

### Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune demyelinating disease of the central nervous system and a common cause of disability in young adults (Carotenuto *et al.*, 2022) <sup>[11]</sup>.

In MS, inflammation and demyelination drive astrocytic proliferation, leading to the formation of glial scars. The increase of the intermediate filament protein glial fibrillary acidic protein (GFAP) is a marker of this reactive process (García-Domínguez, 2025) <sup>[20]</sup>.

In MS, the blood-brain barrier is compromised, peripheral immune infiltrate CNS with lesions in the brain and spinal cord. Pro-inflammatory cytokines disturb the blood brain barrier (BBB). Activated macrophages and microglia worsen tissue damage by releasing oxidative burst mechanisms, reactive oxygen species, and proinflammatory cytokines (Woo *et al.*, 2024).

Astrogliosis is a stress-induced reaction in the central nervous system that causes the release of GFAP and its breakdown products into the cerebrospinal fluid and circulation. Accordingly, GFAP levels in CSF and blood (Park *et al.*, 2024) <sup>[47]</sup>.

Interleukin-6 plays a pivotal role in neuroinflammation by activating astrocytes, which attract immune cells to injured sites by secreting pro-inflammatory cytokines and chemokines. IL-6 also induces structural changes in astrocytes, amplifying the inflammatory response (Kerkis *et al.*, 2024) <sup>[30]</sup>

and activation of microglial lead to produces inflammatory mediators and reactive oxygen species (Zhang *et al.*, 2024). Several studies have reported a substantial reduction in catalase activity in MS patients and experimental MS models, including cuprizone-induced demyelination (Abo Taleb and Alghamdi, 2020; Ghahremani *et al.*, 2025) [1, 22]. This reduction in CAT reflects impaired antioxidant defenses and excessive generation of ROS (Omotoso *et al.*, 2018) [46] which oxidatively damage lipids, proteins, and nucleic acids (Shiri *et al.*, 2021) [58].

Dental pulp stem cells(DPSCs) originate from the ecto-mesenchyme during embryonic development, making them more potent than other MSC sources such as bone marrow(Grawish *et al.*, 2021) [24]. In addition they can also differentiate into neurogenic cell lineages (Salkin *et al.*, 2022) [54].

Conditioned medium (CM) derived from stem cells may offer superior therapeutic advantages over exosome. It is rich in potent immunomodulatory and anti-inflammatory cytokines (Kim *et al.*, 2025), [31] in addition to a wide variety of bioactive compounds, including hormones, chemokines, cytokines, proteins, nucleic acids, and lipids (Ventura *et al.*, 2025) [64].

## Materials and methods

### Materials

#### Chemicals

**Bis-cyclohexanone-oxaldihydrazone (Cuprizone):** White to off white powder,(purity  $\geq 95\%$ ; CAS no. 370-81-0, Product number C9012), was purchased from(Sigma-Aldrich), administrated via dietary supplementation, by

mixing with standard mice chow at concentration of 0.2%(w/w).

### Animals & ethical approval

The present study was carried out on 30 male C57BL/6J mice, weighted 40 to 50 gm, housed in cages and were given free access to water and food. All animal care and experimental methods were carried out in accordance with the guidelines of ethical committee of Medical Research, Faculty of Medicine, Tanta University, Egypt(Approval no 36264MS664/8/24).

### Experimental design and animal grouping

After being acclimatized for one week, thirty mice were categorized randomly into three equal groups: Group I(control): Normal mice were fed standard mice chow. Group II (MS-model): mice were fed standard mice chow mixed with 0.2% cuprizone (w/w) for 6 weeks. Group III (Cuprizone treated with Odontogenic derived- mesenchymal Stem cells- Conditioned media): Mice were fed standard mice chow mixed with 0.2% cuprizone (w/w), After confirmation of the MS model development by assessment of behavioral, motor test and histopathological examination of the brain tissue, the animals were injected by 500  $\mu$ l of P2 odontogenic MSCs-CM (200  $\mu$ g/ml) intravenously in the tail vein once weekly for 3 successive weeks. After one week of the end of the experiment (one week after the last dose of CM), mice were reassessed by behavioral, motor test and the mice were euthanized by injecting 30 mg/kg of pentobarbital intraperitoneally and blood and brain tissues were collected.

