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# Melatonin as a potential adjunct therapy for central nervous system lupus: evidence from the MRL/lpr mouse model

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**Dear Editor,**

Systemic lupus erythematosus (SLE) is a multifaceted autoimmune disorder that affects over a million Americans, predominantly women, with a female-to-male ratio of ∼9 : 1 and higher prevalence rates in African-Americans and Hispanics. Despite advancements in treatment strategies, individuals with SLE still face a significantly reduced life expectancy. Current treatments, such as immunosuppressants and corticosteroids, are effective but carry risks of severe side effects, including malignancies. This highlights the urgent need for innovative therapeutic approaches. Recent research has increasingly acknowledged the influence of lifestyle factors—such as diet, exercise, stress, and sleep—on the progression of SLE [1], suggesting that lifestyle interventions could serve as valuable adjunct therapies to existing treatments.

Exercise is advised by international task forces, while adequate sleep is emphasized due to its role in mitigating fatigue—a common issue in SLE patients. Research indicates a connection between sleep disorders and disease activity in SLE [2], with insufficient sleep and obstructive sleep apnea increasing susceptibility to SLE and correlating with disease severity. Sleep is modulated by melatonin, a hormone linked with circadian rhythms. Melatonin levels are reduced in lupus patients [3] and are associated with disease severity. Studies have shown the positive impact of melatonin on lupus nephritis but its effect on central nervous system (CNS) lupus remains incompletely understood and needs further investigation.

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indoleamine hormone known for its role in regulating circadian rhythms and possessing anti-oxidative and anti-inflammatory properties. Although primarily synthesized in the pineal gland, melatonin is also expressed in other tissues such as the gastrointestinal tract, skin, and retina, as well as by immune cells. Its effects on hematopoiesis, cytokine production, and overall immune function are well documented. It is established that melatonin acts on the sleep/wake cycle by regulating sleep-related neurotransmitters and neural circuits. This study will use melatonin as a tool to elucidate the impact of sleep on CNS lupus using the MRL/lpr mouse model.

The MRL/lpr mouse is a well-established model for studying CNS lupus, demonstrating various clinical and pathological features comparable to human SLE, including skin rashes, kidney dysfunction, and CNS manifestations. While human SLE exhibits variable disease activity, murine SLE progresses consistently, providing a stable framework for study. As mentioned earlier, SLE is more prevalent in females, and therefore our study focused on female mice. Notably, this model shows pathological similarities to human lupus, such as edema and behavioral changes like anxiety and cognitive impairment. One critical protein, aquaporin 4 (AQP4), involved in brain water balance and edema development, is highly conserved and expressed in the plasma membranes of ependymal cells and astrocytes [4]. In this study we will probe the underlying disease-causing mechanisms, focusing on their effects on overall wellness, bone density, complement activation, cytokine levels, and brain function.

Results: In our study, female MRL/lpr mice showed significantly improved behavior and lupus disease pathology when treated with melatonin (1 mg/kg body weight from 12 to 22 weeks). Characteristic disease-associated changes such as necrosis and skin rashes were significantly reduced in melatonin-treated mice. The treated mice had a reduction in spleen weight (lpr 0.4 ± 0.03 mg vs treated 0.28 ± 0.05 mg, Fig. 1A) and improved bone density (Fig. 1B), however, body composition measures did not differ significantly between groups (results not shown). Furthermore, melatonin-treated mice demonstrated good nest-building behaviors (Fig. 1D, supplementary Fig. 1, see online supplementary material), indicative of overall wellness, and lower fecal boli counts (Average number of fecal boli: MRL/lpr mice 5.43 ± 2.2 vs treated 2.14 ± 1.3, $P < 0.05$, Fig. 1C), suggesting reduced anxiety. Behavioral despair tests, including tail suspension (immobility time : MRL/lpr mice 98 ± 56 sec vs treated 43 ± 33 sec, $P < 0.05$, Fig. 1E) and forced swimming (immobility time: MRL/lpr mice 143 ± 23 sec vs treated 67 ± 43 sec, $P < 0.05$, Fig. 1F) tests showed that melatonin treatment decreased immobility times, reflecting reduced depressive-like behavior and indicating improved brain function. The brain is protected from circulating toxins by the blood–brain barrier (BBB). Brain tissue analyses revealed alleviation of altered BBB permeability (supplementary Fig. 2, panel 1, CD31 staining, see online supplementary material) and reduced brain edema (80 ± 1.3 mg vs 77 ± 0.6 mg in treated, Fig. 1I), gliosis (increase in astroglial cells, supplementary Fig. 2, panel 2, Glial fibrillary acidic protein (GFAP) staining) and AQP4

