Original Article

Melatonin Potentiates the Curative Effect of Mesenchymal Stem Cells in Subcortical White Matter Tissues of Cuprizone Treated Mice Model of Multiple Sclerosis

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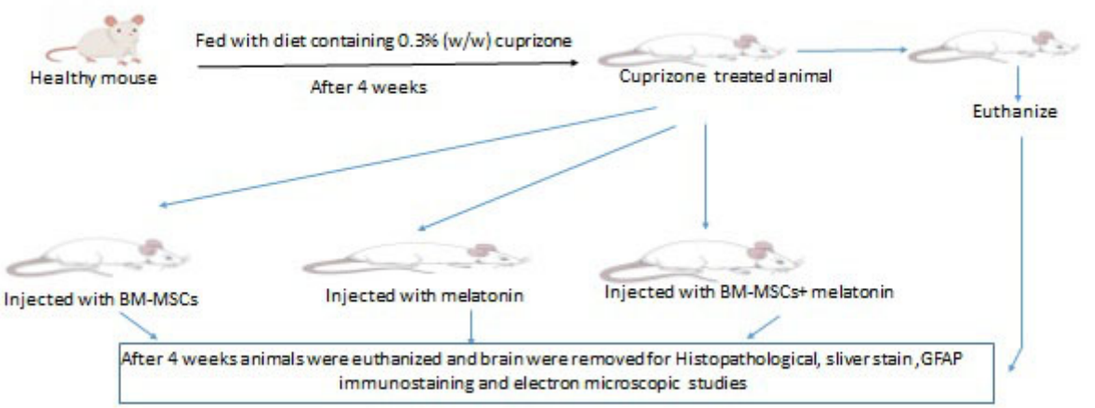
ABSTRACT

Aim of the Work: : The aim of the present study is to examine if melatonin may potentiate the positive effect of Bone Marrow Mesenchymal stem cells BM-MSCs for Induced Multiple Sclerosis (MS) model in subcortical white matter tissues

Materials and Methods: 30 mice were categorized into 5 groups. Group (1), control group. The animals of groups (2, 3, 4 and 5) were nourished with food comprising 0.3% cuprizone for 4 weeks for induction of the demyelination in subcortical white matter. Group (2) was directly euthanized after 4 weeks of cuprizone feeding. Group (3) the animals were intravenously injected via tail $1x10^6$ BM –MSCs and euthanized after 4 weeks. Group (4) the animals were given intraperitoneal daily doses (5 mg/kg) of melatonin and euthanized after 4 weeks. Group (5) the animals were given BM-MSCs, as in Group (3), and melatonin, as in Group (4), and then euthanized after 4 weeks.

Results: The histo-pathological examination of subcortical white matter tissues revealed that cuprizone induced sever alterations in fascicles with notable increase in glial fibrillary acidic protein (GFAP) immunoexpression (IE), loss in sliver impregnation satin, and ultrathin examination showed various morphological changes in myelin sheath. After treatment of animals with BM-MSCs definite improvement could be detected in white matter tissues. Moreover, melatonin administration ameliorates the negative effect of cuprizone. All the findings nearly regressed after coadministration of BM-MSCs + melatonin.

Conclusion: BM-MSc and/or melatonin have a potential remylination effect in MS mouse model.



Graphical Abstract

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Key Words: BM-MSc, cuprizone, melatonin, multiple sclerosis.

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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, neurodegenerative, demyelinating illness of unidentified etiology that occurs to the central nervous system (CNS). It is described by demyelination and axonal loss. MS is considered a significant cause for debility among adults (20- 45 years old)[1]. Now, there are immune-modulatory and anti-inflammatory drugs that are effective in particular types of MS but ineffective in other types. These regimens offer transitory efficiency by decreasing the annualized relapse degree, but patients suffer from side effects in progressive stage of MS[2]. Till now, there are no exact therapeutic agents for progressive types and no definite therapy for MS, mostly due to the mechanisms of pathogenicity are still indistinct and researchers have restricted access to samples from humane[3].

Biscyclohexanone-oxaldihydrazone is a chemical compound known as (cuprizone), it is a copper chelator compound that prompts cell death of oligodendroglia then demyelination, with astrocytes and microglia activation has been occurred. The initial experiment was done by Carlton who found that ingestion of cuprizone for 4-6 weeks stimulated oligodendrocytes destruction and demyelination in definite regions of the white matter in the brain of some rodents. Cuprizone action is achieved by disturbing the energy metabolism in the mitochondria of oligodendrocytes[4].

Under some circumstances, bone marrow mesenchymal stem cells (BM-MSCs) have the ability to multi-lineage distinguish into a numerous types of cells According to certain reports; mesenchymal stem cells (MSCs) can go through neuroectodermal development. MSCs can restore neurological impairments caused by a variety of CNS disorders, including brain injury as MS. Along with their capacity to differentiate and self-renew, stem cells also possess certain advantageous traits, such as the capacity for regeneration and immune system modulation[5].

Melatonin is a hormone generated by the pineal gland and several other organs that controls some of physiological processes, comprising sleep, circadian rhythms, and neuroendocrine effects. One of the strongest endogenous antioxidants is melatonin. It has antioxidant properties both directly and indirectly by removing reactive oxygen species (ROS), and indirectly by motivating many antioxidant enzymes, as an example catalase (CAT) and superoxide dismutase (SOD)[6]. Numerous investigations revealed that MSCs and neural stem cells expressed the melatonin receptors MT-1 and MT-2, which are responsible for the hormone's activity in stem cells. Additionally, it promotes the differentiation and proliferation of brain stem cells[7]. The purpose of the study is to examine the effects of BM -MSCs and melatonin on remyelination in mice as a paradigm of multiple sclerosis, both singly and in combination. In addition, it aims to investigate if melatonin might enhance BM -MSC activity in subcortical white matter tissues.