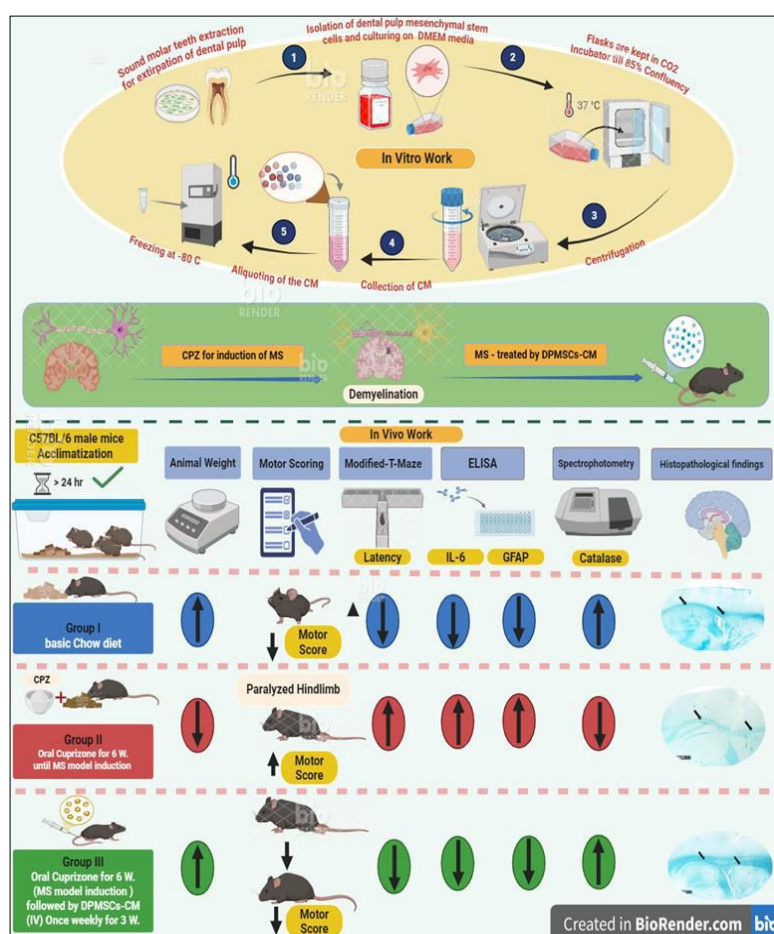


Fig 1: Flow chart of the study.

## Methods

**In vitro work:** Isolation, culturing and identification of dental pulp-mesenchymal stem cells (BM-MSCs) in culture (Aziz et al., 2008) [5]

**a) Isolation and culturing of DP-MSCs (enzymatic digestion):** Dental pulp was isolated from disinfected healthy permanent teeth, enzymatically digested with collagenase, and centrifuged to obtain cells. The cells were cultured in DMEM with fetal bovine serum under standard conditions, with regular medium changes. Spindle-shaped cells appeared within 5-10 days, and second-passage cells at 80-85% confluence were used for experiments (Aziz *et al.*, 2008; Gatti *et al.*, 2011) [5, 21].

**b) Characterization of DP-MSCs:** Characterization of cultured MSCs was done using morphology: MSCs exhibited a spindle-shaped, fibroblast-like appearance in culture. Plastic adherence, and Phenotypic Fluorescence-Activated Cell Sorting (FACS): using the criteria of CD105 positive and CD45 negative (Tondreau *et al.*, 2004; Dominici *et al.*, 2006; Mageed *et al.*, 2007; Szatanek *et al.*, 2017) [17, 38, 62, 63].

### c) Preparation of Mesenchymal Stem Cells (MSCs)-CM

At 80-85% confluence, cells were washed, trypsinized, and centrifuged to obtain first-passage cultures, which were then re-cultured under standard conditions. After reaching confluence again, cells were washed and incubated in serum-free, low-glucose, low-oxygen DMEM for 48 hours to produce conditioned medium. The collected medium was centrifuged, filtered, aliquoted, stored at  $-80^{\circ}\text{C}$  (Simundic, 2014) [59], and its total protein content was measured using the Bradford assay (Bradford, 1976) [8]. All CM preparations were obtained from DP-MSCs at the 2nd passage and under identical culture conditions to minimize batch-to-batch variability (Dominici *et al.*, 2006; Pawitan, 2014) [48]. Cell viability was confirmed at the time of CM collection (Strober, 1997) [61].

**In vivo work:** Animal groups were assessed three times for body weight, motor clinical scoring, assessment working memory by Delayed-non-matching to sample (DNMS) modified T-maze test, biochemical and histopathological changes; at the start of experiment, after induction of MS model to confirm the demyelination and after treatment period.

**Tissue sampling:** After the animals were sacrificed, brain tissues were carefully dissected, weighed, rinsed three times with ice-cold saline, blotted on ash-free filter paper, and cooled on ice. Tissues were fixed in 10% neutral buffered formalin for 72 h, processed through graded ethanol (95%), cleared in xylene, and embedded in Paraplast. Sagittal sections (5  $\mu\text{m}$ ) were cut using a rotary microtome and stained with Harris Hematoxylin and Eosin (H&E) for general examination and Luxol Fast Blue (LFB) to quantify myelinated fibers in the corpus callosum, following the protocol of Culling, 2013 [14].

**Microscopic analysis:** At least six random fields from each corpus callosum sample were analyzed for LFB-positive myelinated fibers. Images were captured using a Leica Full

HD microscope and quantified with Alpha-Imager and Odyssey scanners (Mustafa et al., 2024) [41].

## Biochemical assessment

- The GFAP level and IL6 levels in blood were evaluated by enzyme-linked immunosorbent assay. A commercially available GFAP and IL-6 assay kit (Cat. Nos. 201-11-0971 and 201-12-0091) respectively; was obtained from Sun Red Biotechnology Company Ltd., China.
- Level of catalase enzyme in plasma was determined through colorimetric method by commercial assay kit supplied by bio-diagnostic company, Egypt.

**Statistical analysis:** The data were presented as mean  $\pm$  standard deviation (SD), and p-values were calculated. Differences among groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value less than 0.05 was considered significant.

