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*Original Article*

# The protective effect of melatonin on chronic paradoxical sleep deprivation induced metabolic and memory deficit in rats

Omyma G Ahmed, Ghada S Mahmoud, Selvia S Samy, Sally A Sayed

*Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt*

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**Abstract:** Backgrounds: Impaired sleep is independent risk factor of neurodegeneration and dementia. Chronic insomnia impairs melatonin (MEL) production that is directly proportionate to its duration. The underlying mechanisms linking sleep loss to dementia and the possible therapeutic effect of melatonin have not been fully elucidated. Previous research showed great controversy concerning the effects of paradoxical sleep deprivation (PSD) on body weight, serum lipoproteins, and inflammatory cytokines. Goals: To examine the effect of chronic paradoxical sleep deprivation (PSD) with and without MEL supplementation on memory using RAWM, parameters of metabolic syndrome (MS), liver enzymes, serum cortisol, and inflammatory cytokines as well as liver, colon, and brain histopathology. Methods: Forty rats were divided into four groups ten animals each; C: control, G: grid group, SD: sleep deprivation group, and SD+MEL sleep deprivation treated with melatonin. Results: MEL supplementation reversed PSD-induced memory deficits ($P<0.05$), the elevation of serum cortisol ($P<0.001$), glucose ($P<0.05$), ALT ($P<0.05$), AST ($P<0.001$), TNF-alpha ($P<0.001$), IL-10 ($P<0.01$) and improved colon, liver, and brain architecture. Melatonin reduced body weight ($P<0.05$), total cholesterol, LDL-c, and triglycerides as well as increased HDL-c ($P<0.001$). Conclusion: MEL has a protective effect against chronic PSD-induced metabolic malfunction and cognitive deterioration by reducing stress, improving immunity, and maintaining colonic wall integrity.

**Keywords:** Melatonin, paradoxical sleep deprivation, metabolic syndrome (MS), radial arm water maze (RAWM), prefrontal cortex (PFC), hippocampus, liver, tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10)

## Introduction

Several previous studies highlighted the correlation between sleep loss and neurodegenerative diseases such as Alzheimer disease [1-3]. However, the underlying mechanisms linking sleep deprivation to dementia have not been fully elucidated. On the other side, a sleep laboratory study of old U.S. adults revealed little association between self-reported insomnia and cognitive decline [4]. The central question of this study was to examine the effect of chronic paradoxical sleep deprivation (PSD) on the learning and memory function of the brain. Moreover, to examine the histopathological changes of brain areas responsible for memory and cognition such as the hippocampus and prefrontal cortex respectively.

Metabolic syndrome is considered an important risk factor for Alzheimer's disease [5]. Recently, the relationship between sleep loss and metabolic syndrome (MS) has attracted wide attention. Systematic review and meta-analysis including 18 studies and 75657 participants aged from 18-96 years revealed that a short sleep duration of fewer than 5 hours per day was associated with MS [6]. Moreover, sleep restriction caused systemic inflammatory status that may be a source of metabolic, cardiovascular, and cognitive impairments [3, 7]. Increased production of tumor necrosis factor-alpha (TNF-α) impaired the blood-brain barrier, cognitive function as well as intestinal barrier [7, 8]. Research in the literature demonstrated the association between sleep disturbance and colitis in both animal models and humans [9]. A previous study reported that chronic sleep loss caused low-grade neuroinflammation through the activation of proinflammatory cytokines, astrocytes, and microglia in rat hippocampus and piriform cortex [10]. Up to this point, the

current study was designed to elucidate the effects of chronic PSD on weight gain, glucose homeostasis, lipid profile, liver function, stress level, and immune system. Moreover, to examine the histopathological changes in the brain, colon, and liver.

A review of previous studies showed that MEL modulated the learning and memory process in several animal models. Moreover, a previous study proved the neuroprotective effect of MEL against 5-fluorouracil and methotrexate-induced oxidative stress by increasing the antioxidants and promoting neurogenesis in the hippocampus and prefrontal cortex (PFC) [11]. It was also reported that MEL supplementation restored normal sleep and reduced anxiety thus improving immunity [12]. Melatonin supplementation reduced the elevated TNF-alpha level in pinealectomized rats [13]. Previous studies showed the antiatherogenic effect of MEL regardless of the cause whether metabolic, dietary, or drug-induced [14]. They also reinforced the marked controversy between different studies concerning MEL dose and mode of administration that were effective in lowering cholesterol and highlighted the need for further studies on MEL efficacy in other lipid diseases. Moreover, MEL administration or restoring its expression is considered a good therapeutic strategy for liver disease [15, 16]. It was shown that PSD decreased MEL concentration in the colon tissue and feces of mice, increased colonic microbiota, and caused intestinal dysbiosis that were recovered by MEL supplementation [17]. Therefore, we examined the effect of MEL supplementation on rats exposed to chronic PSD for two weeks on memory function of the brain using a radial arm water maze (RAWM). Moreover, we tested the possible protective effect of MEL treatment for five weeks on the chronic PSD-induced changes in body weight, lipid profile, blood sugar, serum insulin, and liver enzymes as well as liver, colon, and brain histopathology.

## Methods

### *Drugs and chemicals*

Melatonin (Mel Product No. 7903 (n-Acetyl-5-Methoxy tryptamine)) purchased from Puritan's Pride Egypt © 2019, (http://www.puritanspride.com.eg/) 30 mg mashed, dissolved in 30 ml distilled water freshly prepared and given at a concentration of 10 mg/Kg/day orally daily by gavage for 21 days at 5:00 PM (1 hour before the start of induction of sleep deprivation) [18]. Rat golden-hamster cortisol ELISA Kit REF No. 04610049 from SIEMENS, ADVIA Centaur CP. Rat tumor necrosis factor-alpha (TNF-α) ELISA Kit Cat. No. CSB-E11987r from CUSABIO. Interleukin-10 (IL-10) ELISA Kit Cat. No. 201-12013 from Sun Red. Human Interferon Gamma (IFN-γ) ELISA Kit Cat. No. SG-10041 from Sino Gene Clon Biotech. Interleukin-6 (IL-6) ELISA Kit Cat. No. SG-10267 from Sino Gene Clon Biotech. Alanine aminotransferase (ALT-GPT) colorimetric Kit REF No. 264001 from SPECTRUM. Aspartate aminotransferase (AST/GOT) colorimetric Kit REF No. 260001 from SPECTRUM. Cholesterol - Liquizyme CHOD-PAP (Single Reagent) REF No. 230001 from SPECTRUM. High-density lipoproteins (HDL-cholesterol-precipitant) Kit REF No. 266001 from SPECTRUM. Triglycerides-Liquizyme GPO-PAP (Single Reagent) Kit REF No. 314001 from SPECTRUM. Low-density lipoprotein LDL = total cholesterol - (triglycerides/5 + HDL-cholesterol). Glucose-Liquizyme (GOD-PAP Single Reagent) Kit REF No. 250001 from SPECTRUM. Rat insulin (INS) ELISA Kit Cat. No. SG-20161 from Sino Gene Clon Biotech.

### *Animal groupings and experimental design*

Forty adults male Albino rats (100-150 gm) were bought from The Animal Core Facility of Assiut University, Assiut, Egypt. They were brought to the physiology lab 1 week before experimentation for adaptation. They were fed regular rat chow and water ad libitum and exposed to a normal 24 hr light/dark cycle and temperature of 25°C. Rats were divided into four groups ten animals each; C: Home cage control group, SD: Sleep deprivation group; G: Grid group; SD+MEL: Sleep-deprived treated with melatonin. In this study, we wanted to expose the rats to chronic paradoxical sleep deprivation. We used the modified multiplatform technique (MPT) in which the rat is put on a small platform in a tank filled with water to about 2 cm below the edge of the platform. Loss of muscle tone during paradoxical sleep caused the rat to touch the water and be awakened, therefore, selectively deprived of paradoxical sleep. The grid group was exposed to the same conditions as the SD group but allowed to sleep by putting stainless steel grid over the tank floor. The MPT, in which animals

