



Review

Effects of melatonin on the management of multiple sclerosis: A scoping review on animal studies

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Abstract: Background: Multiple sclerosis (MS), is a debilitating neurological disease that currently has various treatments, like disease-modifying therapies, monoclonal antibodies, corticosteroids, and hormonal derivatives. Melatonin has several actions, like regulating circadian rhythms, which are usually used for insomnia. This scoping review aimed to explore the efficacy of melatonin, both as a standalone treatment and in conjunction with other drugs, in the management of MS in animal models. **Methods:** We searched PubMed, Web of Science, EMBASE, and Google Scholar using (“melatonin” OR “melatonin receptor”) AND (“multiple sclerosis”) AND (“animal”). Animal studies that evaluated the effects of melatonin on the development, progression, and outcomes of MS were included. Human studies and other types of studies like case reports were excluded. We used narrative synthesis for reporting the results. **Results:** Overall, 21 studies were included, conducted on mice (n = 15) and rats (n = 6). Mostly, studies reported that melatonin led to normal circadian rhythms in animals. Melatonin in doses of both 50 and 100 mg/kg were useful in nociception latency. Melatonin in combination with other drugs like H-89, diisopropylamine dichloroacetate, gibberellins, and IFN-1 β improved outcomes, while there was not improved cognition in combination with mesenchymal stem cells. In some tests, male subjects showed significantly better responses. There were controversial results regarding the effects of melatonin on cytokines, but overall, it led to a reduction in proinflammatory cytokines. **Conclusions:** Melatonin overall demonstrated favorable outcomes regarding oxidative stress, anti-inflammation, and cytokine levels across various doses. It is recommended conducting systematic reviews and meta-analyses with a larger number of primary studies to provide more comprehensive insights.

Keywords: multiple sclerosis; melatonin; animal; hormone therapy; treatment; scoping review

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system (CNS). It is characterized by inflammation, demyelination, gliosis, and neuronal loss [1]. The symptoms of MS can vary widely among patients, depending on the location and severity of nerve fiber damage in the CNS [1]. Common symptoms include vision impairments such as blurred vision or diplopia, muscle weakness, fatigue, depression, sexual dysfunction, and cognitive problems [1]. The progression of MS is often categorized into four stages, which are clinically isolated syndrome, relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS) [2].

Several types of treatments have been developed for MS. In this regard, disease-modifying therapies (DMTs) aim to target the immune system, each with its own mechanism of action [3]. First-line treatments include interferon (IFN) beta group, glatiramer acetate (GA) or Copaxone, dimethyl fumarate, teriflunomide, and alemtuzumab [3,4]. Second-line treatments consist of natalizumab and fingolimod [3,4]. However, no DMTs have been conclusively proven to affect the long-term prognosis of MS [3,4].

MS is three times more common in women than men, particularly in those of childbearing age [5]. Hormonal differences, such as testosterone and estrogen levels, may contribute to this higher prevalence in women: prior to puberty, MS rates are almost similar between males and females, whereas with advanced ageing and hormonal fluctuations, women develop MS more frequently than men [6]. So, hormones play roles in MS and research on hormone therapy is ongoing [3,4].

Melatonin (N-acetyl-5-methoxytryptamine), a natural hormone derived from tryptophan, is primarily synthesized by pinealocytes in the mammalian pineal gland. It is predominantly secreted during darkness [7,8]. Melatonin and its metabolites play multifaceted roles, including reducing demyelination and promoting remyelination in inflammatory conditions, providing anti-oxidative effects by eliminating reactive oxygen and nitrogen species [7,8]. Additionally, it has been observed to increase anti-immunomodulatory and anti-inflammatory effects, thereby reducing both chronic and acute inflammation [9]. Melatonin has also been noted for its potential in improving lipid profiles [10].

Animal studies have demonstrated that melatonin can decrease the loss of oligodendrocytes and increase levels of microglia and CD4+ T cells, suggesting its potential role to alter the treatment approaches for neurodegenerative disorders and improve patient quality of life [11]. A systematic review on human studies evaluated the effects of melatonin on MS and showed that it is almost a safe intervention that can improve sleep, fatigue, and cognition in MS [12]. Nevertheless, they recommended further studies due to the limited number of included studies [12]. Other previous review articles shed light on different aspects of melatonin roles in MS [13,14]. Very recently, Rezmara et al. conducted a systematic review to investigate the melatonin supplementation efficacy for disease severity and neurobehavioral modification among MS-simulating animal models. In this work, we aim to assess the melatonin mechanism in ameliorating the disease and also consider the effects of intrinsic melatonin level fluctuations and outcomes among animals (Figure 1).

Thus, this scoping review aimed to evaluate the potential of melatonin, as combined with other medications or endogenous ones, on MS in animal models.