## Results

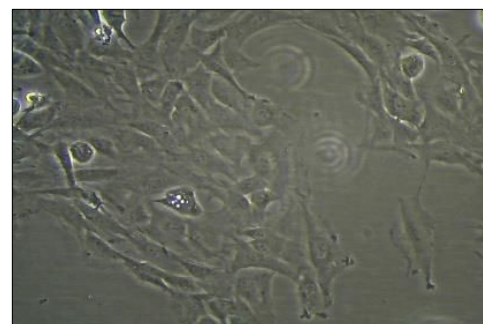


**Fig 2:** Spindle fusiform fibroblast like MSCs.

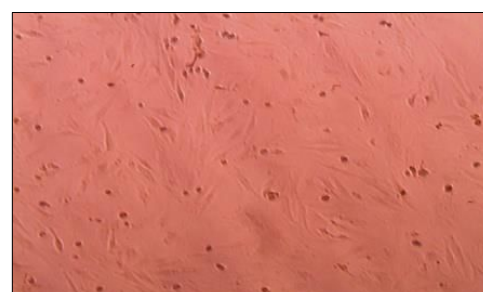


**Fig 3:** MSCs with 30% confluency

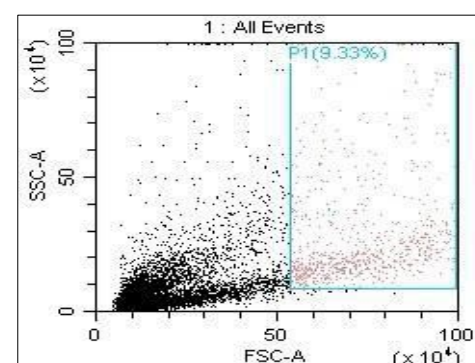


**Fig 4:** Flow cytometry dot plot (2d scatter graph) for characterization of MSCs, show a positive surface marker for CD105 and negative CD45

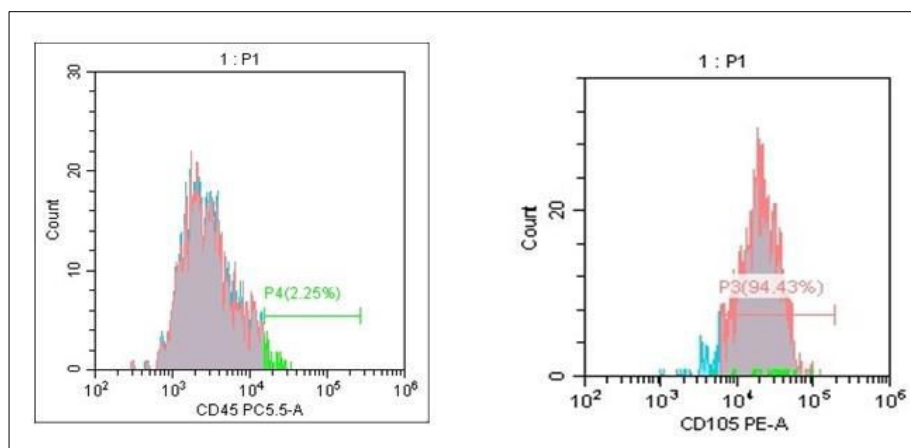


**Fig 5:** MSCs flow cytometric analysis

### Phenotypic FACS- histogram analysis

MSCs are positive for CD105 (94.43%), but negative for surface marker CD45 (2.25%)

### Body weight, motor clinical scoring, and behavioral test

Administration of cuprizone resulted in significant decrease in weight in addition to significant motor impairments progressed to hindlimb paralysis and even death in group II (MS-model) when compared to other groups ( $P < 0.001$ ). These deficits are commonly assessed using motor clinical scoring, with a widely used scale ranges from 0 to 5 described by Aharoni *et al.* (2019) [3]. Notably, these neurological deficits were significantly ameliorated in group III after treatment with odontogenic-derived mesenchymal stem cell conditioned medium injection compared to group II ( $P < 0.001$ ) (Fig 6) (Table1).

Administration of cuprizone significantly impaired cognitive function, particularly working memory in group II

(MS-model) when compared to other groups. A significant decrease in number of correct response and increase in latency (in seconds) in the delayed non-matching to sample (DNMS) modified T-maze test indicated this impairment. These deficits were statistically significantly improved in Group III following treatment with odontogenic-derived mesenchymal stem cell-conditioned medium(MSC-CM) injection compared with Group II(MS-model) ( $P < 0.001$ ) (Fig 7) (Table 1).

A substantial increase in neuroinflammation and oxidative stress was seen after cuprizone administration. The MS group had the highest astrocyte activation levels. The results showed that catalase activity decreased, GFAP and IL-6 levels increased, all of which are indicators of inflammation. The CM treated group had a significantly lower level of GFAP and IL-6, and higher level of catalase activity after injecting Odontogenic derived-mesenchymal Stem cells-CM compared to group II ( $P < 0.001$ ) (Fig 8) (Table 2).