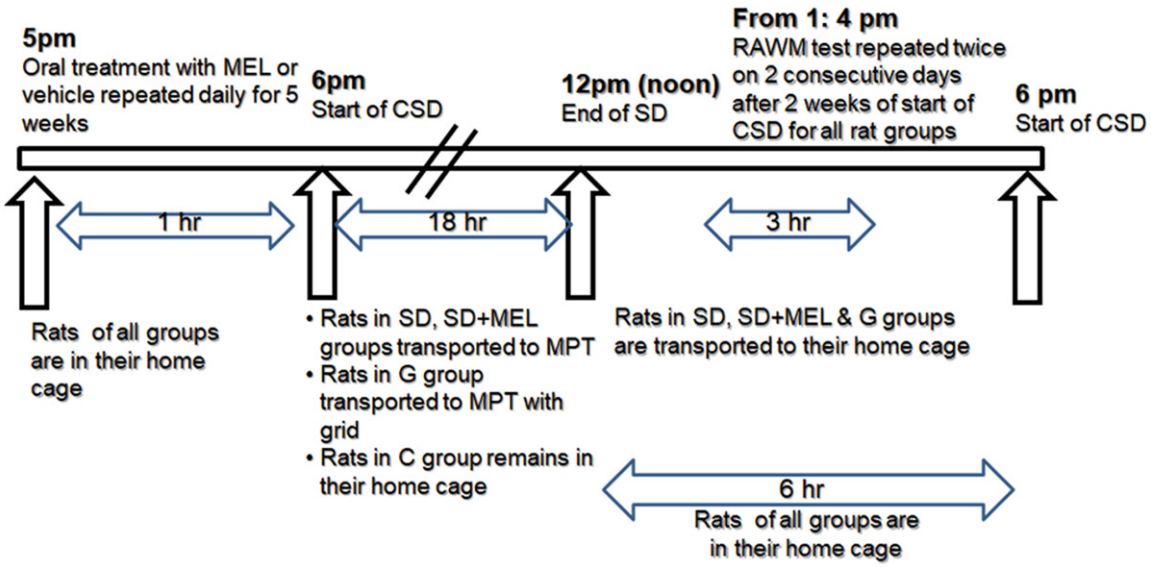


**Figure 1.** Schematic diagram of chronic sleep deprivation (CSD) procedure. MEL: melatonin, CSD: chronic sleep deprivation, RAWM: Radial Arm Water Maze, C: control group, G: grid group, SD: sleep-deprived group, SD+MEL: sleep deprived plus melatonin group, MPT: multiplatform tank.

were placed with new cohorts inside the water tanks, resulted in augmented ACTH and corticosterone responses. Therefore, a home cage control group was used to measure how much stress was added by MPT compared to normal conditions.

*Sample size calculation*

This was calculated according to the resource equation method. E = total number of animals - total number of groups. E = 40 - 4 = 36.

*Chronic sleep deprivation procedure*

Rats of SD and SD+MEL groups were sleep deprived for 18 hours from 6:00 P.M. to 12:00 on the next day and allowed 6 hours of sleep in their home cage per day from (12:00 noon to 6:00 P.M.) (**Figure 1**). Chronic sleep deprivation (CSD) continued for five weeks. We used a multiplatform stainless-steel tank (MPT) 110 cm in length, 20 cm in depth, and 60 cm in width. The tank contained 15 platforms (8.5 cm in height and 6.5 cm in diameter) and was filled with water 6 cm in height. When the rats of the SD group started paradoxical sleep, muscle hypotonia caused them to touch the water and be awakened, therefore, selectively deprived of paradoxical sleep [19]. The rats of the G group were located on a wire mesh steel grid in MPT to prevent them from falling into water under the same conditions. Rats of the SD+MEL group were treated with melatonin (MEL) 10 mg/Kg orally daily for 6 weeks at 5 pm (1 hour before the start of sleep deprivation). During the next 6 hours, SD, G, and SD+MEL group rats were transferred to their home cage. Rats of the C group were housed in their home cage for 5 weeks.

*Feeding conditions*

Rats of all groups had free access to food and water ad-libitum. Rats fed regular rat chow through iron wire nets located at the side of the water tank and water through bottles hanging on the tank cover. Iron nets and bottles were filled with food and water, respectively and the tank was cleaned and filled with water at a temperature of 25°C before 6:00 p.m. every day for 5 weeks. The home cage control group of rats was fed by placing chow pellets in a small container inside the cage and water through bottles hanging on the tank cover. The radial arm water maze (RAWM) test was on two days after two weeks of PSD. Blood sampling from all rat groups was done after 4 weeks of CSD.

*Behavioral tests*

*Radial arm water maze:* Forty rats were tested over fifteen trials after 2 weeks of PSD. Testing was repeated on 2 consecutive days. After test-

ing on the first day, the rats of all groups returned to their home cage. After two hours, rats of the SD group and SD+MEL group returned to the MPT and were subjected to PSD for 18 hours. Rats of the grid group returned to MPT with a grid. The control rat group stayed in their home cage. In each trial, we calculated the time to reach the pedestal (TRT), working memory errors (WME), and reference memory errors (RME). Entering the wrong arm for the first time is considered RME and entering the wrong arm more than once is considered WME. The average of three consecutive trials is presented as five blocks on each day. Failure of the rat to reach the pedestal in 1 minute is considered an incorrect trial. The rat was guided to the pedestal and allowed to explore the surrounding area for 1 min. Rat refusing to stay on the platform, swimming in circles, floating, jumping, or climbing was excluded [18].

*Biochemical parameters*

Blood sampling from 10 rats from each group and serum isolation was done after 4 weeks of the start of CSD and stored at -20°C for further analysis. Serum levels of cortisol, TNF-α, IL-10, IL-6, IFN-γ, and insulin were measured using ELISA and following the manufacturer's instructions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total cholesterol (TC), high-density lipoproteins (HDL-c), Triglycerides (TGs), and glucose were measured using the colorimetric method, and following the manufacturer instructions. Low-density lipoprotein (LDL-c) was calculated using the following equation [LDL-c = TC - (TGs/5 + HDL-c)]. All biochemical analysis was done using Auto Biochemistry Analyzer Stat Fax 3300 and Stat Fax 2100 ELISA reader.

*Histopathology*

After 5 weeks of CSD, rats were anesthetized with ketamine (50 mg/Kg). Animals were euthanized by humane cervical dislocation at the atlantooccipital region and rapid decapitation done by an experienced Staff member who strictly adheres to IACUC-approved protocols and institutional policies to avoid disturbance of brain tissue according to guidelines for the Use of Cervical Dislocation for Rodent Euthanasia the University of Texas at Austin Institutional Animal Care and Use Committee and all efforts were made to minimize suffering. The skulls were opened, and the brains were removed and washed with ice-cold physiologic saline solution (0.9%). Samples from the hippocampus, prefrontal cortex, colon, and liver were taken from six rats per group and fixed in 10% of neutral buffered formalin at room temperature for 24 hours. The samples were dehydrated in increasing concentrations of ethanol and then routinely processed and embedded in paraffin blocks for sectioning. Sections (4 μm thick) were de-waxed, stained with hematoxylin and eosin (H&E) for all samples, and PAS stain for intestinal samples according to standard protocol and examined using Olympus CX31 light microscope. At least six microscopic fields were examined for each group. Tissue samples of the colon were oriented with longitudinally cut crypts to precisely assess alterations in the overall intestinal tissue architecture [20].

*Quantification of goblet cells*

To quantify the number of goblet cells, six randomly chosen microscopic fields were examined under magnification (×400) and the number of goblet cells was counted in each group using image J (IJ-1.46r software).

*Statistical analysis*

GraphPad Prism 5 (GraphPad Software Inc., LA Jolla, CA, USA) was used for data analysis. Data were presented as mean ± SEM. Data were compared among groups using One Way ANOVA or Two-Way ANOVA with Bonferroni Multiple Comparison as a post hoc test as appropriate. A (*P*) value of less than 0.05 was considered to represent a statistically significant difference. Neuronal and goblet cells count were calculated using Image J (IJ-1.46r software).

## Results

*Effect of melatonin and sleep deprivation on rat performance in radial arm water maze*

Significant increase in TRT in SD group ($P<0.05$) when compared to SD+MEL (B1) and G (B1 & B2) on day 1. A significant difference in TRT in the C group ($P<0.05$) compared to G (B1-2) and compared to the SD+MEL group (B1) on day 1. No significant difference between treated groups in TRT on day 1 (B3-5). No significant difference in TRT between the SD group and C