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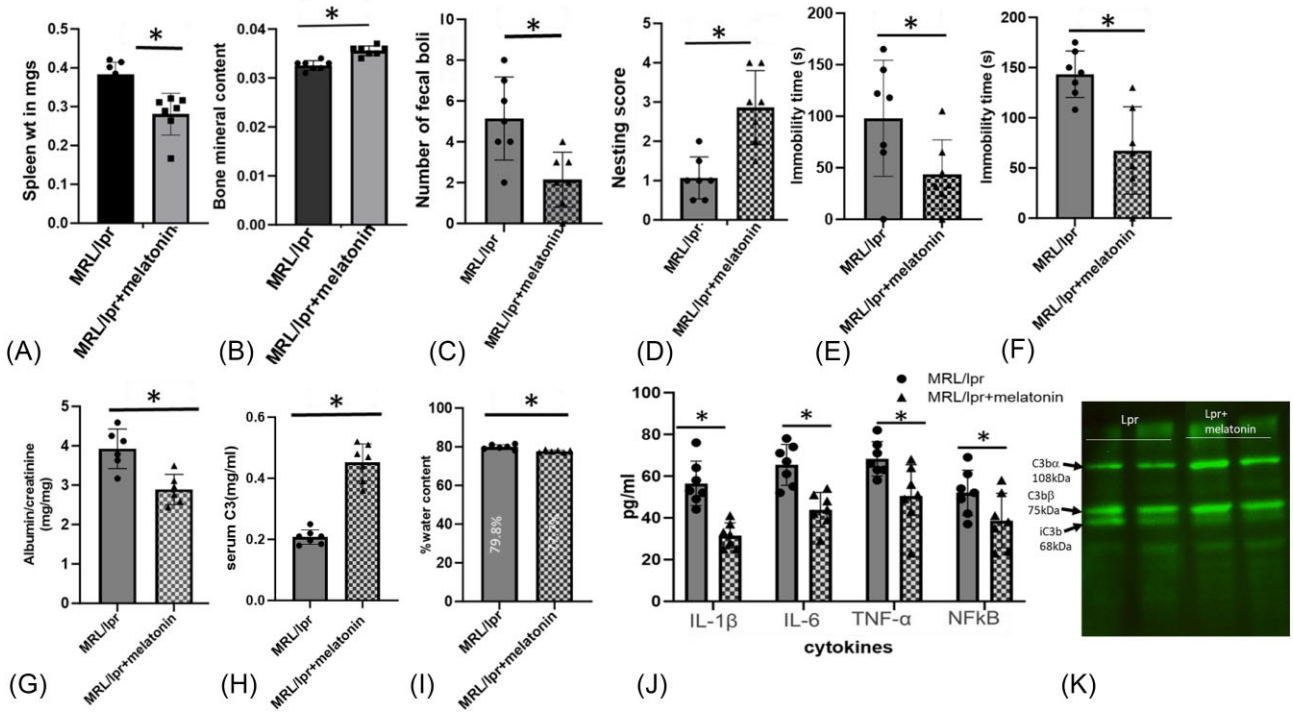


**Figure 1.** Melatonin treatment improves disease pathology in MRL/lpr mice. Compared to the MRL/lpr mice, in mice treated with melatonin: (**A**) spleen weights are reduced; (**B**) bone mineral content assessed by Dexa is increased; (**C**) number of fecal boli is reduced; (**D**) nesting behavior score is increased with noticeable nests constructed; (**E**, **F**) reduced despair behavior, dependent on immobility time is observed using, tail suspension test (E) and forced swimming (F); (**G**) kidney function is improved with reduced albuminaria; (**H**) serum C3 assessed by ELISA is increased; (**I**) water content in brain is decreased indicating edema is decreased; (**J**) concentration of proinflammatory cytokines, IL-1$\beta$, IL-6, TNF-$\alpha$ and NF$\kappa$B are reduced; and (**K**) western blot showing reduced iC3b formation indicating reduced complement activation. $n = 7$/group. Normally distributed data are given as mean ± standard deviation (SD). $^*p < 0.05$.

expression (water channel in brain, supplementary Fig. 2, panel 3, AQP4 staining) in melatonin-treated mice. Melatonin treatment significantly lowered urinary albumin levels ( albumin/creatinine, mg/mg, Fig. 1G), indicating improved renal function. The inflammatory responses normally observed in lupus mice and lupus patients were significantly toned down with reduced complement activation (Fig. 1K), altered serum C3 levels (Fig. 1H), and significantly reduced serum levels of proinflammatory cytokines (interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), tumor necrosis factor(TNF)$\alpha$ , IFN-$\gamma$) (Fig. 1J).