**Table 1:** Comparison regarding weight, motor clinical scoring and number of correct response and latency in(DNMS) modified T-maze test among the studied groups

|                                                           | Groups                         |                               |                                           | ANOVA/<br>tukey's test F P |
|-----------------------------------------------------------|--------------------------------|-------------------------------|-------------------------------------------|----------------------------|
|                                                           | Group I(control group)(n = 10) | Group II (MS-model)<br>(n=10) | Group III (odontogenic MSCs-CM)<br>(n=10) |                            |
| Weight                                                    | 105.8 ± 4.42                   | 27.70 ± 3.23                  | 78.10 ± 5.90                              | 726.601* <0.001*           |
| Motor scoring                                             | 0.0 ± 0.0                      | 3.80 ± 0.79                   | 1.60 ± 0.52                               | 122.850* <0.001*           |
| number of correct responses in(DNMS) modified T-maze test | 5.50 ± 0.53                    | 2.60 ± 0.52                   | 4.50 ± 0.53                               | 79.176* <0.001*            |
| Tukey's test: P1 < 0.001*, P2 < 0.001*, P3 < 0.001*       |                                |                               |                                           |                            |
| latency in(DNMS) modified T-maze test                     | 18.90 ± 3.75                   | 59.20 ± 3.55                  | 22.80 ± 3.22                              | 399.257* <0.001*           |
| Tukey's test: P1 < 0.001*, P2 = 0.049*, P3 < 0.001*       |                                |                               |                                           |                            |

P1: I vs II, P2: I vs III, P3: II vs III

### Statistically significant p value < 0.05

Data are expressed as mean ± SD; n =number of mice per group.

Statistical analysis is carried out using one-way ANOVA followed by Tukey's test.

**Table 2:** Comparison regarding levels of GFAP, levels of IL-6 and catalase activity among groups

|                                                    | Groups                           |                            |                                      | ANOVA/<br>tukey's test F P |
|----------------------------------------------------|----------------------------------|----------------------------|--------------------------------------|----------------------------|
|                                                    | Group I (control group) (n = 10) | Group II (MS-model) (n=10) | Group III(odontogenic MSCs-CM)(n=10) |                            |
| GFAP(ng/ml)                                        | 19.76 ± 2.65                     | 98.26 ± 8.62               | 21.82 ± 2.21                         | 697.011* <0.001*           |
| Tukey's test: P1 < 0.001*, P2 = 0.669, P3 < 0.001* |                                  |                            |                                      |                            |
| IL-6(pg/ml)                                        | 3.49 ± 0.58                      | 30.92 ± 6.34               | 3.58 ± 0.59                          | 183.346* <0.001*           |
| Tukey's test: P1 < 0.001*, P2 = 0.999, P3 < 0.001* |                                  |                            |                                      |                            |
| Catalase activity(U/L)                             | 92.12 ± 7.58                     | 12.65 ± 1.63               | 87.93 ± 2.08                         | 930.238* <0.001*           |
| Tukey's test: P1 < 0.001*, P2 = 0.127, P3 < 0.001* |                                  |                            |                                      |                            |

P1: I vs II, P2: I vs III, P3: II vs III

Statistically significant p value < 0.05

Data are expressed as mean  $\pm$ SD; n= number of mice per group.

Statistical analysis is carried out using one-way ANOVA followed by Tukey's test.

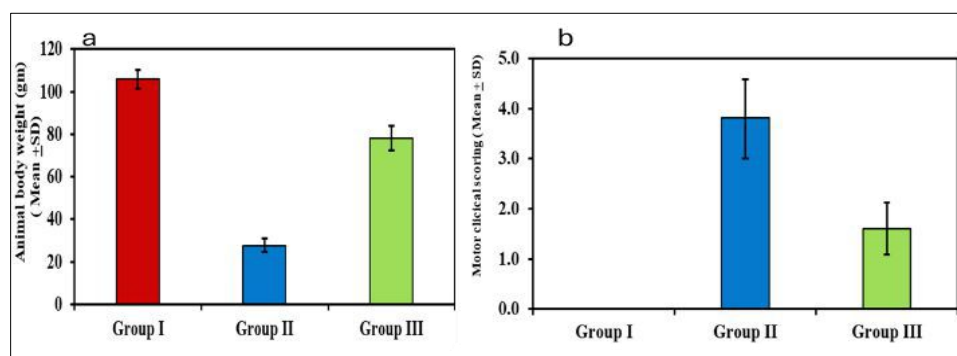


**Fig 6:** Comparison regarding (a) weight and (b) motor clinical scoring in different studied groups along the course of the study

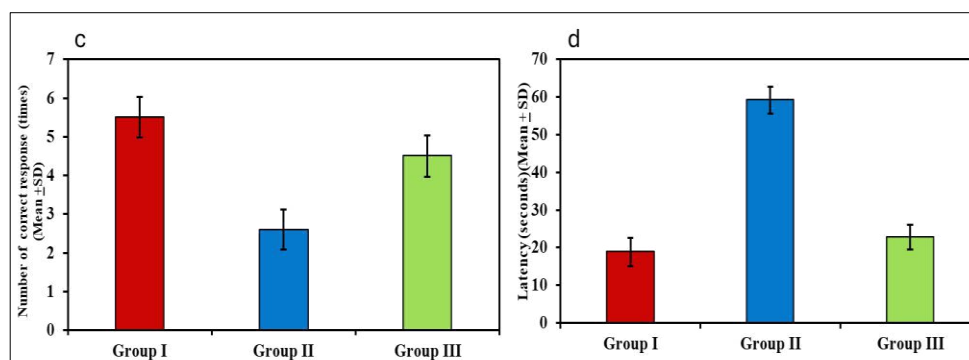


**Fig 7:** Comparison regarding (a) number of correct response and (b) latency in (DNMS) modified T-maze test in different studied groups along the course of the study