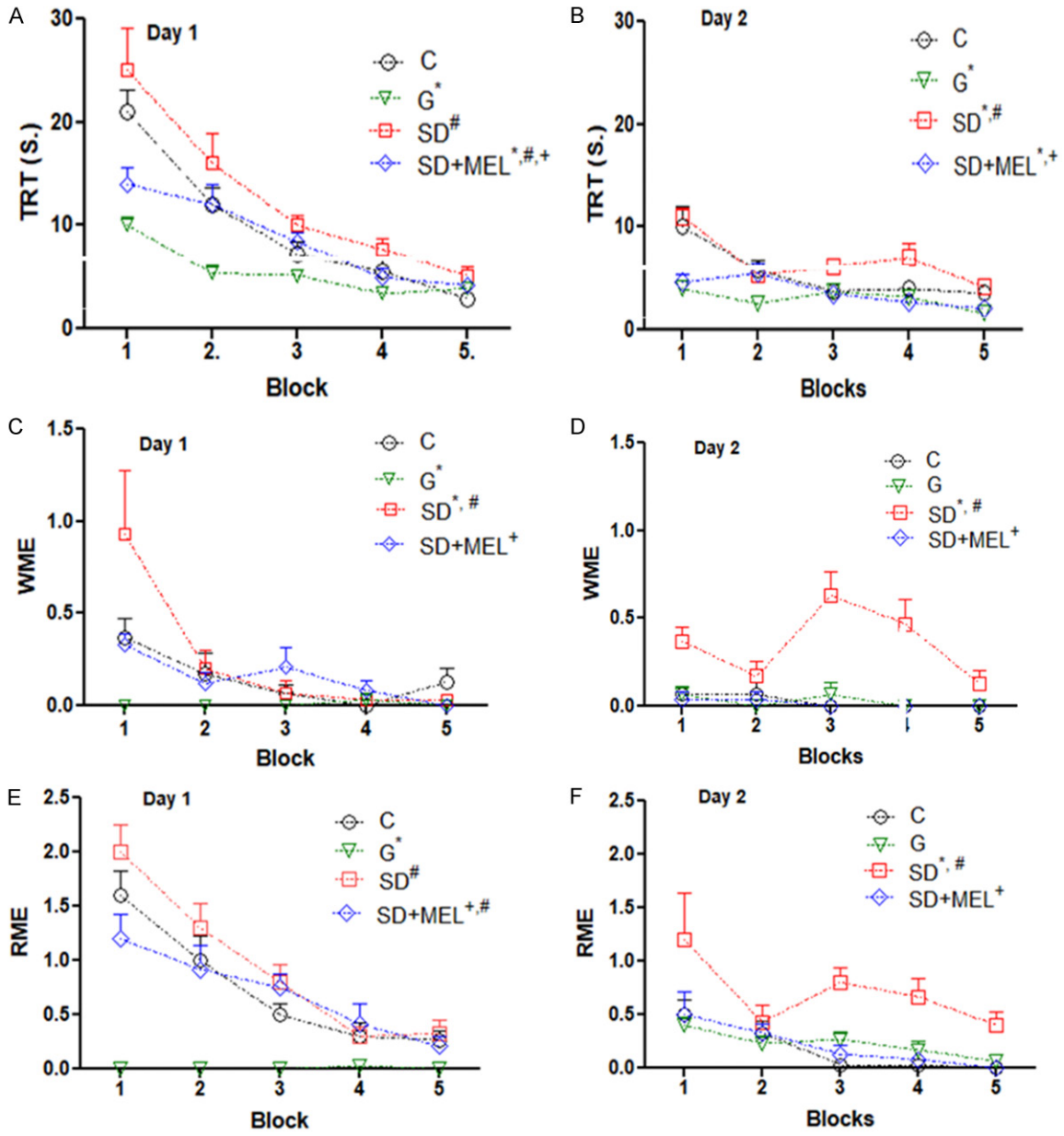


**Figure 2.** Effect of melatonin and sleep deprivation on rat performance in radial arm water maze. TRT: time to reach the target, WME: working memory errors, RME: reference memory errors, C: control group, G: grid group, SD: sleep-deprived group, SD+MEL: sleep deprived plus melatonin group; Two Way ANOVA with Bonferroni posttests; (*P<0.05) any group vs. C group, (#P<0.05) SD or SD+MEL groups vs. G group, (+P<0.05) SD+MEL group vs. SD group, N=10 in each group.

group on day 1 (**Figure 2A**; **Table 1**). A significant increase (P<0.05) in TRT in the SD group when compared to C (B4), G (B1 & B4), and SD+MEL groups (B1 & B4) on day 2. A significant difference (P<0.05) between C and G groups (B1) on day 2. No significant difference between treated groups in TRT on day 2 (B3 & B5) (**Figure 2B**; **Table 1**). Significant increase in WME in the SD group (P<0.05) when compared to C, G, and SD+MEL groups in B1. Significant decrease in WME in the G group (P<0.05) compared to the C group on day 1 (B1). No significant difference in WME between groups on day 1 (B2-5) (**Figure 2C**; **Table 1**). Significant increase in WME in the SD group (P<0.05) compared to the C group, G group, and SD+MEL in (B1, B3, B4) on day 2. An insignificant difference in WME between C, G, and SD+MEL

**Table 1.** The interaction of time and treatment on rat performance in radial arm water maze in different treated groups

| Source of Variation | Df | Sum-of-squares | Mean square | F | % of total variation | *P* value |
|---|---|---|---|---|---|---|
| TRT Day 1 | | | | | | |
| Interaction | 12 | 820 | 68 | 2.8 | 7.77 | 0.0015 |
| Treatment | 3 | 1244 | 415 | 17 | 11.79 | <0.0001 |
| Time | 4 | 4387 | 1097 | 45 | 41.57 | <0.0001 |
| TRT Day 2 | | | | | | |
| Interaction | 12 | 214 | 18 | 2.9 | 10.33 | 0.0010 |
| Treatment | 3 | 405 | 135 | 22 | 19.58 | <0.0001 |
| Time | 4 | 420 | 105 | 17 | 20.29 | <0.0001 |
| WME Day 1 | | | | | | |
| Interaction | 12 | 3.3 | 0.28 | 2.9 | 13.41 | 0.0013 |
| Treatment | 3 | 1.4 | 0.48 | 4.9 | 5.78 | 0.0026 |
| Time | 4 | 3.6 | 0.90 | 9.2 | 14.38 | <0.0001 |
| WME Day 2 | | | | | | |
| Interaction | 12 | 1.3 | 0.11 | 2.8 | 10.56 | 0.0018 |
| Treatment | 3 | 3.9 | 1.3 | 33 | 31.68 | <0.0001 |
| Time | 4 | 0.47 | 0.12 | 3.0 | 3.83 | 0.0195 |
| RME Day 1 | | | | | | |
| Interaction | 12 | 12 | 0.96 | 4.5 | 11.92 | <0.0001 |
| Treatment | 3 | 24 | 7.8 | 37 | 24.22 | <0.0001 |
| Time | 4 | 26 | 6.4 | 30 | 26.41 | <0.0001 |
| RME Day 2 | | | | | | |
| Interaction | 12 | 2.1 | 0.18 | 0.85 | 4.07 | 0.5954 |
| Treatment | 3 | 8.7 | 2.9 | 14 | 16.86 | <0.0001 |
| Time | 4 | 5.9 | 1.5 | 7.2 | 11.43 | <0.0001 |

Data represent mean ± SEM of; TRT: time to reach the target, WME: working memory errors, RME: reference memory errors. Two Way ANOVA with Bonferroni Multiple comparison test P<0.05 is considered significant; N=10 in each group.

groups on day 2 (B1-B5) was found (**Figure 2D**; **Table 1**). Significant increase in RME in the SD group (P<0.01) compared to SD+MEL (B1) and compared to the G group (P<.001) (B1-3) on day 1. Significant decrease in RME in the G group (P<0.001) compared to the C group (B1-2) and SD+MEL (B1-3) on day 1. No significant difference between the C group and SD group or C group and SD+MEL in RME (B1-5) on day 1 (**Figure 2E**; **Table 1**). Significant increase in RME in the SD group (P<0.05) when compared to C, G, and SD+MEL groups in (B1, B3, and B4) on day 2. No significant difference between C, G, and SD+MEL groups in RME (B1-5) on day 2 (**Figure 2F**; **Table 1**).

*Effect of melatonin and sleep deprivation on total body weight*

The present work showed a significant effect of both time (P<0.05) and treatment (P<0.0001) on percent change in body weight as well as significant interaction of both factors (P<0.0001) on body weight. Two Way ANOVA with Bonferroni posttests showed a significant difference in % change in body weight of the control group when compared to the G group at week 3 (P<0.05) and weeks 4 and 5 (P<0.001), SD group at weeks 4 and 5 (P<0.05) and compared to SD+MEL group at week 1 (P<0.05), 2 and 4 (P<0.001), 3 and 5 (P<0.01). Moreover, we found significant differences between the G group and SD group at week 2 (P<0.05) and weeks 3, 4, and 5 (P<0.001). A significant difference between the G group and SD+MEL group (P<0.001) at weeks 2, 3, 4, and 5. A significant difference between the SD group and SD+MEL group at weeks 2 (P<0.001) and 3 (P<0.05) (**Table 2**; **Figure 3A**).

*Effect of melatonin and sleep deprivation on serum lipid profile*

Insignificant differences in serum lipoproteins between C, SD, and G groups were found. Co-administration of melatonin caused a sig-

**Table 2.** Interaction of time and treatment on % change of total body weight of different rat groups

| Source of Variation | Df | Sum-of-squares | Mean square | F | % of total variation | *P* value |
|---|---|---|---|---|---|---|
| Interaction | 12 | 26400 | 2200 | 7.6 | 17.08 | <0.0001 |
| Treatment | 3 | 79476 | 26492 | 91 | 51.42 | <0.0001 |
| Time | 4 | 3057 | 764 | 2.6 | 1.98 | 0.0365 |

Data represent a comparison of mean ± SEM of the percentage change of total body weight. Two Way ANOVA with Bonferroni Multiple comparison test P<0.05 is considered significant; N=10 in each group.