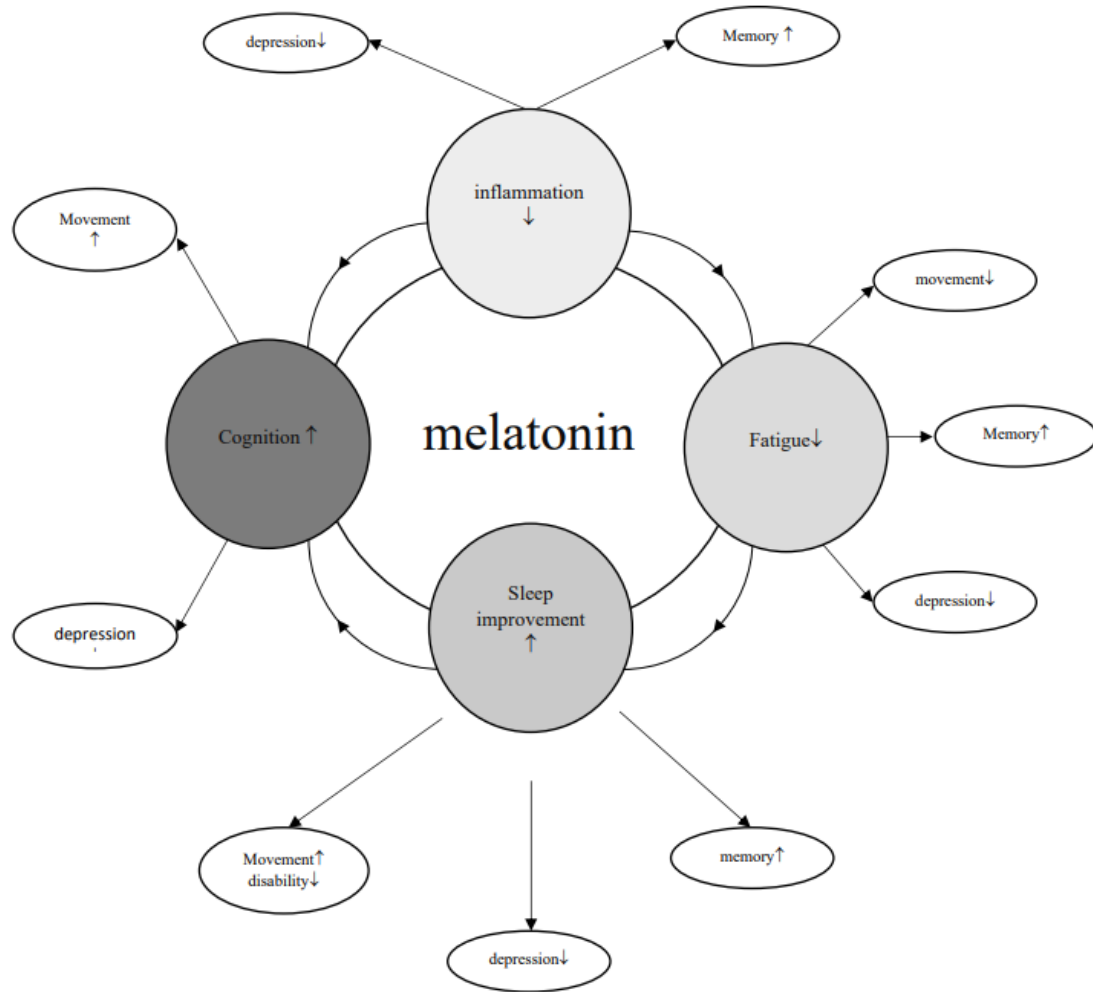


Figure 1. Melatonin effects on sleep, cognition, fatigue, and inflammation among MS patients.

2. Methods

The study was in accordance with preferred reporting items for systematic reviews and meta-analyses extension for scoping reviews [15].

2.1. Database searching

We conducted a search in PubMed, Web of Science, EMBASE, and Google Scholar using a systematic strategy on November 23, 2023. We systematically searched the five aforementioned databases using the terms: (“melatonin” OR “melatonin receptor”) AND (“multiple sclerosis” OR “disseminated sclerosis” OR “acute fulminating”) AND (animals or EAE). The results were imported into EndNote software for duplicate removal.

2.2. Study selection

The inclusion criteria were as follows: (1) Peer-reviewed articles reporting different effects of melatonin on MS occurrence, progression, or outcomes in animal models; (2) studies examining changes in melatonin levels in animals during various phases of the disease; and (3) research involving animals receiving melatonin supplements, either alone or in conjunction with other medications. Studies were excluded if they were other types of studies, such as case reports and case series, reviews, and conference proceedings, articles not published in English, articles without access to full-text articles published before 1990, retracted articles, and articles lacking information on the number of samples studied or study outcomes. Two authors independently reviewed the titles, abstracts, and full texts of the documents, by eligibility criteria. Any discrepancy was resolved by discussion.

2.3. Data extraction

Two authors independently reviewed the selected articles and then imported the data into a Microsoft Excel sheet. The extracted information was the first author's name, year of publication, MS simulation induction, sex distribution, phase of disease, disease duration, concomitant drugs and dosages, treatment duration, and outcomes for each study. We used discussion between those authors to resolve discrepancies.

2.4. Data synthesis

We used a narrative synthesis for reporting the results of the study due to the high heterogeneity between studies.

3. Results

3.1. Study selection and characteristics

Overall, 519 studies were found during literature search. Following the removal of 97 duplicates, 422 remained. In title/abstract screening, 401 studies were excluded. The full text of the remaining 21 studies were evaluated for eligibility and all of them met the eligibility criteria. Finally, 21 studies were included in the qualitative synthesis [16–36] (Figure 2).