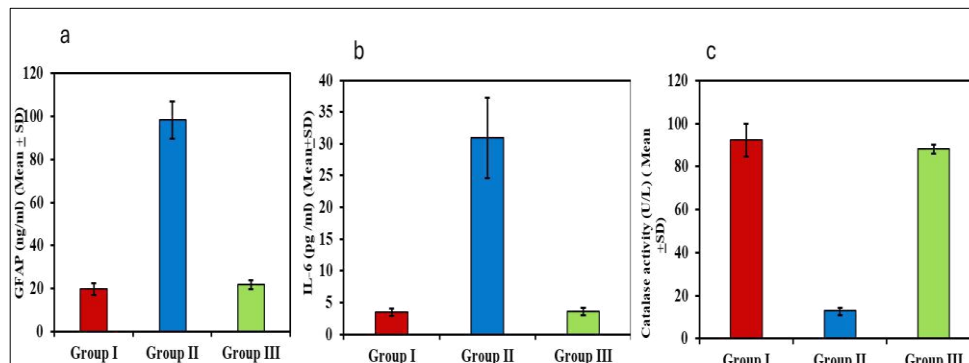


**Fig 8:** Comparison regarding (a) levels of GFAP, (b) levels of IL-6 and (c) catalase activity in different studied groups along the course of the study

**Histopathological results:** The biochemical and behavioral evaluations were supplemented by the analysis of H&E and LFB stained corpus callosum brain sections.

**H&E results (figure 9):** Control group demonstrated normal histological architecture of corpus callosum. It was formed of densely packed nerve fibers as well as normally organized oligodendrocytes with small dense nuclei arranged in short chains. There were number of glial cells with deeply stained nuclei (A&B). Cuprizone treated group showed disrupted histological architecture of corpus callosum with loosely arranged degenerated nerve fibers. There was decrease in the number of oligodendrocytes with scattered small dense pyknotic nuclei. In addition, the number of glial cells increased together with interstitial hemorrhage (C&D). Odontogenic derived mesenchymal

stem cell -CM treated group showed a significant improvement in the histological architecture of the corpus callosum with densely packed nerve fibers except for localized vacuolation of a few axons. There was increased number of oligodendrocytes with small dense nuclei arranged in short chains together with glial cells with deeply stained nuclei (E&F).

#### Luxol fast blue results (figure 10)

Control group demonstrated strong positive reaction in closely packed myelinated nerve fibers (A&B). Cuprizone treated group showed weak positive reaction in lightly myelinated nerve fibers (C&D). Odontogenic derived mesenchymal stem cell -CM treated group demonstrated strong positive reaction in closely packed myelinated nerve fibers more or less similar to control group (E&F).