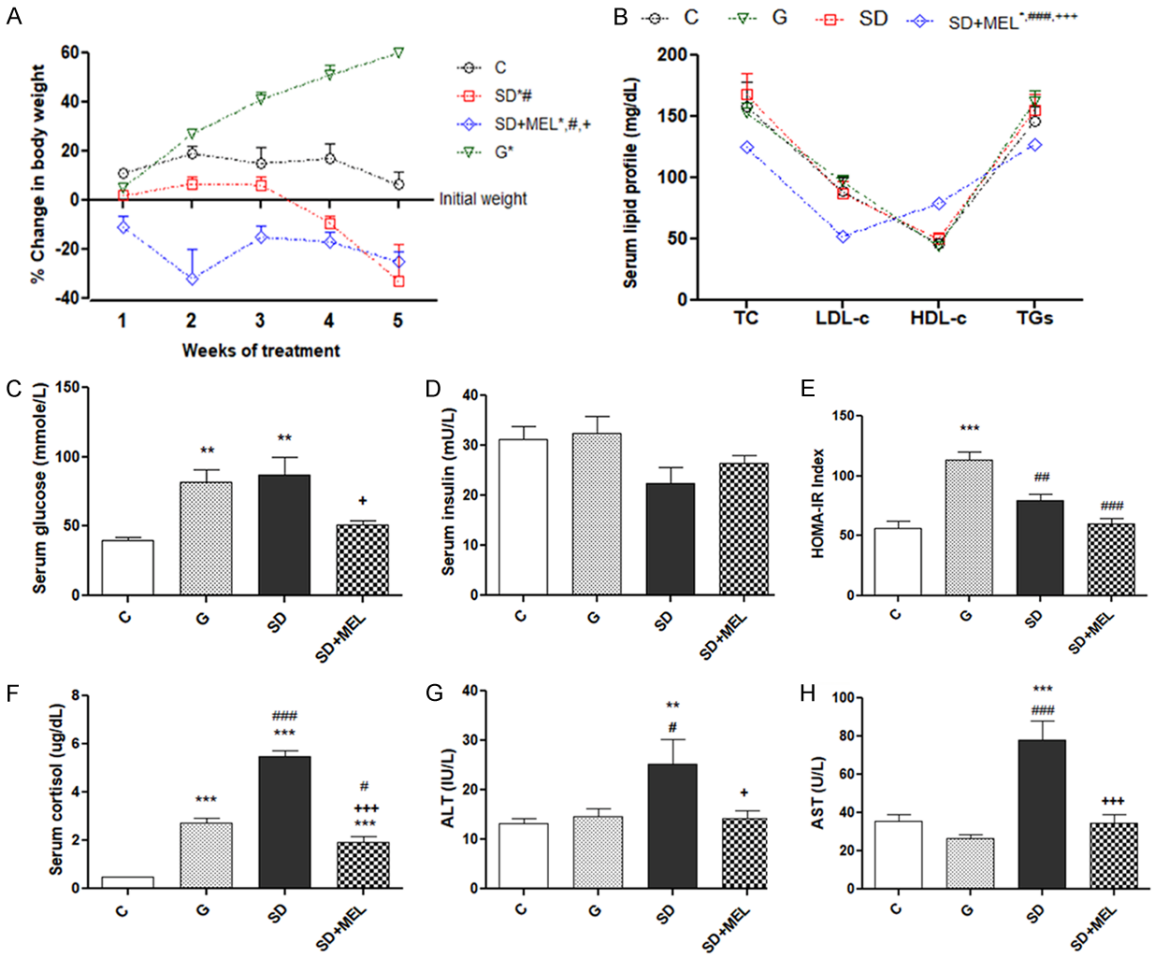


**Figure 3.** Effect of sleep deprivation and melatonin administration on (A) Total body weight. Data represent the percent change of total body weight with time compared to initial weight (Final weight - initial weight)/initial weight * 100). Serum level of (B) Lipoproteins; TC: total cholesterol, LDL-c: low-density lipoproteins, HDL-c: high-density lipoproteins, TGs: triglycerides. (C) Glucose. (D) Insulin. (E) HOMA IR (HOMA-IR index = Glucose (mmole/L) * Insulin (pmole/L)/22.5). (F) Cortisol. (G) Alanine amino transferase (ALT). (H) Aspartate amino transferase (AST). C: control group; G: grid group; SD: sleep deprived group; SD+MEL: sleep deprived treated with melatonin. Two Way ANOVA with Bonferroni posthoc in (A and B); ($^{+++}$P<0.001) SD+MEL group vs. SD group, ($^{+}$P<0.05) SD+MEL group vs. SD group, ($^{\#\#\#}$P<0.001) SD+MEL group vs. G group, ($^{\#}$P<0.05) SD and SD+MEL group vs. G group, ($^{*}$P<0.05) any group vs. C group. One Way ANOVA with Bonferroni posthoc in (C-H); ($^{+++}$P<0.001) SD+MEL group vs. SD group, ($^{+}$P<0.05) SD+MEL group vs. SD group, ($^{\#\#\#}$P<0.001) SD+MEL group vs. G group, ($^{\#\#}$P<0.01) SD+MEL group vs. G group, ($^{\#}$P<0.05) SD+MEL group vs. G group, ($^{***}$P<0.001) any group vs. C group, ($^{**}$P<0.01) G and SD groups vs. C group, n=10 in each group.

nificant lowering in serum total cholesterol, LDL-c, and triglycerides compared to C (P<0.05), G (P<0.001), and SD (P<0.001) groups. Significant rise in serum HDL-c in the SD+MEL

group when compared to C (P<0.05), G (P<0.001), and SD (P<0.001) groups (**Figure 3B**).

*Effect of melatonin and sleep deprivation on glucose homeostasis*

*Effect of melatonin and sleep deprivation on serum glucose level:* Significant rise in serum glucose levels in the SD group and G group compared to the C group (P<0.01). Co-administration of melatonin caused a significant decrease in serum glucose levels in the SD+MEL group compared to the SD group (P<0.05). No significant difference in serum glucose levels between the SD group and the G group (P>0.05) (**Figure 3C**).

*Effect of melatonin and sleep deprivation on serum insulin level:* An insignificant difference in serum insulin levels between treated groups was found (**Figure 3D**).

*Effect of melatonin and sleep deprivation on the HOMA-IR index:* Significant rise in HOMA-IR Index in the G group compared to the C group (P<0.001), SD+MEL group (P<0.001), and SD group (P<0.01). Co-administration of melatonin caused a significant decrease in HOMA-IR Index in SD+MEL compared to the G group. Insignificant difference between the HOMA-IR index in C, SD, and SD+MEL groups (**Figure 3E**).

*Effect of melatonin and sleep deprivation on serum cortisol level*

Significant rise in serum cortisol levels in G, SD, and SD+MEL groups compared to the control group (P<0.001). Significant rise in serum cortisol levels in the SD group when compared to the G group (P<0.001). Co-administration of melatonin caused a significant decrease in serum cortisol levels in the SD+MEL group compared to the SD group (P<0.001) and G group (P<0.05) (**Figure 3F**).

*Effect of melatonin and sleep deprivation on serum liver enzymes*

*Effect of melatonin and sleep deprivation on serum alanine aminotransferase (ALT):* Significant rise in serum ALT levels in the SD group when compared to the C group (P<0.01) and G group (P<0.05). Co-administration of melatonin caused a significant decrease in serum ALT level in the SD+MEL group when compared to SD (P<0.05). No significant difference in serum level ALT between C, G, and SD+MEL (P>0.05) (**Figure 3G**).

*Effect of melatonin and sleep deprivation on serum aspartate aminotransferase (AST):* Significant rise in the serum level of AST in the SD group when compared to the C and G groups (P<0.001). Co-administration of melatonin caused a significant decrease in serum AST level in the SD+MEL group when compared to SD (P<0.001). No significant difference in serum level AST between C, G, and SD+MEL groups (P>0.05) (**Figure 3H**).

*Effect of melatonin and sleep deprivation on serum inflammatory cytokines*

*Effect of melatonin and sleep deprivation on serum tumor necrosis factor alpha (TNF-α):* Significant rise in serum TNF-α levels in G, SD, and SD+MEL groups compared to the control group (P<0.001). Significant rise in serum TNF-α level in the SD group when compared to the G group (P<0.01). Co-administration of melatonin caused a significant decrease in serum TNF-α level in the SD+MEL group compared to the SD group (P<0.001) and G group (P<0.001) (**Figure 4A**).

*Effect of melatonin and sleep deprivation on serum IL-6:* Significant increase in IL-6 in the SD group compared to the G group (P<0.001). Insignificant difference in IL-6 between the C group, SD, and SD+MEL groups (P>0.05). Significant decrease in serum IL-6 in the G group compared to the C group (P<0.001) and SD+MEL group (P<0.05) (**Figure 4B**).