MATERIAL AND METHODS

Preparation of bone marrow derived – mesenchymal stem cell (BM-MSCs)

Stem Cell Research Unit, Biochemistry department, Qasr Al-Ainy, Faculty of Medicine, Cairo University providing stem cell isolation, culture according to[8] followed by identification according to[9].

Experimental design

30 male Swiss albino mice (7-8 weeks old) from the National Organization for Drug Control and Research (NODCAR) animal House were kept in plastic cages with unrestricted access to water, standard pellet food, and enough ventilation under normal circumstances at a temperature of 25°C (12°F). All animal procedures comply with the guidelines of the National Guide for the Care and Use of Laboratory Animals which was followed by the NODCAR Research Ethics Committee (NODCAR-REC/III/43/19).

Induction of demyelination

Animals were fed on diet containing 0.3% (w/w) cuprizone (Sigma, USA) to induce demyelination of nerve fibers (experimentally induced multiple sclerosis) for 4 successive weeks[10].

Mice were allocated into 5 groups, 6 mice for each.

Group (1): Control Group: The animals were nourished with standard food for 4 successive weeks directly euthanized and served as control.

Group (2): Cuprizone Treated Group: The animals were fed cuprizone, as previously mentioned, then directly euthanized after being fed on cuprizone.

Group (3): BM -MSCs Group: MS induced animals were intravenously injected with 100 µm of media containing $1x10^6$ BM -MSCs as a single dose[11] injected via the tail vein then euthanized 4 weeks later.

Group (4): Melatonin Group: MS induced animals were injected with an intraperitoneal daily dose (5 mg/kg) with melatonin according to[12] then euthanized after 4 weeks.

Group (5): BM-MSCs + Melatonin Group: MS induced animals were injected with combination of BM-MSc and melatonin then euthanized after 4 weeks.

Finally, the animals were euthanized under intraperitoneal anesthesia with sodium pentobarbital (50 mg/kg) and brain tissues were removed then exposed to the following studies: -

A. Hematoxylin and Eosin (H &E) for routine histological study according to[13].

B. Sliver impregnation stain for detection of nerve fiber according to[13].

C. Immunostaining of galial fibrillary acidic protein (GFAB) for astrocytes according to[14].

D. Electron microscopic study for detection of ultrastructure changes in myelin sheath according to[15] at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, used a JEOL-JEM 1010 transmission electron microscope to analyze stained sections at an electron vacuum of 80 KV.

E- Morphometric analysis

1. Counting the number of pyknotic nuclei of oligodendrocytes in the H&E stained sections. The measurements were done in 10 fields from 5 sections with power x400) of each rat of each group using the interactive measurements menu.
2. The number of oligodendrocytes in the sliver impregnation stained section. The measurements were done in 10 fields from 5 sections with power (x400) of each rat of each group using the interactive measurements menu.
3. Measurement of area % of the +ve GFPA stained section. The measurements were done in 10 fields from 5 sections with power (x400) of each rat of each group using binary mode.

Statistical analysis

The data obtained from different groups were compared for statistical significance using Graph Pad Prism (version 5.00 for Windows, Graph Pad software, San Diego California USA, www.graphpad.com). The differences among different groups were evaluated by one-way analysis of variance (ANOVA). Probability values lower than 0.05 were considered statistically significant ($P<0.05$).

RESULTS

Mesenchymal stem cells (MSC) in culture were identified by their adhesiveness and fusiform shape (Figure 1)

Histopathological results

Microscopic examination of the H&E stained section of subcortical white matter from control mice showed that the white matter formed mainly from well-organized nerve fibers and neuroglia cells (astrocytes, microglia and oligodendrocytes) (Figure 2 a,b), in contrast extensive histo-pathological changes, were seen in subcortical white matter tissues of the cuprizone treated group represented in multiple vacuolated areas in neurofibrillary network among nearby highly disorganized fibers and widespread cellular infiltrate mainly monocytes with congested and dilated blood vessels. Most of oligodendrocytes appeared with highly dense pyknotic nuclei. Also gliosis (microglosis and astrogliosis) could be detected (Figure 2 c-h). When animals were injected with BM-MSCs, remarkable improvement was observed in subcortical white matter tissues where mild vacuolated area and localized cellular infiltrate among the organized nerve facials were observed (Figure 3 a,b). After treatment with melatonin, the subcortical white matter tissue demonstrated moderate vacoulation area with mild cellular infiltrate (Figure 3c,d). BM-MSCs + melatonin group was nearly comparable to control in some extent, in which marked improvement could be noticed in the form of highly organized nerve fibers and normal appearance of neuroglia cells (Figure 3e).

Histochemical results

Examination of the silver impregnation stained section showed a dark brown coloration of densely packed nerve fibers in the control group (Figure 4a). While the examination of the cuprizone treated group showed severe loss of silver satin this indicated notable loss of nerve fibers with significant decrease in mean count of oligodendrocytes (Figure 4 b). After treatment of animals with BM-MSCs alone or melatonin alone, there was marked restoration of silver stain with significant increase in mean count of oligodendrocytes (Figure 4c,d). In the BM-MSCs + melatonin group, the silver stained section appeared nearly comparable to that of the control group with no statistically difference in the mean count of oligodendrocytes (Figure 4e).

Immunohistochemical results

In the GFAP immunostained stained sections, the control group exhibited nearly –ve immunoexpression in astrocytes (Figure 5 a), but in the cuprizone treated group there was a highly +ve immunoexpression with highly significant increase in mean area % of GFAP +ve cells when compared to the control group (Figure 5b). Examination of the BM-MSCs group showed mild +ve immunoreactvity in astrocytes with no statistical difference matched to the control group (Figure 5c). The Melatonin group showed mild to moderate +ve immunoreactvity with mid significant increase in mean area % of GFAP +ve cells as compared to the Control Group (Figure 5d). The BM-MSCs+ Melatonin group revealed nearly comparable with that of the control group a picture similar to that of the control group with no significant difference in mean area % as in comparison with the control group (Figure 5e).