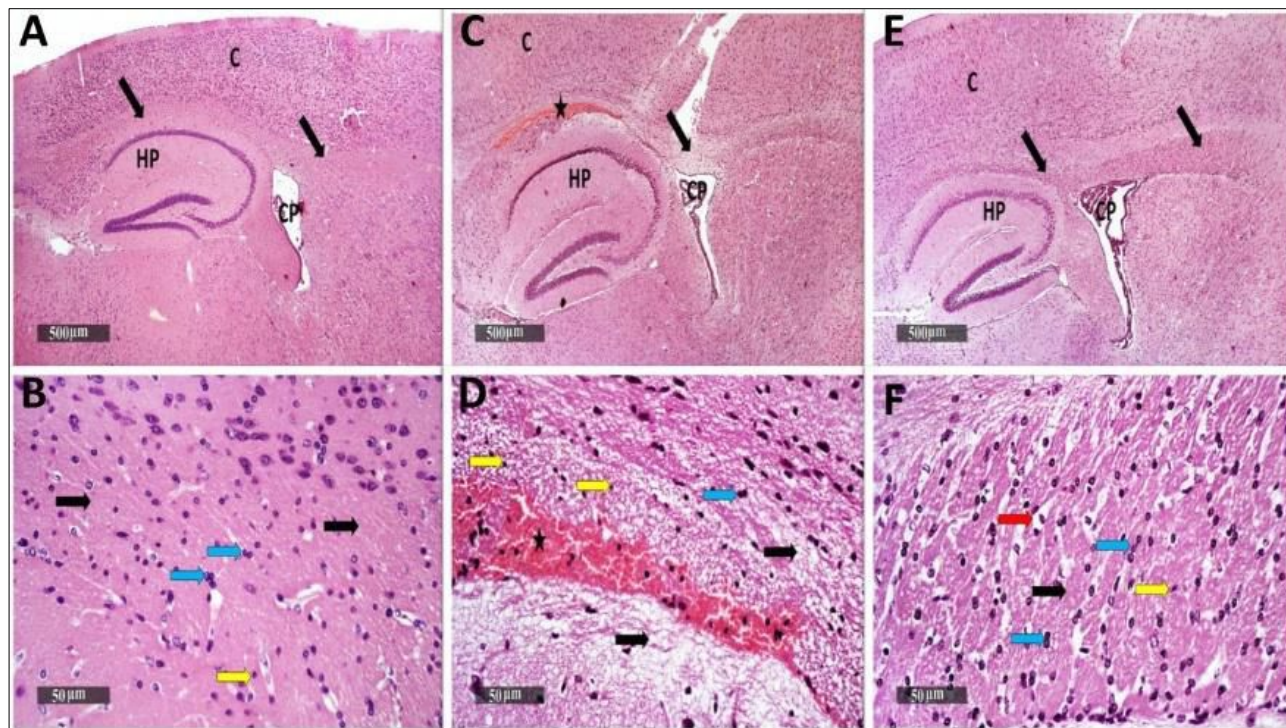


**Fig 9:** Photomicrographs of a section of corpus callosum of all studied groups by H&E.

Fig (9): photomicrographs of a section of corpus callosum of studied groups; Control group shows normal histological architecture of corpus callosum formed of densely packed axons, small dense nuclei of oligodendrocyte arranged in short chains and deeply stained nuclei of glial cells (A and B). Cuprizone treated group shows disrupted histological architecture with loosely arranged degenerated nerve fibers, decrease number of scattered small dense pyknotic nuclei of oligodendrocytes, increased number of glial cells and interstitial hemorrhage (C and D). Odontogenic derived mesenchymal stem cell -CM treated group shows

improvement of histological architecture of corpus callosum similar to control group except for focal vacuolation of some axons (E and F).

Densely packed axons (black arrows), vacuolation of axons (red arrows), oligodendrocyte (blue arrows), glial cells (yellow arrow), cerebral cortex (C), choroid plexus (CP), hippocampus.

**(HP) and interstitial hemorrhage**  
**(H&E, scale bar 500 and 50µm)**

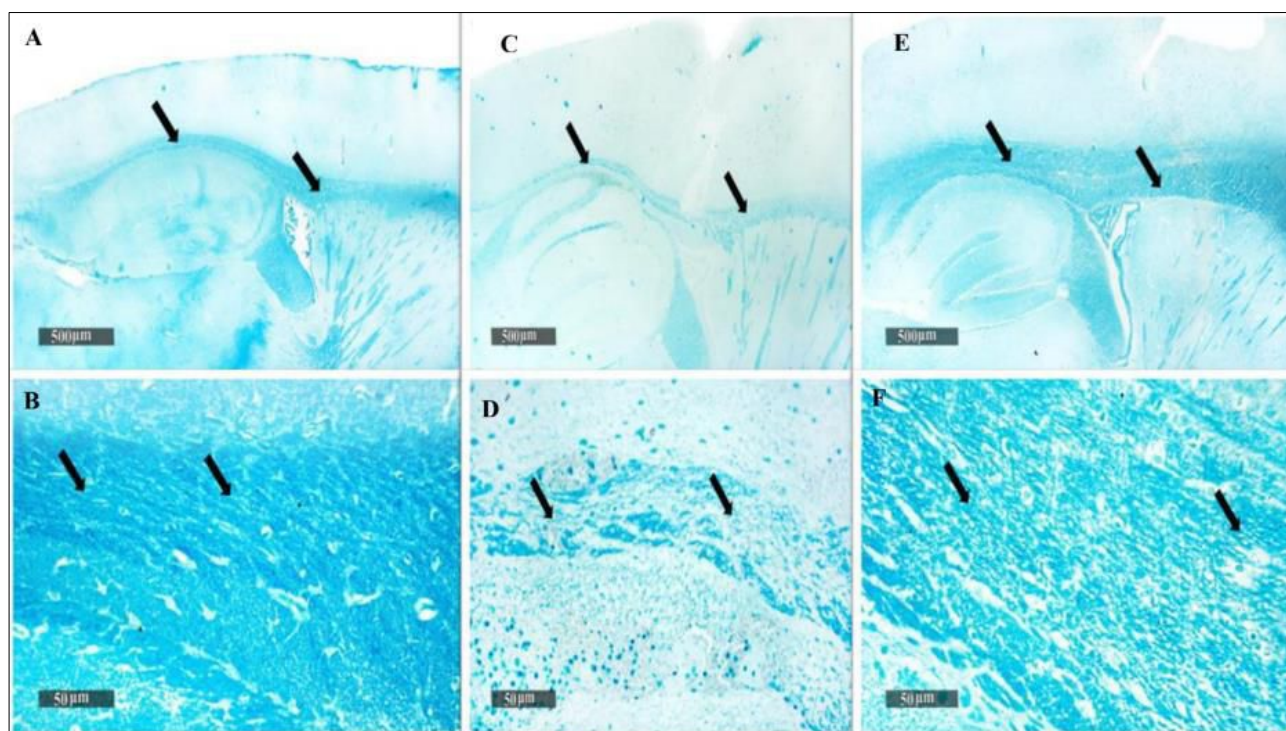


**Fig 10:** Photomicrographs of a section of corpus callosum of all studied groups by luxol fast blue

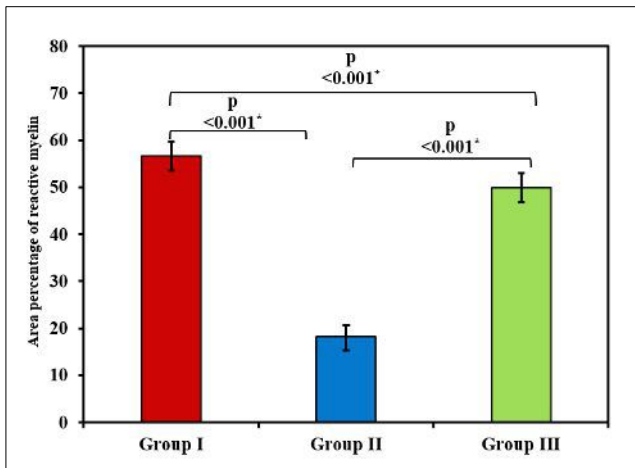


**Fig 11:** Comparison between the three studied groups according to the area of percentage of myeline nerve fibers -corpus callosum by LFB staining