*Effect of melatonin and sleep deprivation on serum INF-gamma:* Insignificant difference in serum INF-gamma level between treated groups (**Figure 4C**).

*Effect of melatonin and sleep deprivation on serum IL-10 level:* Significant increase in IL-10 in the SD group compared to the G group (P<0.001). Melatonin caused a significant decrease in serum IL-10 level in the SD+MEL group compared to the SD group (P<0.01). Insignificant difference in serum IL-10 in the C group when compared to SD and SD+MEL groups (P>0.05). Significant decrease in serum IL-10 in the G group compared to the C group

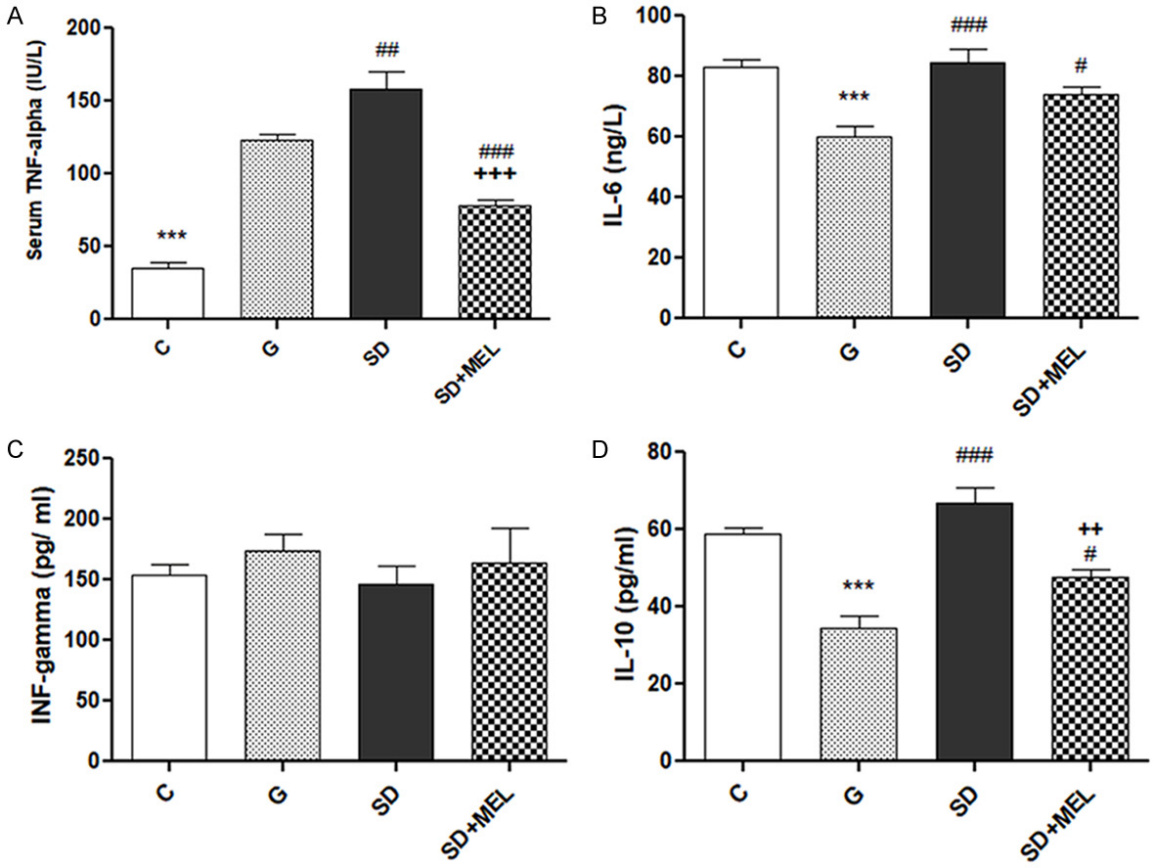


**Figure 4.** Effect of melatonin and sleep deprivation on serum inflammatory cytokines. A: Tumor necrosis factor alpha (TNF-α); B: Interleukin-6 (IL-6); C: Interferon gamma (INF-γ); D: Interleukin-10 (IL-10). C: control group, G: grid group, SD: sleep deprived group, SD+MEL: sleep deprived plus melatonin group; One Way ANOVA with Bonferroni posttests; (*) any group vs. C group, (#) SD or SD+MEL groups vs. G group, (+) SD+MEL group vs. SD group, One Way ANOVA with Bonferroni posttests; ($^{***}$P<0.001) G group vs. C group, ($^{++}$P<0.01) SD+MEL group vs. SD group, ($^{+++}$P<0.001) SD+MEL group vs. SD group, ($^{\#\#}$P<0.01) SD group vs. G group, ($^{\#\#\#}$P<0.001) SD group vs. G group, ($^{\#}$P<0.05) SD+MEL group vs. G group, N=10 in each group.

(P<0.001) and SD+MEL group (P<0.05) (**Figure 4D**).

*Effect of melatonin and sleep deprivation on liver histopathology*

Photomicrographs of liver sections from the control group showing preserved lobular architecture and portal areas (asterisk). Hepatocytes had a polyhedral shape with vesicular nuclei, and granular cytoplasm and arranged in cords radiating outwards from a central vein (thin arrow). They revealed no evidence of any specific pathology (fatty changes, hydropic changes, feathery changes, cholestasis, lymphocytic infiltration, or fibrosis). Hepatic sinusoids (S) between the strips of hepatocytes drained into the central vein (**Figure 5A**, **5B**). Liver sections from the grid G group showed unremarkable pathology (**Figure 5C**, **5D**). Liver sections from the SD group showed congested blood vessels with hemorrhagic areas (Hge). Hepatocytes showed diffuse hydropic degeneration in the form of clear vacuolated cytoplasm with centrally located darkly stained nuclei, preserved lobular architecture with no significant portal area inflammation or fibrosis (**Figure 5E**, **5F**). Liver sections from sleep-deprived rats treated with melatonin SD+MEL group showed unremarkable pathology (**Figure 5G**, **5H**).

*Effect of melatonin and sleep deprivation on colon histopathology*

Sections of the colon of the control rat group showed the mucosa lined by simple columnar epithelium with a thin brush border and numerous goblet cells. The crypts of Lieberkühn are

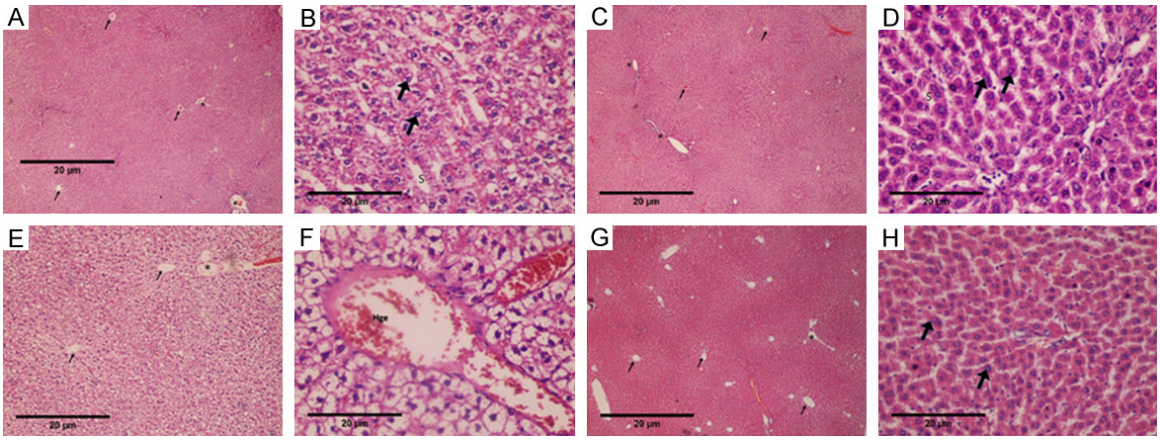


**Figure 5.** Liver sections of rats from different treated groups. (A, B) Control group showing the normal histological structure of the hepatic lobule. (C, D) Grid group. (E, F) Sleep-deprived group. (G, H) Sleep-deprived treated with melatonin group. Nuclei of hepatocytes (thick arrows), central vein (thin arrows), sinusoids (S). H&E images magnification (A, C, G) (40X); (E) (100X); (B, D, F, H) (400X), N=6 in each group.

straight and unbranched and lined with goblet cells. The lamina propria showed abundant leukocytes (**Figure 6A**, **6B**). Sections of the colon of the grid group (**Figure 6C**, **6D**) showed normal colon architecture. Sections of the colon of sleep-deprived rats (SD) group showing multifocal mucosal and submucosal (bracket) mixed infiltrate of inflammatory cells (**Figure 6E**, **6F**). Sections of the colon of sleep-deprived rats treated with melatonin group showing normal colon architecture (**Figure 6G**, **6H**).