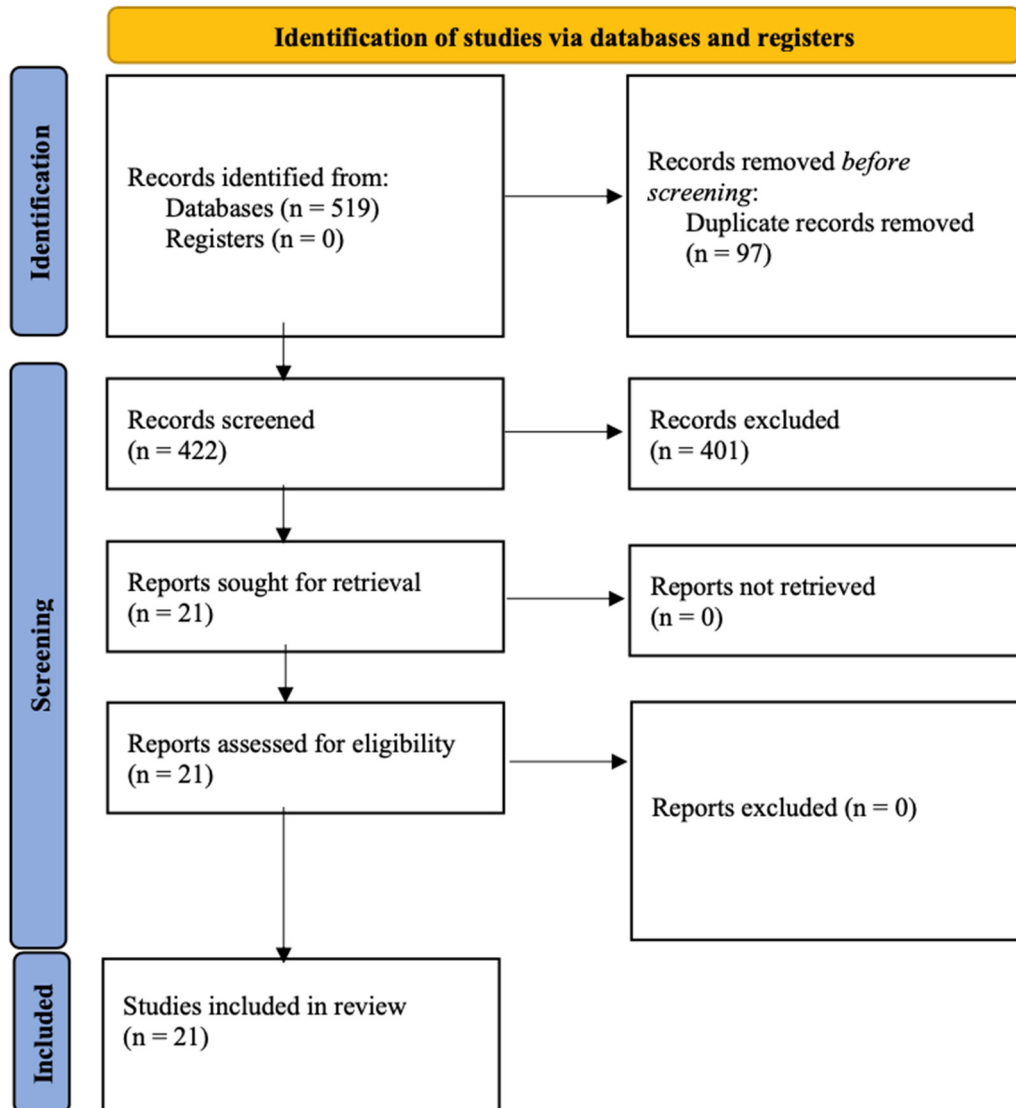


Figure 2. Study selection process.

Studies were published between 2015 and 2023. Seven studies were case-controls, two were cohorts, seven were experimental, and five were other types of observational studies. Eight studies were conducted in Iran, two in the countries of Mexico, Saudi Arabia, Spain, and Ukraine, and one in each of the countries of Canada, China, Egypt, Taiwan, and the United States. Fifteen studies were conducted on mice, and the remaining six were conducted on rats. The treatment duration was between three days and nine weeks. Concomitant medications included luzindole, cuprizone, IFN, GA, and methylprednisolone (Table 1).

3.2. Multiple sclerosis induction approaches

Different methods for induction were used in the studies. Cuprizone was among the most applied approaches for simulating experimental allergic encephalomyelitis (EAE). Cuprizone was administered whether at 0.2%, 0.2 mL orally, or 400 mg/kg/day. All the experiments took 5-week

cuprizone administration to reach the EAE approvable extent, except Labunets et al. [27] that took three weeks. Also, in the article by Abo Taleb et al. [16], cuprizone induced the demyelination stage for five weeks, followed by spontaneous remyelination stage for four weeks (Table 2).

3.3. Melatonin pretreatment administration

Pretreatment [3 days after myelin oligodendrocyte glycoprotein (MOG) injection] and post-treatment (day 10 after EAE induction) of melatonin was used in the study by Wen et al. [17]. Primary microglia cells were pretreated with melatonin at concentrations of 0.3 and 3 mM starting 30 min before and 6 h after the start of lipopolysaccharide (LPS, 100 ng/mL) exposure. Treatment of LPS-stimulated microglia with melatonin at a concentration of 0.3 mM did not significantly affect inducible nitric oxide (iNOS) expression. However, treatment with melatonin at 3 mM significantly inhibited the production of iNOS [17].

Animals pretreated with melatonin spent a similar time in the target quadrant proximity as compared to the control group [34]. In another study, the prophylactic and therapeutic treatments with melatonin significantly protected against EAE, but not as potently as daily administration. Again, levels of IL-1a, IL-6, IL-2, IFN, TNF, IL-17 α , and IL-22 were reduced with melatonin and IL-10 production was significantly augmented. However, melatonin could not change the onset of MS [28].