### Figure 10

A photomicrograph of sections of corpus callosum of studied groups; Control group shows strong positive reaction in closely packed nerve fibers (black arrows) (A and B). Cuprizone treated group shows weak positive reaction in nerve fibers (black arrows) (C and D). Odontogenic derived mesenchymal stem cell -CM treated group shows strong positive reaction in closely packed nerve fibers (black arrows) more or less similar to control group (E and F).

### (Luxol Fast Blue, scale bar 500 and 50µm)

Figure(11) shows statistical analysis of the mean area% of myelinated nerve fibers showed that Cuprizone treated group revealed statistically highly significant decrease in the mean area percentage of myelinated nerve fibers compared with control and CM treated groups CM treated group showed non-significant change in the mean area percentage of myelinated nerve fibers when compared with control group. There was a highly significant increase in the mean area percentage of myelinated nerve fibers in control and odontogenic mesenchymal stem cell -CM treated groups compared to the Cuprizone treated group.

### Discussion

Multiple sclerosis(MS) is a chronic neuroinflammatory and neurodegenerative disorder characterized by immune-mediated demyelination, axonal damage, astrocytic activation and oxidative stress. The Cuprizone model is a well-established non-autoimmune experimental model widely used to study demyelination and remyelination mechanisms in MS (Mey *et al.*, 2023; Mirmosayyeb *et al.*, 2022; Nicola *et al.*, 2024; Oliva Ramirez *et al.*, 2021) [39, 40, 44, 45]. In this study, the neuro-modulatory and immunoregulatory potential of odontogenic mesenchymal stem cell-conditioned medium was investigated using CPZ-induced mouse model of MS. Their effects are mediated by secretome which includes cytokines, growth factors, enzymes, lipids and extracellular vesicles such as exosomes (Hade *et al.*, 2021) [28]. Even though exosome-based methods have been extensively researched, CM provides better therapeutic benefits because of its composition of bioactive ingredients that can target several disease pathways (Das and Sloan, 2023; Ghasemi *et al.*, 2023) [16, 23].

In the present study, cuprizone administration successfully induced an MS-like demyelinating phenotype characterized by body weight loss, increased motor clinical scores, and neurological impairments, consistent with previous reports (Li *et al.*, 2019; Aharoni *et al.*, 2019) [3, 36]. These effects are mainly attributed to cuprizone's copper-chelating action, which disrupts oligodendrocyte function, increases oxidative stress, and induces demyelination (Zirngibl *et al.*, 2022; Yong and Yong, 2022).

Neurobehavioral assessment using the modified T-maze showed reduced correct responses and prolonged latency in cuprizone-treated mice, indicating cognitive and motor impairment related to cortico-hippocampal damage and neuroinflammation (Navarro *et al.*, 2023; Omotoso *et al.*, 2018) [43, 46]. Treatment with odontogenic mesenchymal stem cell-conditioned medium significantly improved body weight and neurobehavioral performance, consistent with previous studies demonstrating functional recovery following MSC-derived secretome therapy (Zhang *et al.*, 2022; Brown *et al.*, 2021; Farinazzo *et al.*, 2018) [10, 19].

On a histopathological level, brain tissue examination of MS mice (group II) using hematoxylin and eosin (H&E) and Luxol Fast Blue (LFB) revealed disorganized morphology in the midbody zones of the corpus callosum, with demyelination of nerve fibers and significant oligodendrocyte loss. These findings are consistent with previous studies (Pyka-Fosciak *et al.*, 2023; Hassani, 2021) [29, 50], which reported axonal damage, myelin loss, and inflammatory lesions in MS models. The observed demyelination can be explained by the recruitment of activated CD4 T cells and monocyte-derived macrophages, which phagocytize myelin and promote inflammatory lesions (Hassani, 2021) [29].

OMSC-CM-treated mice (group III) showed improved myelin integrity, higher nerve fiber density, and partial restoration of normal corpus callosum architecture, with abundant oligodendrocytes and minimal focal infiltrates. These results align with earlier findings of (Kråkenes *et al.*, 2023; Gugliandolo *et al.*, 2021) [26, 34] and are likely due to MSCs' ability to migrate to affected brain regions, reduce inflammation, and enhance myelin content. Additionally, MSC-derived extracellular vesicles secrete neurotrophic factors and exert paracrine effects on subventricular zone oligodendrocytes, promoting oligodendrogenesis, remyelination, and oligodendrocyte progenitor differentiation (Cruz-Martinez *et al.*, 2016) [13]. Secretome components also likely downregulate microglial inflammatory activity, contributing to structural recovery in the corpus callosum (Kråkenes *et al.*, 2023) [34].