*Effect of melatonin and sleep deprivation on goblet cell count:* Photomicrograph of colon sections stained with PAS stain showing a marked decrease in glandular goblet cells in the SD group compared to other groups (**Figure 6I-L**). Sleep deprivation caused a significant decrease in goblet cell count in the SD group compared to the C group and G group ($P<0.01$). Treatment with melatonin caused a significant rise in goblet cell count in the SD+MEL group compared to the SD group ($P<0.05$) (**Figure 6M**). Sleep deprivation caused 88% goblet cell loss compared to controls. Melatonin decreased goblet cell loss to 18% compared to the control group. We found an increase in goblet cells to 67% in the grid group when compared to the control group (**Figure 6N**).

*Effect of sleep deprivation and melatonin on prefrontal cortex histology*

Parasagittal H&E sections of the internal layers prefrontal cortex of the control rat group showed larger neuronal cell bodies with open-face vesicular nuclei (N) and prominent nucleoli and a rim of basophilic cytoplasm, lightly (lg) and deeply stained nuclei (dg) of glial cells with normal blood capillaries (bc) observed in acidophilic neuropile (*) (**Figure 7A**). Sections of the grid group showed a good number of open-face vesicular nuclei (N) with prominent nucleoli and a rim of basophilic cytoplasm, lightly (lg), and deeply stained nuclei (dg) of glial cells. It also showed some dark shrunken neurons (pn) with perinuclear haloes (h) or flame-like pointed ends (thick arrow) and dilated blood capillaries (*bc) (**Figure 7B**). Sections of the sleep-deprived group showed many dark shrunken neuronal cell bodies with deeply stained pyknotic nuclei (pn) and perinuclear haloes (h), some of these dark cells appear as flame-like with pointed ends (thick arrow), dilated blood capillaries (*bc) with many lightly (lg) and deeply (dg) stained glial cells in the vacuolated neuropile (V) (**Figure 7C**). Sections of the sleep-deprived group treated with MEL showed a good number of open-face vesicular nuclei (N) with prominent nucleoli and a rim of basophilic cytoplasm, lightly (lg), deeply stained nuclei (dg) of glial cells, some dark shrunken neurons (pn) with perinuclear haloes (h), vacuolated neuropile (V) and dilated blood capillary (*bc) (**Figure 7D**). Count of large neurons with open face nuclei (**Figure 7E**).

*Effect of sleep deprivation and melatonin on hippocampus histology*

Histopathological examination of sections of control rat brain A: showed the different parts

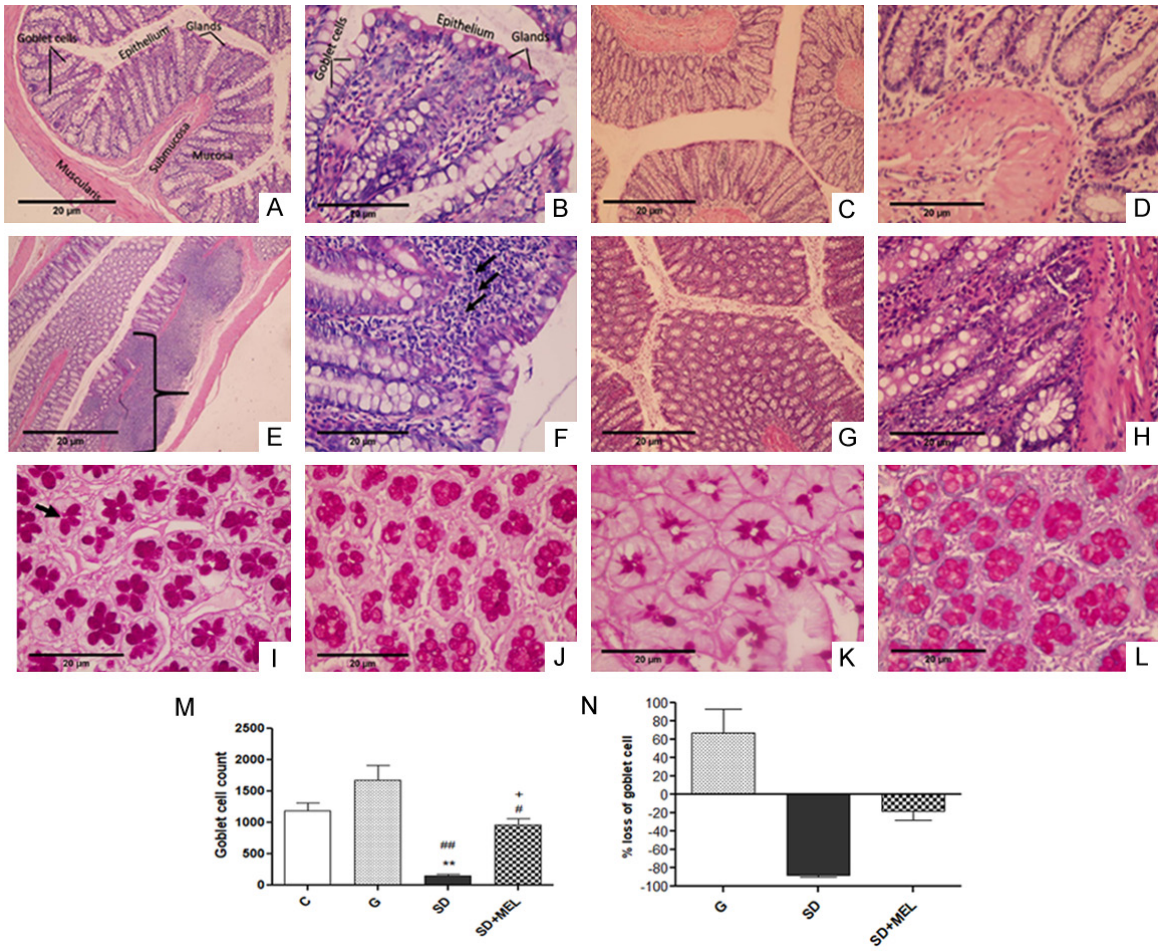


**Figure 6.** Photomicrographs of colon sections stained with H&E from; (A, B) Control rat group showing normal appearance of all layers of the colon; mucosa, submucosa, and Musculosa. (C, D) Grid group showing normal colon architecture. (E, F) Sleep-deprived rat group showing multifocal mucosal and submucosal mixed infiltrate of inflammatory cells (bracket). (G, H) Sleep-deprived rat group treated with melatonin showing normal colon architecture. (I-L) Representative photomicrograph of colon glands; (I) Control group, (J) Grid group, (K) Sleep-deprived group, (L) Sleep-deprived plus melatonin group. The arrow indicates three PAS-positive glandular goblet cells. Magnification; (A, C, E, G) (100X) and (B, D, F, H) (H&E, 400X), (I-L) (PAS, 400X). (M) Quantification of glandular goblet cells of treated groups. (N) Percentage of goblet cell loss ((C group - treated group)/C group * 100). One Way ANOVA with Bonferroni posttests; ($^{**}$P<0.01) SD group vs. C group, ($^{+}$P<0.05) SD+MEL group vs. SD group, ($^{\#\#}$P<0.01) SD group vs. G group, ($^{\#}$P<0.05) SD+MEL group vs. G group, N=6 in each group.

of the hippocampal formation; the hippocampus, subiculum (SU), and entorhinal cortex (EC). The hippocampus is formed of; cornu ammonis (CA) and dentate gyrus (DG), these two parts are separated by a hippocampal sulcus (HS). CA is further partitioned into; CA1, CA2, CA3, and CA4. The choroid plexus (CP) of the lateral ventricle was observed (**Figure 8A**). B, D and H: Higher magnification of the squared area of CA1 region of control, grid, and sleep-deprived rats treated with melatonin groups respectively exhibited well-defined three layers; polymorphic layer (POL), pyramidal cell layer (PCL), and molecular layer (ML). The pyramidal cell layer (PCL) exhibited closely packed cell bodies of the pyramidal neurons (arrowheads) that were regularly arranged in 3 to 4 rows and appeared small with vesicular nuclei, prominent nucleoli, and scanty cytoplasm. The POL and ML showed deeply (dg) and lightly (lg) stained nuclei of glial cells with normal blood capillaries (bc) (**Figure 8B**, **8D**, **8H**). C, E and I: Higher magnification of the squared area of the dentate gyrus (DG) showed well-defined three layers; ML, granule cell layer (GCL), and POL. The GCL had aggregation of rounded to oval granule cell bodies. Small oval deeply stained nuclei (thick arrow) of the immature neurons could be detected in the