Ultrastructure results

Examination of the ultrathin sections from the control animals showed myelinated nerve fiber with dense ordered myelin sheath of uniform thickness (Figure 6a). While in the cuprizone treated group, most of nerve fibers appeared demyelinated with complete splitting of myelin sheath from axons in addition to focal areas with loss of nerve fiber and focal splitting in myelin lamella. Also, some of the nerve fibers appeared with morphological changes as discontinuation, irregularity, deformation and formation of myelin loop (Figure 6 b-e). Treatment of animals with BM-MSCs alone or melatonin alone showed moderate to marked improvement, where some of morphological alterations were restored for intact appearance. Few nerve

fibers showed a degree of thin myelin sheath (Figure 6f,g). Examination of the ultrathin section obtained from BM-MSc+ Melatonin Group revealed marked improvement, and some of nerve fibers appeared normal, few with mild discontinuation of myelin sheath (Figure 6 h).

Morphometric and statistical results

The statistically evaluated data for number of pyknotic nuclei of oligodendrocytes, number of oligodendrocytes and area % of +ve GFAP cells in control and experimental groups were summarized in (Table 1).

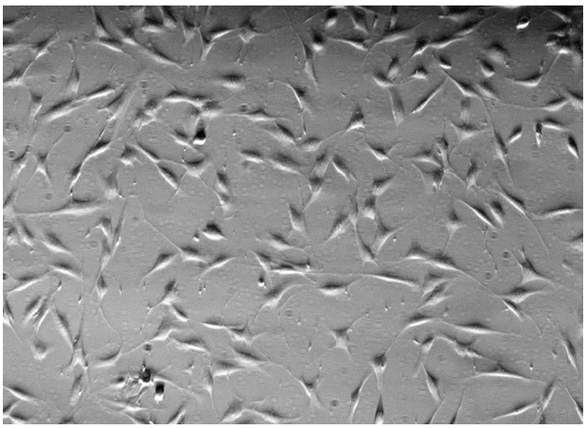

Fig. 1: photomicrograph of Mesenchymel stem cells (MSC) in culture were identified by their adhesiveness and fusiform shape (x: 400)

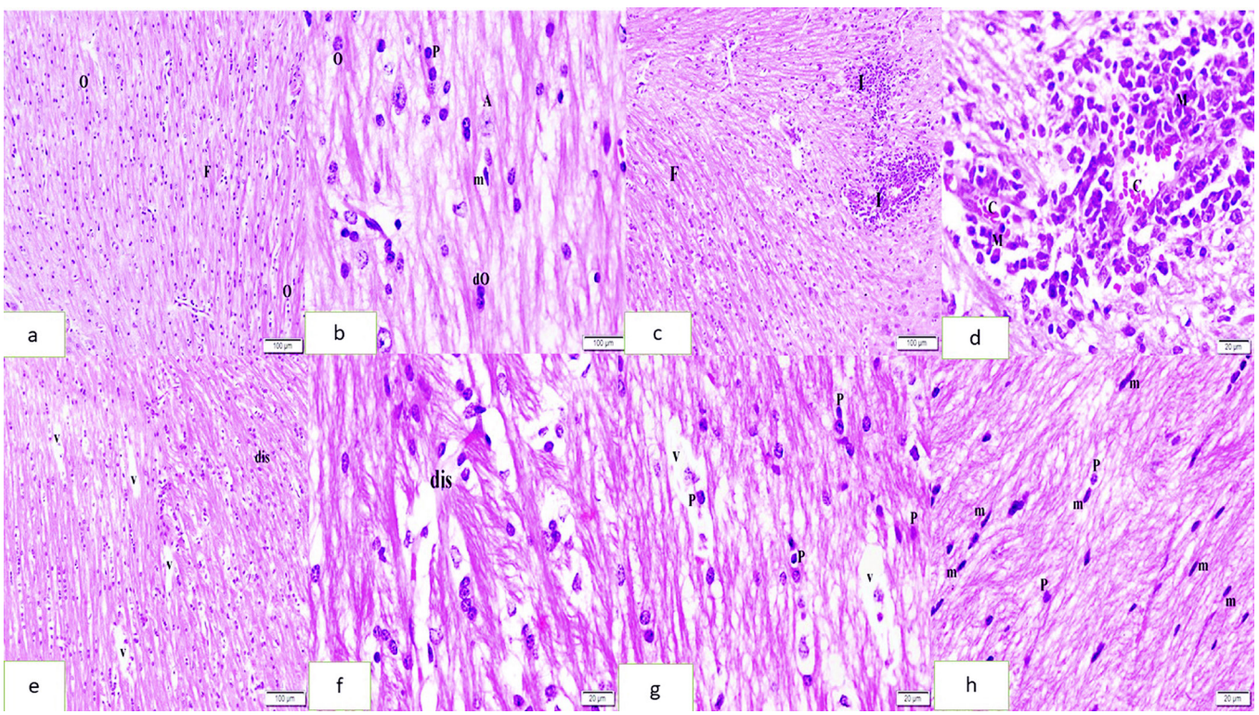

Fig. 2: photomicrograph of H&E stained section of mouse in subcortical white matter. (a&b) from control group. (a): showing normal fascicles fiber (F) and oligodendrocyte (o). (b): Showing oligodendrocytes with dark nucleus (o), dividing nucleus of oligodendrocytes (do), few dense pyknotic nucleus (p), astrocytes (A) and bizarre nuclei of microglia (m). (c-h): from cuprizone treaded group. (c): showing focal cellular infiltrate (I) among the fascicles fiber (F). (d): Higher magnification of the pervious figure (c) showing a mononuclear cellular infiltration (M), congested, blood vessel (c). (e): multiple vacuolated areas (V) and disorganized area (dis). (f): higher magnification of area of the pervious figure (e): showing disorganized nerve fascicles (dis). (g): Higher magnification of the figure (e) showing vacuolated areas (v) and multiple highly dense pyknotic nuclei (p) of oligodendrocytes. (h): showing multiple microglia (m) and highly dense pyknotic nuclei of oligodendrocytes (p) among the fascicles. (a, c, e, g, x:100, scale bar: 100 µm) (b, d, f, h x:400, scale bar: 20 µm)