3.4. Melatonin and circadian rhythms

Nine studies showed that the circadian rhythms of mice who received melatonin were normal (i.e., 12/12 h light/dark cycle) [16,18,20,21,23,30,33,34,36]. In the study by Ghareghani et al., 24 h/day bright light and 24 h/day darkness were defined as constant light and darkness, respectively. The mice exposed to constant light and darkness experienced that condition for 3 days, followed by a rest day of normal 12/12 h light/dark [22]. Five weeks of cuprizone feeding for mice showed enhanced melatonin levels compared with controls. Constant light and darkness significantly decreased and increased melatonin levels, respectively. No significant changes were reported in melatonin level with the administration of melatonin at 80 mg/kg and luzindole at 30 mg/kg. The oligodendrocyte precursor cells (OPCs) were significantly higher in vehicle mice than in control mice ($p < 0.05$). Immature OPCs did not increase response to constant light or luzindole in comparison to those in the vehicle group; however, the maturation of OPCs into oligodendrocytes increased significantly in response to constant light or luzindole in comparison to that in the vehicle group ($p < 0.001$ and $p < 0.05$, respectively). Despite mature oligodendrocytes, immature OPCs were significantly reduced by constant darkness but not by melatonin in comparison to the vehicle group ($p < 0.01$) [22] (Table 1).

Table 1. Study characteristics of included studies and their main outcomes.

Study ID	Study type	Country	Subjects	Sex and sample size	Interventions	Treatment duration	Results
Alghamdi et al. 2020	Observational	Saudi Arabia	Mice	All male = 30	Cuprizone (400 mg/kg) and melatonin (80 mg/kg) daily	5 weeks	Enhanced the memory defects, increased the nociception latency, antagonists for toxic effects of cuprizone on motor activity, enhanced memory outcomes
Dokoohaki et al. 2017	Observational	Iran	Lewis rats	All female = 18	Methylprednisolone 30 mg/kg	6 days	Decrease in melatonin level ($p < 0.05$), diminished IFN- γ ($p < 0.01$), increased serum IL-4 levels ($p < 0.01$)
Ghareghani et al. 2022	Case control	Iran	C57BL/6 mice	All female = 32	Melatonin (10 mg/kg/day) DADA (50 mg/kg/day, orally)	10 days	Improved neurological disability scores for melatonin, combination of melatonin + DADA improved neurological disability scores more than melatonin alone, significant increase of IL-4 and IL-10 levels, and reduction in IL-1B and TNF levels for combination therapy ($p < 0.000001$)
Ghareghani et al. 2023	Case control	Canada	C57BL/6 J wild-type mice	All males	30 mg/kg Luzindole, 80 mg/kg melatonin	5 weeks	Reduction in total Olig2 + oligodendroglia ($p = 0.051$), neural stem/progenitor cells proliferation inhibition, no influence on Olig2 + /SOX2 + cells, decreased number in melatonin did not cause modified percentage of Ly6Clow monocytes
Gharib et al. 2022	Case control	Egypt	Mice	All males = 50	Cuprizone 0.2% MSC-MVs 0.2 mg/kg, A single melatonin 10 mg/kg for three weeks	3 weeks	Increase in the duration of maintained balance, melatonin did not change the cognitive performance but MSC-MVs did.

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Study ID	Study type	Country	Subjects	Sex and sample size	Interventions	Treatment duration	Results
González et al. 2021	Case control	Mexico	Sprague-Dawley rats	All male = 128	IFN-® 1b (dosed at 8000 U), every third day, intramuscularly Glatiramer acetate 0.50 mg/kg/day, subcutaneously Melatonin at 10 mg/kg/day, i.p	12 days	The least level of TNF was shown in melatonin + EAE group that was 0 pg/mL
Ghareghani et al. 2018	Case control	Iran	C57BL/6 mice	All female = 24	10 mg/kg/day i.p	13 days	Decrease in mean clinical scores ($p < 0.05$), procalcitonin reduced augmented concentrations of 25-hydroxyvitamin D ($p < 0.05$)
Jand et al. 2022	Observational	Iran	KP. C57BL/6 mice	All female = 68	Melatonin 0.1, 1.0, 5.0, 10 mg/kg	9 days	Melatonin significantly decreased the EAE clinical scores, kynurenine pathway regulatory enzyme mRNA expression reduction inhibits the enzyme Nicotinamide N-Methyltransferase overexpression ($p < 0.001$)
Labunets et al. 2023	Case control	Ukraine	Mice	Young = 79 Aged = 79 All female	Melatonin at 6 p.m. daily, 1 mg/kg, i.p	Days of 7, 21, and 60	Astrocyte activation in the young and aging mice reduced the percentage of neurons with destructive variations, behavioral reactions in young and aging mice improved level of thymulin in blood, diminished only in younger mice ($p < 0.05$)