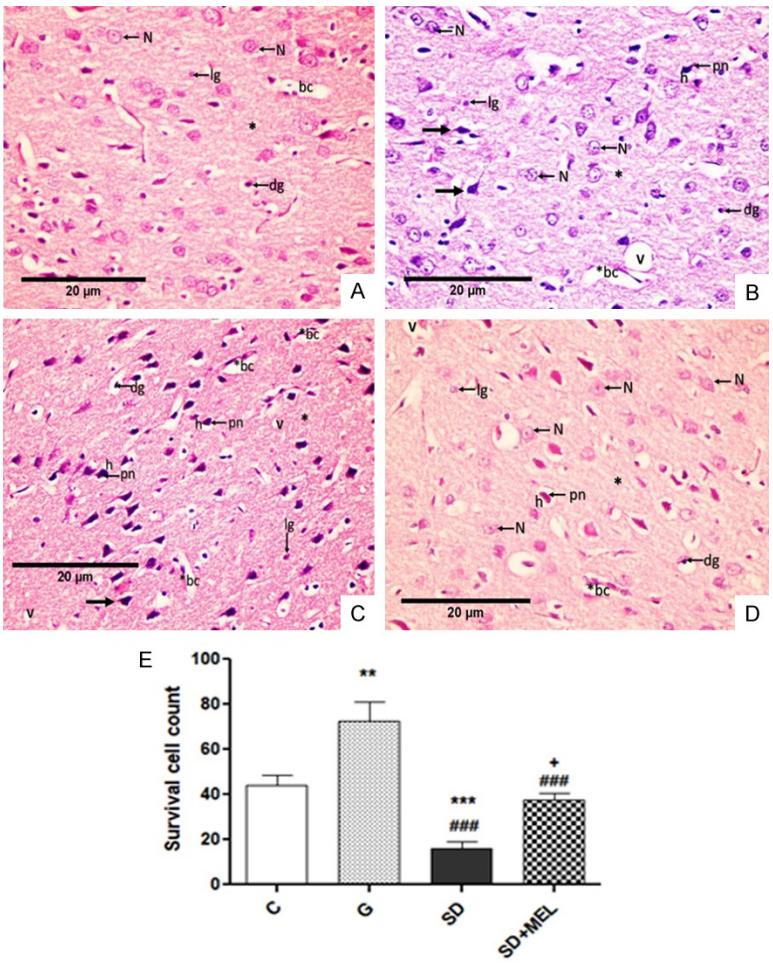


**Figure 7.** Representative photomicrograph of a parasagittal section of the internal layers prefrontal cortex of; (A) control rat group, (B) rat of grid group, (C) sleep deprived rat, and (D) sleep deprived rat treated with melatonin. Larger neuronal cell bodies with open-face vesicular nuclei (N) and prominent nucleoli and a rim of basophilic cytoplasm, dark shrunken neuronal cell bodies with deeply stained pyknotic nuclei (pn) with perinuclear haloes (h) or flame-like pointed end (thick arrow), lightly (lg) and deeply stained nuclei (dg) of glial cells, normal blood capillaries (bc), dilated blood capillary (*bc), acidophilic neuropile (*), vacuolated neuropile (V), Magnification (A-D) (H&E 400X). (E) Count of large neurons with open face nuclei; One Way ANOVA with Bonferroni posttests; ($^{***}$P<0.001) SD group vs. C group, ($^{**}$P<0.001) G group vs. C group, ($^{+}$P<0.05) SD+MEL group vs. SD group, ($^{###}$P<0.01) SD and SD+MEL groups vs. G group, No. of animals used =6 in each group.

subgranular zone (SGZ). The POL and ML had deeply (dg) and lightly (lg) stained nuclei of glial cells with normal blood capillaries (bc) (**Figure 8C**, **8E**, **8I**). F: higher magnification of the squared area of the CA1 region of the sleep-deprived rat group exhibited most of the cell bodies of the pyramidal neurons in PCL are disarranged and loosely packed; they appeared dark, shrunken, and having pyknotic nuclei (pn) with pericellular haloes (h) (**Figure 8F**). G: Higher magnification of the squared area of the DG of the sleep-deprived group displayed dark shrunken granule cell bodies having pyknotic nuclei (pn) with pericellular haloes (h) and few apparently normal granules cell bodies (thick arrows) in GCL. Widening of SGZ showing few immature neurons (thin arrows) was observed (**Figure 8G**). Count of large neurons with open face nuclei in CA1 area of hippocampus in different groups (**Figure 8J**).

## Discussion

Paradoxical sleep deprivation (PSD) is implicated in impairment of spatial learning and memory function of the brain. We found that eighteen hours of PSD after 15 trials of training in the radial arm water maze (RAWM) caused a significant increase in latency to reach the pedestal (TRT) in the SD group when compared to the C group (B4) and G group (B1 & B4). The present work showed that co-administration of melatonin (MEL) improved PSD-induced spatial learning and memory deficits. We observed that MEL significantly decreased TRT in the first three trials (B1) as well as in trials 7 to 9 (B3) in the SD+MEL group when compared with the SD group. Moreover, posttraining PSD caused a significant increase in reference (RME) and working (WME) memory errors in the SD group compared to both the C group and G group (B1, B3, B4) on day 2. Melatonin significantly decreased RME and WME in the first 3 trials (B1) and trials 7 to 12 (B3 & B4) in the SD+MEL group when compared with the SD group on day 2. Also, we found no significant difference between C, G, and SD+MEL groups in escape latency and search errors throughout the fifteen trials (B1-5) on day 2. Results of day 2 testing especially in the first three trials (B1) indicated a lack of memory retention from day

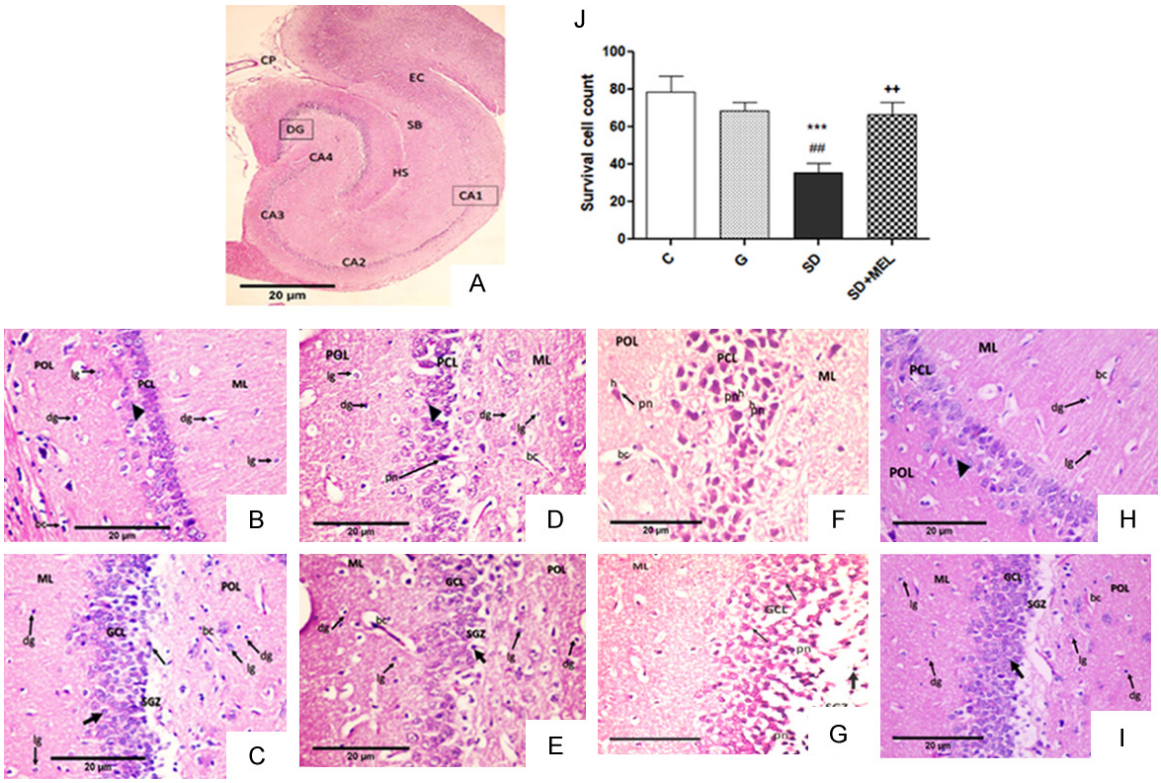


**Figure 8.** Representative photomicrographs of parasagittal sections of hippocampus of (A-C) control rat, (D, E) grid rat group, (F, G) sleep deprived rat and (H, I) sleep deprived rat treated with melatonin. (A) Showing different parts of hippocampal formation, (B, D, F, H) higher magnification of squared region of CA1 area and (C, E, G, I) higher magnification of squared region of dentate gyrus. Subiculum (SU), entorhinal cortex (EC), Cornu Ammonis (CA); CA is further divided into; CA1, CA2, CA3 and CA4, dentate gyrus (DG), hippocampal sulcus (HS), choroid plexus (CP) of the lateral ventricle, polymorphic layer (POL), pyramidal cell layer (PCL) and molecular layer (ML), pyramidal neurons (arrowhead), deeply (dg), lightly (lg) stained nuclei of glial cells, normal blood capillaries (bc), granule cell layer (GCL). small oval deeply stained nuclei (thick arrow) of the immature neurons, subgranular zone (SGZ), dark, shrunken and pyknotic nuclei (pn) with pericellular haloes (h). H&E (Magnification; A: 40X; B-I: 400X, Scale bar 20 µm). (J) Count of large neurons with open face nuclei in CA1 area of hippocampus in different groups; C: control, G: grid group, SD: sleep deprived and SD+MEL: sleep deprived treated with melatonin. One Way ANOVA with Bonferroni posttests; ($^{***}$P<0.001) SD group vs. C group, ($^{++}$P<0.05) SD+MEL group vs. SD group, ($^{\#\#}$P<0.01) SD group vs. G group, No. of animals used =6 in each group.