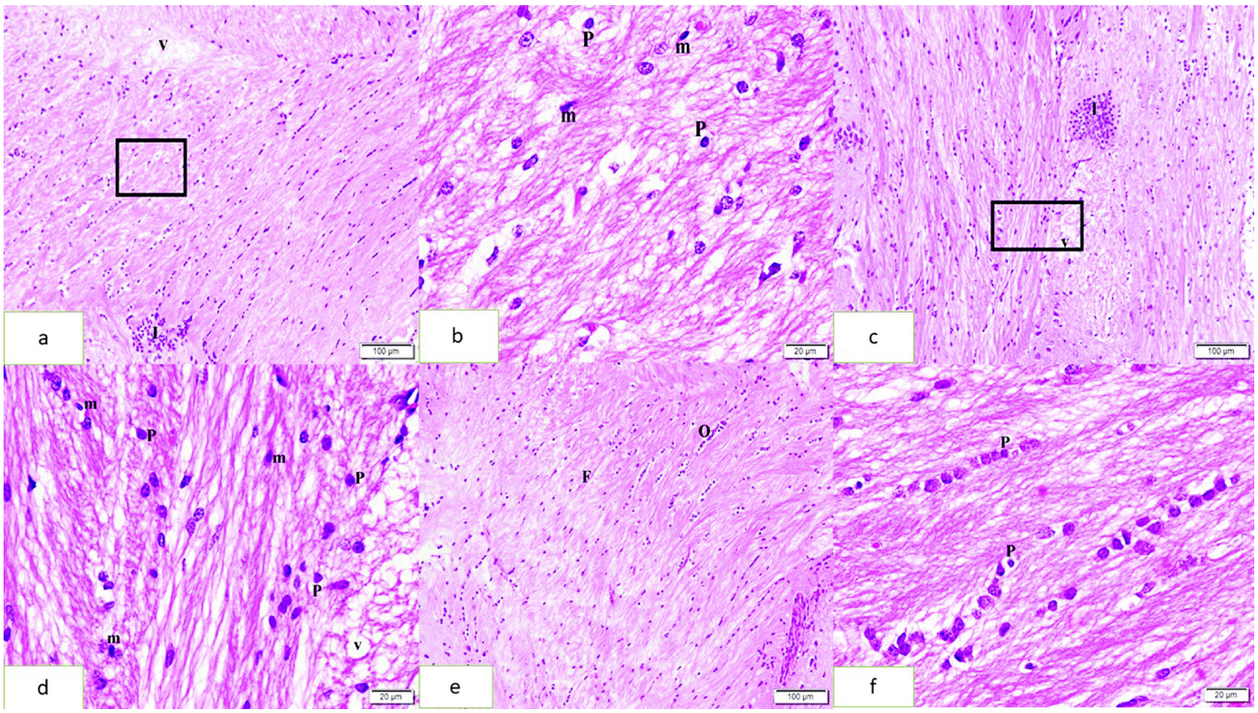

Fig. 3: photomicrograph of H&E stained section of mouse in subcortical white matter. (a&b) from BM-MSc group. (a): showing a vacuolated area (v) and localized cellular infiltrate (I) among organized fascicles. (b): Higher magnification of the pervious figure (a) of the boxed area showing few microglia (m) and few pykontic nuclei (p). (c&d) from melatonin treated group. (c): showing a localized cellular infiltrate (I) and a wide vacuolated area (V) among the fascicles. (d): Higher magnification of the pervious figure (c) of the boxed area showing some microgalia (m) and pykontic nuclei(p) and vacuolated area(V) among the fascicles. (e&f) from BM-MCSc +melatonin group. (e): showing organized fascicles (F) and oligodendrocytes (o). (f): showing few pyknotic nuclei (p) among the fascicles. (a, c, e, x:100, scale bar: 100 µm) (b, d, f, x:400, scale bar: 20 µm)