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Study ID	Study type	Country	Subjects	Sex and sample size	Interventions	Treatment duration	Results
Labunets et al. 2021	Experimental	Ukraine	Mice	NA	Melatonin 1 mg/kg, at 18:00, daily, Cuprizone diet 0.5×10^6 cells, once intravenously	11 days	Cuprizone elevated the malondialdehyde levels and reduced the activity of antioxidant enzymes in the brains of mice; there was a decrease observed in locomotor, emotional, and orientational-exploratory activity, and muscle tone
Álvarez-Sánchez et al. 2015	Experimental	Spain	C57BL/6 mice	All female	80 mg/kg melatonin daily	NA	Reduced the severity of the disease ($p < 0.001$), diminished the incidence of EAE ($p < 0.05$), reduced frequencies of absolute CD4+ T macrophages ($p < 0.05$)
Chen et al. 2016	Experimental	Taiwan	C57BL/6 mic	All female	200 mg/kg injection	Days 9, 11, and 13 after EAE induction	Improvement in clinical scores ($p < 0.01$), leukocyte infiltration reduction, inhibiting the T-cell proliferation, and reduced IL-10 and IL-17 expressions
Long et al. 2018	Experimental	China	C57BL/6 mice	All female	Melatonin 10 mg/kg, i.p	Day 0 post-immunization of EAE	Decrease in neurological deficit scores at the peak stage of EAE ($p < 0.05$); decrease in ROS concentrations ($p < 0.05$)
Sharif et al. 2017	Experimental	Iran	Wistar rats	All males	Melatonin (50 and 100 mg/kg/side), H-89 (10 micro-M) intra-hippocampally 30 min before each day of training	4 days	Improved escape latency compared with H-89 alone ($p = 0.008$); low-dose melatonin pretreatment significantly decreased H-89-induced cytochrome c release compared to control group ($p < 0.05$), and high-dose melatonin completely impeded such event by leaving no significant difference compared with control group

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Study ID	Study type	Country	Subjects	Sex and sample size	Interventions	Treatment duration	Results
Ghareghani et al. 2018	Experimental	Iran	C57BL/6 mice	All females = 40	10 mg/kg/day baclofen 10 mg/kg/day melatonin	10 days	Reestablishing original normal levels of IL-4 and IFN- γ in combination therapy ($p < 0.001$), increase in antioxidant enzymes catalase activity in combination ($p < 0.001$)
Abo Taleb et al. 2019	Case control	Saudi Arabia	Mice	Females = 39 Males = 39	Cuprizone 400 mg/kg/day oral+ melatonin 80 mg/kg/day i.p at 12 p.m.	9 weeks	Reduced MDA in males, increased CAT, SOD, GPx, and GSH, improvement in TNF and IL-1B levels
Escribano et al. 2022	Observational	Spain	Rats	All males = 25	Melatonin 1 mg/kg oral 5 days a week	51 days	Reduction in oxidative stress biomarkers levels ($p < 0.001$), reduction in EAE severity ($p < 0.001$)
Ghareghani et al. 2016	Observational	Iran	Rats	All females = 15	Melatonin 10 mg/kg	18 days	Increase in serum lactate ($p < 0.01$) and number of infiltrated cells ($p < 0.01$)
Gabriel Ortíz et al. 2022	Experimental	Mexico	Rats	All males = 48	Melatonin 20 mg/kg of body weight/day, GA 10 mg/kg of body weight/day, IFN-b1b 9000 IU/kg of body weight every other day	20 days	Reduced cumulative index score, reduced levels of IL1 β , IL-6, and TNF-a, reduced MDA+4-OHA ($p < 0.05$)
Vakilzadeh et al. 2015	Cohort	Iran	Mice	N = 20	Cuprizone, melatonin 50 mg/kg, melatonin 100 mg/kg	7 days	Increase in moved distances for melatonin 50 mg/kg ($p < 0.01$) and 100 mg/kg ($p < 0.001$)
Wen et al. 2016	Cohort	United States	Mice	All females	28 days	Melatonin 20 mg/kg i.p	Attenuated clinical severity ($p < 0.05$), less CD4+ positive cells ($p < 0.001$)

Note: CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GSH: Glutathione; MDA: Malondialdehyde; EAE: Experimental autoimmune encephalomyelitis; GA: Glatiramer acetate; MDA: Malondialdehyde; 4-OHA: 4-hydroxyalkenals; i.p: Intraperitoneally; DADA: Diisopropyl amine dichloroacetate; MSC-MVs: Mesenchymal stem cells microvesicles; TNF: Tumor necrosis factor; CSF: Cerebrospinal fluid; ROS: Reactive oxygen species; IU: International unit.

Table 2. Multiple sclerosis simulation methods.