GFAP is a key marker of astrocyte activation and reactive astrogliosis, central to MS pathology. In CPZ-treated mice (group II), GFAP levels were significantly elevated compared to controls, consistent with previous studies (Azzolini *et al.*, 2022; Saraste *et al.*, 2021; Zahoor *et al.*, 2025) [6, 55]. This increase likely results from astrocyte structural and functional changes, BBB disruption, and glymphatic transport of GFAP into circulation (Dalenogare *et al.*, 2023) [15]. OMSC-CM group show significantly reduction in GFAP, indicating decreased astrocytic reactivity and restored homeostasis. These findings align with Allegrretta *et al.*, 2022 [4] and Laso-García *et al.*, 2018 [35] who reported that MSC-derived extracellular vesicles (EVs) reduce GFAP levels, reduce astrocyte hypertrophy, modulate inflammatory responses and promote a

neuroprotective astrocytic phenotype. CM may provide additional benefits over isolated EVs due to soluble factors that exert anti-inflammatory, neurotrophic, and angiogenic effects (Chen *et al.*, 2020) [12].

IL-6, a pleiotropic cytokine, promotes Th17 differentiation, inhibits regulatory T cells, activates astrocytes and microglia, and disrupts BBB integrity (Grebenciucova and VanHaerents, 2023) [25]. In group II, IL-6 was significantly elevated, reflecting a pro-inflammatory state, which is consistent with the findings of (Brown *et al.*, 2021; Haase and Linker, 2021) [10, 27]. This elevation contributes to glial scar formation and M1 microglial polarization, enhancing demyelination (Brambilla, 2019) [9]. OMSC-CM significantly decreased IL-6 levels, suggesting immunomodulatory effects, reduction of astrocyte/microglial activity, and potential remyelination, consistent with prior studies on MSC-CM and EVs (Selmaj *et al.*, 2017; Perets *et al.*, 2020; Müzes and Sipos, 2022; Kråkenes *et al.*, 2024) [33, 42, 49, 56].

CPZ-induced oxidative stress impairs antioxidant defenses by generating ROS that exceed the capacity of SOD and catalase (CAT), damaging myelin, mitochondria, and cytoskeletal proteins (Omotoso *et al.*, 2018; Shiri *et al.*, 2021) [46, 58]. In this study, catalase activity was significantly reduced in MS-model mice, consistent with previous reports (Ghahremani *et al.*, 2025; Esmaeilzadeh *et al.*, 2022; Abo Taleb and Alghamdi, 2020; Omotoso *et al.*, 2018; Kumar *et al.*, 2021) [1, 18, 22, 32, 46]. Astrocytes and other CNS and immune cells further contribute to ROS/RNS production, aggravating oxidative damage in demyelinating lesions (Brambilla, 2019) [9]. OMSC-CM treatment may help restore antioxidant balance and protect against ROS-mediated CNS damage.

In contrary, research by Shen *et al.*, 2024 [57] revealed that active MS lesions have been shown to destroy the oxidative system, as seen by the increase levels of glutathione, catalase, superoxide dismutase. The observed increase in catalase levels in multiple sclerosis may represent a compensatory antioxidant response to ongoing neuroinflammation and oxidative stress. (Baud *et al.*, 2004; Qi *et al.*, 2007) [7, 51].

According to the current study, catalase activity increased after OMSC-CM treatment, indicating that endogenous antioxidant capacity had been restored. MSCs have been proposed to reduce oxidative injury via scavenging free radicals, enhancing host antioxidant defenses and modulating the inflammatory response (Stavely and Nurgali, 2020) [60]. The antioxidative effects of MSCs often occur in a paracrine manner.

These findings support the potential of MSC-CM to modulate neuroinflammation effectively. The therapeutic benefits of MSCs in neurological disorders, including multiple sclerosis, are largely mediated through paracrine mechanisms.

Under conditions of excessive oxidative stress, as occurs in the cuprizone-induced MS model, hydrogen peroxide H<sub>2</sub>O<sub>2</sub> is markedly increased leading to allosteric and oxidative modifications of the catalase enzyme that impair enzyme kinetics and reduce catalytic efficiency, even when total enzyme protein levels are preserved or upregulated as a compensatory response.

Moreover, inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , and nitric oxide derivatives can further compromise catalase function through post-translational modifications causing

disruption of enzyme conformation, substrate binding, and turnover rate, resulting in decreased enzymatic activity independent of protein abundance.

Therefore, the reduced catalase activity observed in our MS model does not contradict earlier findings but rather complements them by highlighting that oxidative stress in MS impairs antioxidant defense at the functional level, even when antioxidant enzymes are upregulated. This functional insufficiency contributes to the persistence of oxidative damage and disease progression.

Restoration of catalase activity following therapeutic intervention would thus indicate not only enhanced antioxidant expression, but also recovery of enzyme functionality, representing a more meaningful indicator of redox homeostasis.

This study is among the first to demonstrate that mesenchymal stem cell-conditioned medium derived from teeth can serve as a cell-free therapy in a cuprizone-induced demyelination model. Unlike prior studies relying on direct stem cell transplantation or EAE models, this work combines a secretome-based approach with a non-immune demyelination paradigm. By simultaneously assessing oxidative stress (catalase), neuroinflammation (IL-6), astrocytic activation (GFAP), and histology, the study shows that dental stem cell-derived CM can modulate multiple MS-related pathological pathways, highlighting its translational therapeutic potential.

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