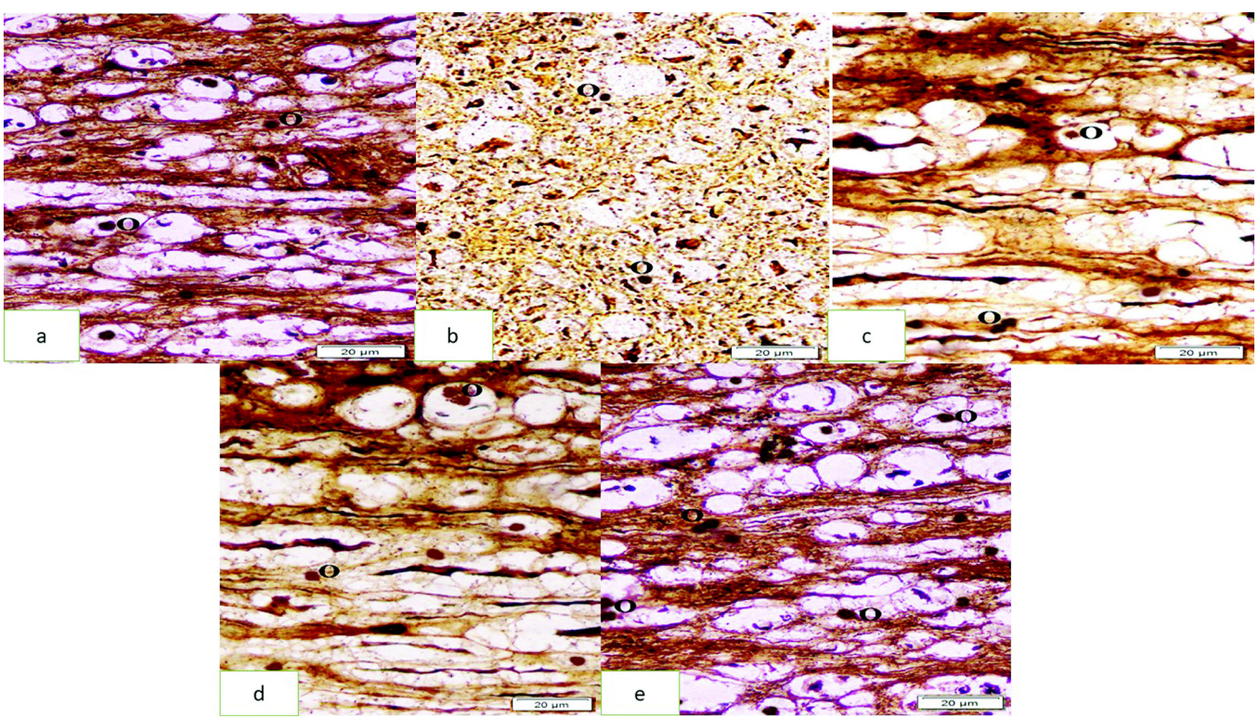

Fig. 4: photomicrograph of silver impregnation stained sections of mouse in subcortical white matter. (a): From control group showing normal dark brown coloration of densely packed nerve fibers and multiple oligodendrocytes (o) among organized fascicles. (b): from cuprizone treated group showing decrease of silver satin few oligodendrocytes (o) among disorganized fibers. (c): from BM-MCs group showing marked restoration of silver stain and multiple oligodendrocytes (o) among organized fibers. (d): from melatonin group showing some oligodendrocytes (o) among organized fibers. (e): from BM-MCs+ melatonin group showing numerous oligodendrocytes (o) among organized fibers. (X: 400, scale bar:20 µm)

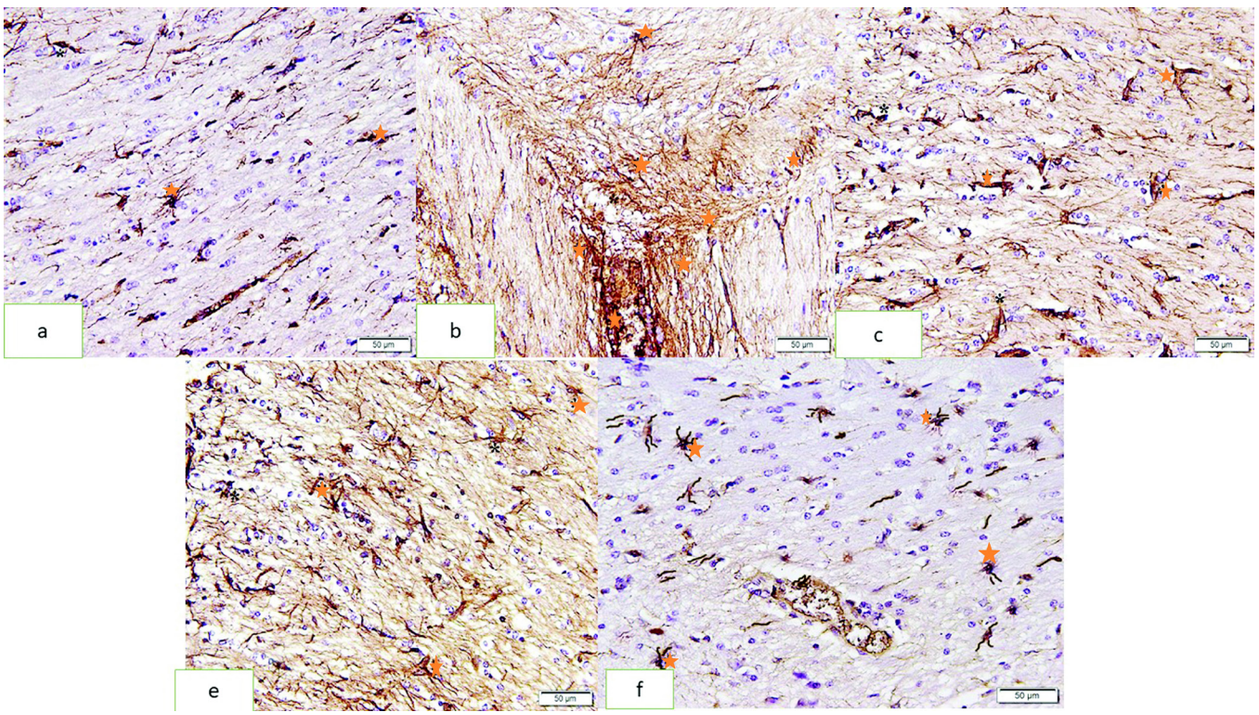

Fig. 5: photomicrograph of GFAP immunostained section of mouse in subcortical white matter. (a): from control group showing nearly-ve GFPA immunoexpression (IE). (b): from cuprizone treated group showing strongly +ve GFPA (IE). (c): from BM-MSCs group showing mild +ve GFPA (IE). (d): from melatonin treated group showing mild to moderate +ve GFPA (IE). (e): from BM-MSCs+ melatonine group showing nearly -ve GFPA (IE). Immunoreaction (Red star) (x: 200, scale bar: 50 µm)