Study ID	Approach	Simulation evidence	Duration
Abo Taleb et al. 2019	400 mg/kg/day cuprizone (oral gavage with 1% CMC)	Progressive decline in locomotor activity	5 weeks
Vakilzadeh et al. 2016	Diet containing 0.2 % cuprizone mixed into ground standard rodent chow	NA	5 weeks
Wen et al. 2016	Injected subcutaneously with 200 µg of MOG 35–55 peptide emulsified in complete Freund's adjuvant containing 500 µg <i>Mycobacterium tuberculosis</i>	When an animal showed a clinical score ≥ 0.5	7 weeks
Escribano et al. 2022	100 µL of a solution containing 150 µg of MOG	NA	14 days
Ghareghani et al. 2017	Subcutaneously over the flank with 200 µL of a 1:1 mixture of 1 g GPSC in 1 mL PBS and complete Freund's adjuvant (CFA, Sigma-Aldrich, USA) and 1 mg/mL <i>Mycobacterium tuberculosis</i> bacteria enriched	NA	NA
Ortíz et al. 2022	Sterile physiological saline solution and emulsified with 50 µL of complete Freund's adjuvant containing 1 mg/mL heat-killed <i>Mycobacterium tuberculosis</i> strain H37Ra	NA	NA
Ghareghani et al. 2022	Immunization solution containing the immunogenic epitope MOG-35-55 was emulsified with complete Freund's adjuvant (CFA, Sigma-Aldrich) and <i>Mycobacterium tuberculosis</i> , and was injected subcutaneously over the flank	NA	NA
Ghareghani et al. 2023	Cuprizone (0.2% wt/wt) was incorporated into standard irradiated ground rodent chow	NA	NA
Gharib et al. 2022	Ad libitum and fed 0.2% (w/w) cuprizone (Sigma) in ground breeder chow	Behavioral stress	5 weeks
Ramos González et al. 2021	150 µL of a homogenate containing 25 µL of pig spinal cord homogenate, 25 µL of pig brain homogenate, and 100 µL of Freund's complete adjuvant	EAE, achieving at least grade 2 of the disease	NA
Ghareghani et al. 2018	Emulsified in complete Freund's adjuvant (CFA, Sigma-Aldrich), enriched <i>Mycobacterium tuberculosis</i> bacteria	NA	NA
Jand et al. 2022	Injection of MOG35-55, 300 ng of pertussis toxin (List Biological Labs, Campbell, CA, USA) intraperitoneally, into all animals, on days 0 and 2	NA	NA
Labunets et al. 2023	Cuprizone at 0.2% (w/w)	NA	3 weeks

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Study ID	Approach	Simulation evidence	Duration
Álvarez-Sánchez et al. 2015	Immunized subcutaneously in each hind leg with 100 µg of MOG35–55 (Cambridge Research Biochemicals), emulsified in CFA (Sigma) containing 50 µg of heat-killed <i>Mycobacterium tuberculosis</i>	NA	NA
Chen et al. 2016	MOG was purified to greater than 95% purity using high-performance liquid chromatography. Each C57BL/6 mouse was immunized with 100 µg of MOG in 100 µL of an emulsion of complete Freund's adjuvant and 400 µg of <i>Mycobacterium tuberculosis</i> H37Ra	NA	NA
Long et al. 2018	Injected subcutaneously at one side of the flank with 250 µg MOG35–55 peptide in complete Freund's adjuvant and 64 mg/mL of heat-killed <i>Mycobacterium tuberculosis</i> . At 0 h and 48 h after MOG injection, 500 ng pertussis toxin in 100 µL PBS was injected intraperitoneally	Neurological deficit	NA
Ghareghani et al. 2018	Immunized with MOG	Clinical signs of the disease	NA
Dokoohaki et al. 2017	Injected subcutaneously over the flank with 200 µL of a 1:1 mixture of 1 g of GPSC in 1 mL PBS and complete Freund's adjuvant and 1 mg/mL enriched <i>Mycobacterium tuberculosis</i> bacteria	Clinical signs	NA
Alghamdi et al. 2020	0.2 mL cuprizone orally	NA	5 weeks

Note: MOG: Myelin oligodendrocyte glycoprotein; GPSC: Guinea pig spinal cord; PBS: Polybutylene succinate; EAE: Experimental autoimmune encephalomyelitis.

3.5. Dosages of melatonin consumption and outcomes

The concentration of 3 mM significantly reduced iNOS production [27]. Also, melatonin blocked the expression of reactive oxygen species generation in reactive microglia [27]. Melatonin at both 50 and 100 mg/kg significantly increased the nociception latency ($p < 0.01$). Moreover, melatonin at 100 mg/kg significantly diminished the mean number of TUNEL-positive cells in the medial region of the corpus callosum compared to the cuprizone group ($p < 0.01$) [36]. Melatonin at 50 mg pretreatment significantly decreased H-89-induced cytochrome C release compared to control group ($p < 0.05$), and melatonin at 100 mg showed no significant changes [34] (Table 1).

3.6. Melatonin and other concomitant therapies

Melatonin treatment, alone ($p < 0.01$) or in combination with H-89 ($p < 0.001$), remarkably augmented target quadrant occupancy in comparison with H-89-treated animals [34]. Combination of diisopropylamine dichloroacetate (DADA) and melatonin further amended melatonin's effects, and it led to a positive effect on diminishing the disability. Another positive interaction occurred in the course of decreasing the loss of oligodendrocytes assessed by levels of myelin basic protein (MBP) and myelin-associated oligodendrocytic basic protein (MOBP) ($p < 0.0001$) [21]. Melatonin had significant influence on lactate reduction ($p < 0.01$) and DADA positive interaction with melatonin for that effect ($p = 0.01$) [21].

Melatonin did not lead to cognitive enhancement, contrary to the influence of mesenchymal stem cells macrovesicles (MSC-MVs) [23]. To some extent, it inhibited the positive effect of MSC-MVs on cognition. In Gharib et al. [23], melatonin significantly improved locomotor activity at the same extent as MSC-MVs+melatonin. Melatonin and MSC-MVs both improved psychomotor status compared with EAEs but there was synergism between them ($p < 0.0001$) [23]. Cognitive abilities improved significantly with MSC-MVs but decreased significantly with melatonin, and the combination made no difference compared with controls and EAE [23]. Tissue malondialdehyde (MDA) decreased significantly compared with EAE in both melatonin and with MSC-MVs. Tissue glutathione (GSH) in both melatonin and MSC-MVs increased significantly compared with EAE but it was still lower than controls [23].