Discussion: Earlier studies showed that melatonin treatment reduced the disease pathology in lupus nephritis, suggesting that melatonin might be an effective therapeutic target for lupus. In the present study, we investigated whether melatonin treatment could alleviate CNS manifestation of lupus along with the possible behavioral and molecular mechanisms involved, a facet that remains incompletely understood. Earlier studies from our lab revealed an increase in AQP4 expression and loss of BBB leading to edema and inflammation in the MRL/lpr brain. Our present findings show for the first time that melatonin treatment ameliorated brain pathology, and reduced inflammatory markers in the MRL/lpr mouse model, thereby leading to improvements in behavioral outcomes.

Cytokines play a critical role in lupus pathology leading to organ damage and tissue injury. In a pristine induced lupus model, melatonin reduced IL-6-reducing antibodies [5]. Our results show that melatonin reduces the cytokines TNF-$\alpha$, IL-6, IL-1$\beta$, and NFkB. These inflammatory molecules activate different pathways such as the inflammasome and canonical NFkB pathways. However, since, NFkB is involved in both the onset and resolution of inflammation further research is needed to gain insight into the underlying mechanisms in the setting of melatonin treatment. In addition, our results show reduced complement activation that will lead to limited generation of proinflammatory molecules such as iC3b, C3a, and C5a, thereby reducing the infiltration of inflammatory cells.

Recently there have been several reports of lupus cases with hypertension and loss of BBB integrity with cerebral edema ([6], [7]). Earlier studies from our lab showed that these changes could be reduced by complete complement inhibition in MRL/lpr mice [8]. Complement participates in an increasing list of non-canonical functions. Therefore, its inhibition could alter homeostasis, and also leave the patient open to infections. Here we show that melatonin alleviates BBB alterations and AQP4 expression resulting in limited edema. Whether melatonin is regulating these changes through complement modulation needs further investigation.

Conventional therapies for lupus such as cyclophosphamide and prednisolone, significantly alleviate disease symptoms and enhance the life quality for these patients, but is accompanied by toxic effects that include neurological manifestations such as anxiety, depression, and cognitive deficits. Our results show for the first time, using a battery of tests that have been developed to understand the equivalent of human pathological anxiety and depression in rodents, that melatonin is protective in a lupus setting, protecting the brain and its function.

These findings support the potential of melatonin as an adjunct to existing therapies and highlight the importance of lifestyle interventions in managing SLE. Melatonin, through its regulation of circadian rhythms was shown to have numerous significant benefits. Melatonin holds promise as an adjunctive therapy for CNS

lupus and our studies provide a foundation for future research to integrate melatonin and lifestyle modifications into comprehensive SLE treatment strategies. The impact of melatonin is dependent on the time of administration and sex of the mouse ([9], [10]). Since SLE is a chronic disease with flares and periods of quiescence, further research is warranted to explore the optimal timing and dosage of melatonin, its effects during different disease phases, and its role in other SLE-related complications.

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## Author contributions

Jaivarshaan Rajkumar (Data curation, Investigation, Methodology), Alexandra Spath (Data curation, Investigation, Methodology, Validation), Hyndevi Dokuri (Data curation, Methodology), Alexander Jacob (Methodology, Supervision, Validation, Writing—original draft), Richard J. Quigg (Conceptualization, Data curation, Resources, Writing—review & editing), and Jessy Alexander (Conceptualization, Methodology, Project administration, Supervision, Validation, Writing—original draft, Writing—review & editing)

## Supplementary data

Supplementary data are available at *PCMEDI* online.

## Conflict of interest

None declared. As an Editorial Board Member of *Precision Clinical Medicine*, the corresponding author J.J.A. was blinded from reviewing and making decisions on this manuscript.

## Ethics statetment

All mice procedures and work described in this study were approved by the Institutional Animal Care and Use Committee. The protocol complies with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals, and the ethical principles established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011).

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