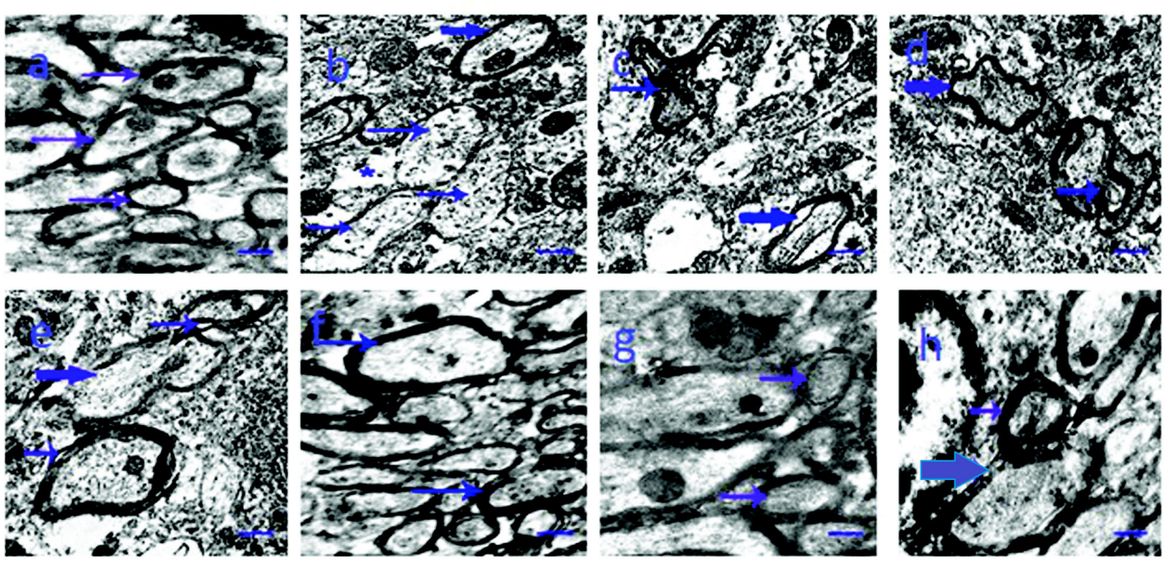

Fig. 6: Electron micrograph of ultrathin section of mouse in subcortical white matter. (a): from control group showing normal myelin sheath (arrow). (b-e) from cuprizone treated group. (b): showing myelinated nerve fiber (thick arrow), demyelinated nerve fiber (thin arrow). focal area with loss of nerve fiber (*). (c): showing splitting of myelin sheath from axon (thick arrow), deformed myelin sheath (thin arrow). (d): showing irregularity of myelin sheath (thick arrow), myelin loop formation (thin arrow). (e): showing discontinuation of myelin sheath (thick arrow), focal splitting of myelin lamella (thin arrow). (f), (g) From BM-MSc group, from melatonin treated group showing normal appearance of some of myelin sheath (arrow) among thin myelinated nerve fiber. (h): from BM-MSCs+melatonin group showing intact myelin sheath (thin arrow), showing mild discontinuation of myelin sheath (thick arrow), (x: 25000, scale bar 500 nm) (g: x: 3000, scale bar 500 nm)

Table 1: Showing Mean count of pyknotic nuclei, Mean count of oligodendrocytes and Mean area% of +ve IE of GFAP between control and experimental groups

Group	Mean count of pyknotic nuclei	Mean count of oligodendrocytes	Mean area% of +ve IE of GFAP
Control	1.7±0.21	10.0± 0.25	4.90±0.08
Cuprizone	6.4±0.37***	5.2±0.24***	28.37±0.62***
BM-MCs	2.7±0.26	9.4±0.26	14.540±0.15
Melatonin	3.0±25	8.5±0.26	17.82±0.11
BM-MCs+ Melatonin	1.8±24	10.0±0.25	6.09±0.17

***Significant $P \leq 0.05$ versus other groups

DISCUSSION

The current study has demonstrated the possible therapeutic effect of BM-MCs, melatonin individually and combined in experimental model mimicking demyelinating aspect of MS to prompt demyelination. Cuprizone mouse model has been chosen as it is pronounced by low mortality as well as high reproducibility in demyelination[16]. In this study, the Cuprizone administration resulted in multiple vacuolated areas in neurofibrillary network among highly disorganized fibers. A widespread cellular infiltrate mainly monocytes with congested, dilated blood vessel. Most of oligodendrocytes appeared with highly dense pyknotic nuclei with gliosis (microglosis and astrogliosis). The histochemical staining of nerve fiber with silver impregnation stain revealed severe loss of silver satin which indicated notable loss of nerve fiber with significant decrease in the mean count of oligodendrocytes.

Cuprizone, also known as (bis-cyclohexanone oxaldihydrazone), is a copper chelating chemical that harms adult oligodendrocytes, which are in charge of producing the myelin sheath that surrounds nerve cell axons[17]. Decrease in copper concentrations have been found in some neurodegenerative diseases with oligodendrocyte depletion in the cortex, as well as reactive astrocytes and microglia activation, which occupy the demyelination area many metalloproteases require copper to operate[18]. Cuprizone consumption causes a drop in the levels of myelin basic protein, phospholipases A2, myelin-associated glycoprotein, ceramide, and galactosyltransferase, which signals the start of demyelination[19]. Additionally,[20] reported that long-term cuprizone consumption causes white matter pathology with the same pattern of MS III lesions, which may be characterized by increased local oxidative stress, down regulated mitochondria-encoded genes expression level, and altered intra-axonal mitochondrial density. According to reports, persistent inflammation, disruption of the blood-brain barrier (BBB), and lymphocyte infiltration into the CNS cause impairment of the myelin sheath, axonal loss and gliosis in MS

In cuprizone treated group microglial activation and monocyte infiltration were evident. This outcome was in concomitant with[21] who documented that the primary pathogenic abnormalities that underlie the progression of debility in the progressive form of MS are mononuclear phagocytes (MPs), which make a substantial influence in the innate immune response in progressive form of MS. chronically activated MPs include both resident tissue (i.e., macrophages entering the CNS via the circulation) and infiltrating MPs (i.e., microglia and non-parenchymal macrophages). In P-MS, MPs congregate in various brain regions and secret proinflammatory cytokines, proteases, and (ROS), finally, lead to neurodegeneration and cortical atrophy.