Therapeutic intervention with methylprednisolone 30 mg/kg resulted in a highly significant decrease ($p < 0.001$) in mean clinical scores [32]. Methylprednisolone caused a significant decrease ($p < 0.01$) in serum IFN- γ and increase in IL-4 [32].

Therapy with GA and IFN-1 β both non-significantly improved cumulative disease index score compared with EAE [20]. Melatonin alone or in combination with GA or IFN-1 β significantly ameliorated the disease clinical score ($p < 0.05$) [20]. Treatment with melatonin alone caused significant reduced plasma levels of TNF α , IL-1 β , and IL-6 compared with EAE ($p < 0.05$) [20]. Melatonin, however, did not alter the amount of IL-1 β after EAE compared with controls [20].

In midbrain, levels of nitrite–nitrate significantly decreased compared with EAE in all medications except GA and melatonin combination, which was even higher than controls [20]. End products of lipid peroxidation significantly decreased compared with EAE in all combinations in midbrain and motor cortex except for IFN- β alone, which was significantly higher compared with controls and unchanged compared with EAE in motor cortex [20].

Synergistic effects were shown in the combination of melatonin and H-89 as the mean of time spent in target quadrant ($p < 0.001$) compared with H-89 alone. Melatonin also inhibited the H-89-induced mitochondrial dysfunction ($p < 0.0001$). Melatonin at 50 and 100 g/kg alone or in combination with H-89 significantly decreased the mitochondrial swelling in comparison with H-89 [34]. Regarding escape latency, the main effects of melatonin ($p = 0.0001$) and the combination of H-89 and melatonin ($p = 0.008$) were significant, while the effect of only H-89 was not remarkable ($p < 0.13$). On the other hand, the main effects of H-89 ($p < 0.02$), melatonin ($p = 0.0001$), and the combination of H-89 and melatonin ($p = 0.004$) were significant for traveled distance [34]. Furthermore, combination groups showed a significant decrease in both time and distance of finding a hidden platform compared with the corresponding H-89 group ($p < 0.0001$) [34].

Baclofen did not make any significant changes on IL-4 and IFN- γ levels; however, melatonin significantly increased IL-4 and reduced IFN- γ [31]. Also, mean cumulative clinical score decreased significantly with melatonin compared with EAE [31]. Baclofen and melatonin individually reduced MDA ($p < 0.05$) and the combination was synergetic ($p < 0.01$) [31].

MS treatment with methylprednisolone caused a significant decrease in the levels of serum melatonin, whereas the decrease in polybutylene succinate (PBS)-treated EAE group was not significant [32]. Methylprednisolone injection on day 1 caused a significant ($p < 0.05$) reduction of melatonin levels on day 2, which was further decreased ($p < 0.01$) on day 3, after a second injection on day 2 [32] (Table 1).

3.7. Sex and age effects

Melatonin led to a significant improvement in total distance movement ($p = 0.0003$) and grip strength ($p < 0.0001$) in male mice compared with females [16].

Melatonin diminished numbers of ratio of CD3+CD4+/CD3+CD8+ cells, independently of the influence of cuprizone, in both aged and young mice. The number of activated macrophages decreased after melatonin supplementation for both ages, reaching that of controls. After melatonin administration, MDA content decreased to that of controls in young mice and even lower compared with the control group among aging ones [27] (Table 1).

3.8. Effect of melatonin on cytokines

Application of melatonin for the last 7 days of cuprizone feeding did not change IL-6 level compared to the cuprizone group [36]. IL-4 levels in PBS- and melatonin-treated EAE rats were significantly lower ($p < 0.001$) than those of controls. The ratio of IFN- γ /IL-4, which acts as an indicator of Th-1/Th-2, was found to be significantly higher in PBS- ($p < 0.001$) and melatonin-treated ($p < 0.001$) subjects [19]. DADA exhibited no changes in serum levels of IL-1B, TNF, IL-4, and IL-10, while melatonin reduced the proinflammatory and enhanced the anti-inflammatory serum levels in mice [21]. There were no significant changes in the percentages of CD69+ cells within the CD4, CD8, or CD19 lymphocyte subset, pointing that melatonin primarily plays its role independently of T-cells count differentiation. Th1 cells in the CNS were slightly lower, and there were no significant differences in the percentages of these cells in either the spleens or CNS of the melatonin-treated and solvent-treated mice [29] (Table 1).

4. Discussion

Our findings showed that melatonin administration contributed to the restoration of normal circadian rhythms in animals. Notably, both 50 and 100 mg/kg doses of melatonin demonstrated efficacy in enhancing nociception latency. Combining melatonin with other substances, such as H-89, diisopropylamine dichloroacetate, gibberellins, and IFN-1 β , yielded varying effects, with no observed improvement in cognition when combined with mesenchymal stem cells. Interestingly, male subjects exhibited a significantly more favorable response in certain tests. Some results differed for aged and young animals, which requires more attention for future studies. Findings regarding the impact of melatonin on cytokines were contradictory; yet, on the whole, melatonin administration tended to reduce proinflammatory cytokines.