In contrast to the control animals, the cuprizone-treated group revealed activation of glial cells in addition to a significant rise in the mean area% of GFPA immunoexpression. Similar result were informed by[22], who found that cuprizone did not create a significant microglial reaction after 14 days of ingestion but did cause a noticeable migration and accumulation of microglia toward the myelin sheath after 28 days. The production of the proinflammatory inerleukin-1 (IL-1) and interleukin-6 (IL-6) increased in the brain extract, indicating a microglia- and astrocytes mediated inflammatory microenvironment in the brain, which may participate in BBB damage and T cell invasion. These mediators also may affect the differentiation of oligodendrocyte progenitor cells (OPCs) into mature myelin sheath.

The electron microscopic results in cuprizone treated group showed various degree of degeneration in myelin sheath. These findings were in consistent with those of an earlier study by[23] who revealed that the changes in myelin basic protein were responsible for the loss of the myelin sheath. Additionally, intramyelinic edema and edematous splitting at various levels of the myelin lamella may be caused by an increase in water content in degrading cells, which leads to demyelination. Similar results were previously recorded by[24] who reported that myelin loops were distinct during the first stage of myelin recycling and that a rise in the frequency of myelin loop distortion is correlated to an early response of axonal atrophy.

Current approved MS treatments (such as natalizumab, cladribine, ocrelizumab, and glatiramer acetate) are effective in preventing the gathering of focal inflammatory mediators and reducing the number of relapses, but they don’t stimulate tissue repair or act to stop the progression of the disease. Despite significant advancements in MS therapies over the past ten years, there is currently no cure for this incredibly weakening condition. Additionally, these treatments can have serious side effects by increasing a

patient's propensity for cancer and infections. Furthermore, the therapeutic possibilities for the more destructive types of MS are really inadequate[25].

The finding of the current study has exhibit that the treatment with BM-MCs showed improvement in subcortical white matter to great extent. This result was previously documented by[26] who stated that stem cell therapies can be utilized to restore injured or destroyed neural tissue, to substitute a deficient immune system, and to offer immunosuppressive, reparative, and neuroprotective processes. MSCs have several advantages over other types of stem cells, including easy culture methods, easy collection, and high accessibility, low immunogenicity allowing allotransplant, immunomodulatory capability, no oncogenic transformation, and slight ethical concerns. MSCs can be obtained from different types of tissues, but the two most often used sources are bone marrow and adipose tissues. Clinical experiments found prominent changes in the clinical course of MS after stem cell therapy, demonstrating the ability of MSCs to considerably alter the pathology in known animal models of MS[27]. Also,[28] found that MCs may offer a level of protection against continuing axon injury through a mechanism containing keeping microglia in an inactive state.

The therapy with melatonin improved the pathological findings in a moderate to marked way. Melatonin is a tiny, molecule that can penetrate any barrier in the body, including cell membranes, mitochondrial membranes, and blood brain barrier (BBB). Melatonin is a main antioxidant agent against neurodegenerative illnesses because it is generated in the brain and released in the cerebrospinal fluid (CSF)[6].

Melatonin has been shown to infiltrate cell membranes and primarily settle in a region of lipid bilayers close to the polar heads of membrane phospholipids. Melatonin is known to operate as a free radical scavenger in this situation, and it may be a covert method for membranes to prevent oxidative damage. Melatonin functions as a direct scavenger and regulates mitochondrial oxidative phosphorylation enzymes and Adenosine triphosphate (ATP) creation to maintain control over mitochondrial homeostasis[29]

According to[30], oxidative stress is one of the pathophysiological characteristics of MS. Extreme production of pro-inflammatory cytokines and (ROS), including hydroxyl radicals, superoxide, hydrogen peroxide, and hydrogen sulphide, is caused by T cell and macrophage infiltration of the (CNS), activation of astrocytes and microglia inside the CNS. Under normal circumstances, the actions of antioxidant defense enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in conjunction with glutathione (GSH) counterbalance the generation of ROS; these enzymes neutralize ROS and enhance mitochondrial function[31].

Numerous studies have shown that astrocyte stimulation under various circumstances, such as cuprizone feeding, results in NF κB upregulation and oligodendrocyte mortality. According to some studies, HO-1 is one of the isoforms of HO, a microsomal enzyme that under normal conditions is expressed at low levels but may be produced in large quantities during times of oxidative stress. In the current study, cuprizone feeding decreased HO-1 level, causing NF κB activation can indirectly down regulate HO-1, which in turn declines the expression of antioxidant genes[32].

Several studies suggest that melatonin and the immune system interrelated identifying melatonin receptors in immune cells such as natural killer cells (NK), T lymphocytes, thymus, and bone marrow cells. Additionally, melatonin may have a role in the pathophysiology of several autoimmune disorders, where dysregulation in melatonin production has an influential role in autoimmune pathogenesis[33].

Combining BM-MSCs and melatonin led to a noticeable improvement and remyelination of nerve fiber. Some data suggested that melatonin regulates a number of MSC in *vitro* properties. Melatonin can influence BM-MSCs through receptor-mediated or receptor-independent mechanisms[34]. Melatonin, regulate MSC differentiation and function. In a study on rats, night-time melatonin concentrations in the bone marrow were double as high as those in peripheral blood, and high melatonin concentrations that were still identified in the bone marrow of pinealectomized rats specified that some melatonin can be produced in the bone marrow[35]. The inflammatory internal microenvironment abates the therapeutic efficacy of MSCs in *vivo*, but recent study has revealed that melatonin can rise MSC survival and create a synergistic effect that lessens inflammation, apoptosis, and oxidative stress Melatonin can act as a highly effective natural antioxidant with multiple mechanisms of action. Oxidative stress mentions to a state of imbalance between oxidation and antioxidation, and it is the harmful effect of free radicals in the body[36]. Melatonin and some of its metabolites, including 6-hydroxymelatonin, cyclic 3-hydroxymelatonin, and N-acetyl-5-methoxykynuramine, can directly bind to reactive oxygen and reactive nitrogen species. Cyclic 3-hydroxymelatonin is considered as a biomarker for in *vivo* detection of hydroxyl radicals. Also, melatonin can stimulate the activity of antioxidant enzymes while suppressing pro-oxidant enzymes. Melatonin can also utilize its antioxidant functions by chelating transition metals[37].

CONCLUSION

The conclusions of this study proposed that cuprizone is a useful model for studying the demyelination of nerve fiber in mice, and BM-MCs and melatonin have positive therapeutic effects for the treatment of multiple sclerosis. These effects may be related to the regenerative properties of BM-MCs and the antioxidant action of melatonin. Melatonin may potentially be enhancing the effects of BM-

MCs. Melatonin administration with BM-MCs therapy may be a safe course of MS treatment.

Future research is advised to clarify the mechanism of action behind the antioxidant benefits of melatonin as well as the precise role that melatonin plays in boosting BM-MSC activity during MS treatment.

ABERRATIONS

(ATP): Adenosine triphosphate, **(BBB):** Blood brain barrier, **(BM-MSCs):** Bone Marrow Mesenchymal stem cells, **(CAT):** Catalase, **(CNS):** Central nervous system, **(CSF):** Cerebrospinal fluid, **(CSF):** Galial fibrillary acidic protein, **(CSF):** Glutathione (CSF), **(GPx):** Glutathione peroxidase, **(H &E):** Hematoxylin and Eosin, **(IL-1):** Inerleukin-1, **(IL-6):** Interleukin-6, **(MPs):** Mononuclear phagocytes, **(MS):** Multiple Sclerosis, **(NK):** Natural killer cells, **(OPCs):** Oligodendrocyte progenitor cells, **(ANOVA):** One-way analysis of variance, **(P-MS):** Progressive form of MS, **(ROS):** Reactive oxygen species, **(RA):** Rheumatoid arthritis, **(SOD):** Superoxide dismutase, **(SLE):** Systemic lupus erythematous.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور الميالتونين في تقويه التاثير العالجي للخلايا الجذعيه الوسيطه في انسجه الماده البيضاء تحت القشريه للفئران المعالجة بالكوبريزون كنموذج للتصلب المتعدد

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المقدمة والهدف من الدراسه: التصلب المتعدد هو مرض التهابي مزمن يحدث في الجهاز العصبي المركزي. هناك الكثير من االهتمام الستخدام الخاليا الجذعية الوسيطة لنخاع العظام كعالج لمرض التصلب المتعدد. الميالتونين ، المعروف بانه من أقوى مضادات أألكسدة الموجوده داخل الجسم. اجريت الدراسة الحالية لفحص ما إذا كان الميالتونين قد يحفز التأثير العالجي لـالستخدام الخالياالجذعية الوسيطة لنخاع العظام لعالج التصلب المتعدد.

الطرق والمواد: تم تقسيم ٣٠ فأرا إلى ٥ مجموعات. المجموعة (١) بمثابة الممجموعه الضابطه. تم تغذية حيوانات المجموعات ٢) ، ٣ ، ٤ ، (٥ بنظام غذائي يحتوي على ٠,٣٪ كوبريزون لمدة ٤ أسابيع لتحريض إزالة الميالين في المادة البيضاء تحت القشرية. ذبحت المجموعة (٢) مباشرة بعد إطعام الحيوانات مع كوبريزون. تم حقن المجموعة (٣) من الحيوانات عن طريق الوريد في الذيل بالخاليا الجذعية الوسيطة لنخاع العظام]$١x١٠^{٦}$ [ثم القتل الرحيم بعد ٤ أسابيع. أعطيت المجموعة (٤) من الحيوانات جرعات يومية داخل الصفاق ٥) ملجم / كجم(من الميالتونين وتم قتلها رحيما بعد أسابيع. المجموعة (٥) تم حقن الحيوانات بالخاليا الجذعية الوسيطة لنخاع العظام ، كما في المجموعة (٣) ،والميالتونين ، كما في المجموعة (٤) ، ثم الموت الرحيم بعد ٤ أسابيع.

النتائج: كشف الفحص النسيجي المرضي ألنسجة المادة البيضاء تحت القشرية أن كوبريزون تسبب في تغيرات شديدة في الحبيبات مع زيادة ملحوظة في التعبير المناعي للبروتين الليفي الدبقي((GFAP ، ، أظهر تغيرات مورفولوجية مختلفة في غمد المايلين. بعد عالج الحيوانات بالخاليا الجذعية الوسيطة لنخاع العظام كان هناك تحسن واضح في أنسجة المادة البيضاء. عالوة على ذلك ، فإن إعطاء الميالتونين يخفف من التأثير السلبي للكوبريزون. تراجعت جميع النتائج تقريبا بعد التناول المتزامن لـالخاليا الجذعية الوسيطة لنخاع العظام + الميالتونين

الاستنتاج: الخاليا الجذعية الوسيطة لنخاع العظام و / أو الميالتونين لهما تأثير محتمل إلعادة التشوه في نموذج التصلب المتعدد في الفار.