There are several suggested mechanisms for MS to lead to disruptions in circadian rhythms in humans. In this regard, ganglion cell atrophy and compromised integrity of the retino-hypothalamic tract result in the disruption of central clock synchronization by light/dark signals [37]. Also, there are disruptions in the rhythms of melatonin secretion, potentially contributing to sleep disturbances and fatigue in individuals with MS, that may be attributed to altered central clock rhythmicity [37]. Peripheral rhythm changes in MS have been observed for certain metabolic hormones like leptin and corticosterone. Together with variations in gut microbiome composition, these changes might contribute to alterations in metabolic functions in MS. Furthermore, disturbances in immune rhythms could potentially contribute to immune dysregulation in MS [37]. Notably, MS is associated with genetic polymorphisms within various clock genes, underscoring the role of genetic factors in MS predisposition [37]. Human studies showed that melatonin levels are lower in patients with MS compared with controls [13]; this can also be one of the explanations for circadian rhythms in MS. In our scoping review on animal studies, melatonin led to normal circadian rhythms in most subjects, whether it was administered as a single therapy or combined with other medications. In the same way as with our findings, a systematic review on observational and interventional clinical studies concluded that melatonin could affect inflammation and oxidative stress, but its application in clinical practice and its impacts on sleep, cognition, and fatigue in patients with MS need further investigations [12].

We revealed that melatonin can be useful in the different doses used in animal studies, like 50 and 100 mg/kg, through its effects on iNOS expression and OPCs. Similarly, previous studies on the roles of melatonin in MS showed that elevation of proinflammatory cytokines triggers an excessive generation of reactive oxygen and nitrogen species. They initiate the activation of nuclear kappa B factor in various cells within the CNS, including T cells, microglia, macrophages, astrocytes, oligodendrocytes, and neurons. Nuclear kappa B factor positively regulates the expression of factors implicated in the pathogenesis of MS, such as TNF- α , iNOS, IL-1 α , vascular adhesion molecule 1, and several growth factors [14,38].

Our findings showed that melatonin administration attenuated H-89-induced memory deficit and lowered escape latency and distance traveled to a control level. To the best of our knowledge, this mechanism has not been described in previous in vitro or clinical studies, and it was solely mentioned in the study by Sharif and colleagues [34]. Together, these data imply that melatonin could reverse the H-89-induced memory deficits, inferring a protective role of melatonin. Regarding other medications, one of our included studies showed corticosteroids significantly reduced melatonin in both rats and humans with MS [32]. In this regard, cortisol, a glucocorticoid hormone, has also been associated with the progression and severity of MS; reduced cortisol levels are associated with feelings of fatigue and

urinary dysfunction, whereas elevated levels have been associated with increased anxiety and depression [39].

The study by Gharib et al., which was included in our scoping review, showed that melatonin did not lead to improvements in cognition, in spite of the positive effects of MSCs [23]. Existing evidence shows the combined effects of melatonin and MSCs on different diseases like MS [40]. In detail, melatonin has the capacity to decrease the release of inflammatory cytokines, boost the proliferative capabilities of MSCs, and prevent the apoptosis of MSCs [41]. Moreover, given the increasing interest in melatonin, it also plays roles in regulating the expression of genes related to both reactive oxygen species generation and antioxidant activity [41].

The study by Farhadi et al. on patients with MS and healthy controls showed a decreased level of melatonin and increased levels of cytokines like TNF- α , which can highlight the roles of melatonin in MS [42]. In accordance with the mentioned findings, we also found that levels of different cytokines like IL-1a, IL-6, IL-2, IFN, TNF, IL-17 α , and IL-22 decreased in those receiving melatonin.

To the best of our knowledge, this is a pioneer scoping review conducted on animal studies to evaluate the effects of melatonin, alone or combined with other drugs, on outcomes of animals with MS. So, the findings of this study can be used for designing future animal studies or randomized controlled trials on humans to assess their effects. It can also shed light on the mechanisms of action of melatonin on MS and possible use as an adjuvant treatment in the future. However, the study has several limitations that should be considered while interpreting its findings. First, there is a possibility that our search strategy may have overlooked certain studies, although we performed searches in several databases. Second, as it was a scoping review, we did not perform a quality assessment of included studies. This point can be considered in following systematic reviews. Third, we were unable to perform a meta-analysis across diverse study designs and outcomes, so we used a narrative approach to report the findings. Fourth, there is a likelihood of publication and reporting bias, although we could not quantify such biases. Fifth, most details about the methodological aspects of animal studies, which were included, were inadequately reported. Sixth, the studies were conducted on mice and rats, while the effects on other types of animals were not evaluated. Finally, because of the fact that the study was conducted on animal studies, the findings cannot be generalized to humans. So, the application of these findings in clinical settings needs to be evaluated in future clinical trials and systematic reviews.

5. Conclusions

Melatonin, in different doses and in combination with other drugs or alone, was shown to have generally improved results in terms of oxidative stress, anti-inflammation, and cytokine levels. Considering the limitations, future primary studies should be conducted on other types of animals. Moreover, systematic reviews and meta-analysis with a higher number of studies are recommended to be performed.

Author contributions

Heliya Bandehagh: Conceptualization and study design; Heliya Bandehagh and Farnaz Gozalpour: Search; Farnaz Gozalpour, Ali Mousavi, Mahdi Hemmati Ghavshough: Data acquisition; Heliya Bandehagh and Ali Mousavi: Data analysis/interpretation; Mahdi Hemmati Ghavshough: Supervision/mentorship; All authors: Drafting of the manuscript. All authors had a critical role in

revision of the manuscript for important intellectual content. The final manuscript was read and approved by all authors.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflict of interests

The authors declare that they have no conflict of interests.

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