1. Moreover, increased errors of the SD group when compared with other groups in B3 and B4 indicated disturbed learning with repeating trials on day 2. Therefore, MEL treatment improved both memory retention from day 1 as well as learning on day 2 in the SD+MEL group when compared with the SD group. Along with us, it was found that MEL administration (100 mg/Kg orally daily for 4 weeks) in rats decreased PSD-induced spatial memory errors in RAWM through its antioxidant effect [21]. Moreover, it was reported that MEL treatment ameliorated PSD-induced delayed escape latency, increased search errors, and path length in the Morris water maze (MWM) task that was associated with decreased glial activation in rat hippocampus [22]. We found that PSD for two weeks before training in RAWM caused no significant difference in escape latency and search errors between treated groups on day 1. Most of the differences in TRT, WME, and RME between groups were in the first 6 trials (B1 & B2) that were abolished with repeating trials 7-15 (B3-5) on the first-day testing. In agreement with us, a previous study in rats showed that post-training PSD for 24 hours caused impairment of task acquisition, and survival function and prolonged the latency of completion in dry radial arm maze (RAM) with marked effect in the first four hours [23]. Another study reported that post-training PSD for 48 hours caused significant impairment of retention of reference memory in MWM task [24]. In contrast to us, it was reported that pretraining PSD

for 24 hours using modified MPT caused short-term memory impairment tested half an hour after 12 learning trials in the RAWM test [25]. This apparent controversy may be explained by the shorter pretraining period of PSD (24 hours) compared to the longer period (2 weeks) in our study which might cause adaptation and increased synthesis of proteins necessary for learning and memory.

Previous studies showed conflicting results concerning the effect of sleep loss on body weight. The present study revealed a significant drop in body weight in the SD group compared to the C group on weeks 4 and 5 and compared to the G group on weeks 2 through 5. This result is supported by previous experimental studies using MPT for PSD and explained by long periods of sleep deprivation, heavier stress, increased sympathetic activity, and energy expenditure [26, 27]. In contrast to us, other studies reported increased body weight with sleep deprivation [28, 29]. This apparent conflict may be explained by a different method of sleep restriction that was induced by noise in the study of Coborn and being a clinical study in the study of Cooper. The present study showed that MEL co-administration didn't recover the PSD-induced reduction of body weight. Moreover, MEL caused a significant drop in body weight in the SD+MEL group compared to other studied groups. Along with us, MEL did not recover the weight loss induced by sleep deprivation in rat models of colitis [30]. Moreover, it was reported that chronic MEL administration in drinking water for 8-11 weeks caused a reduction in weight gain and serum cholesterol, as well as increased cholesterol excretion in obese rats, fed high-fat and high-calorie diets [31].

The present study demonstrated that MEL administration significantly reduced TC, TGs, and LDL-c and increased HDL-c in the SD+MEL group when compared with C, G, and SD groups. In line with our study, it was reported that MEL (50 mg/kg for 8 weeks) elevated plasma HDL-c and lowered plasma LDL-c in rat models of streptozotocin-induced diabetes [32]. We found insignificant differences in serum levels of lipoproteins between SD, C and G groups. In agreement with us, one study in rats reported insignificant change in serum lipids with chronic PSD for 3 months (18 h/day) compared to their controls [33]. In contrast to us, it was found that sleep deprivation for 72 h using a randomly moving platform induced a significant increase of serum cholesterol, a decrease of HDL-c while triglycerides and free fatty acids levels kept unchanged when compared with the control group in both rats and mice [34]. This apparent controversy may be explained by a different method and short time of sleep restriction in the previous study 72 hours compared to our study 5 weeks.

We found that MEL significantly lowered the PSD-induced rise of serum glucose and cortisol levels in the SD+MEL group when compared with both SD and G groups. In agreement with us, it was reported that IP injection of MEL (5 mg/kg) or its agonist piromelatine (20 mg/Kg) reduced plasma glucose, and improved insulin sensitivity and lipid profile in a rat model of sleep restriction using intermittently rotating cages 20 hours per day for 8 days [35]. We found a significant rise in serum glucose and cortisol levels in both SD and G groups compared to the C group. Significantly higher serum cortisol in the SD group compared to the G group was found indicating that PSD augmented stress induced by being in the MPT. No significant difference in the serum glucose levels between the SD group and the G group was observed. We found insignificant differences in the serum insulin levels between treated groups. In the current study, PSD didn't change insulin sensitivity as indicated by insignificant differences in the HOMA-IR indices between the C, SD, or SD+MEL groups. Along with us, it was reported that PSD for 4-8 weeks in rats caused a significant rise in plasma glucose and corticosterone and an insignificant change in plasma insulin or HOMA-IR compared to controls [26]. Moreover, it was found that sleep disturbance for 72 hrs. caused an elevation of serum corticosterone and an insignificant change in serum insulin level in both rats and mice [34]. In addition, chronic PSD for 12 weeks in rats caused a significant rise in serum glucose and a lowering of body weight when compared to controls [33]. In contrast to us, Xu and his colleagues found a significant increase in HOMA-IR and TGs compared to controls. This might be explained by the longer duration of PSD (12 weeks) compared to ours (5 weeks). In the current study, we observed a significant rise in the HOMA-IR Index in the G group that was

associated with a significant increase in weight gain compared to C, SD and SD+MEL groups. We suggest that the marked increase of body weight in the G group may be the cause of increased insulin utilization and decreased insulin sensitivity.

Clinical and experimental evidence showed that sleep loss is associated with impaired immune function which might be a risk factor for metabolic and cognitive deterioration. We found that co-administration of MEL caused a significant decrease in serum TNF-α and IL-10 levels in the SD+MEL group compared to the SD group. Along with the current result, It was reported that MEL treatment at a dose of 20-40 mg/Kg reduced the acute 3 days SD-induced rise of serum corticosterone, TNF-alpha, IL-6, IL-4, and IL-10 in mice [36]. Moreover, MEL administration decreased serum IL-6, TNF-alpha, and corticosterone levels that were induced by 3 days of continuous PSD in mice through its antioxidant activity [37]. We observed a significant rise in serum TNF-α in the SD group when compared to both C and G groups. Moreover, we found a significant rise in IL-6 and IL-10 levels in the SD group when compared to the G group. In agreement with this result, one study in rats reported that 96 hours of PSD induced inflammation and elevation of TNF-alpha immuno-expression in both rat temporal and masseter muscles [38]. Moreover, five days of sleep restriction using a periodically rotating wheel caused a significant rise of IL-1β and TNF-α expression in the frontal cortex, hippocampus, and basal forebrain in rats that were associated with neurocognitive malfunction [39]. We found insignificant differences in serum INF gamma levels between treated groups. In contrast to us, it was reported that PSD decreased TNF-alpha, INF-gamma, and IL-6 as well as impaired T cell function in experimental mice model of malaria [40]. In addition, 12 hrs of sleep loss decreased IL-10 expression in the brain and peripheral tissues of rats [41]. This apparent controversy may be explained by different experimental models, duration, and methods of sleep deprivation. We found that MEL caused a significant decrease in serum TNF-alpha, IL-6, and IL-10 in SD+MEL when compared to the G group which was associated with decreased cortisol, glucose, and improvement in memory function in the RAWM task. Up to this point, we may suggest that the beneficial effect of MEL on memory was due to its anti-inflammatory effect and its ability to decrease serum cortisol and glucose levels.

The current work revealed that co administration of MEL caused significant decrease in PSD-induced rise of serum ALT and AST levels in the SD+MEL group when compared to SD. We found a significant rise in serum ALT and AST levels in SD group when compared to both C and G groups. Moreover, liver sections from the SD group showed congested blood vessels with hemorrhagic areas and diffuse hydropic degeneration. Along with us, a previous study reported that 24 hrs of PSD in mice caused a significant rise of ALT and AST that was associated with hepatocyte ballooning and necrosis [42]. Moreover, it was found that 22 hrs of PSD per day for 21 days in rats caused a significant rise of liver enzymes ALT, AST, and ALP that was associated with hydropic degeneration of liver cells [43]. MEL caused significant improvement in liver histopathology in the SD+MEL group when compared to SD. In line with us, it was found that 3 days of PSD significantly reduced MEL, raised liver enzymes, and exacerbated liver injury induced by lipopolysaccharides and D-galactose in mice [44]. In addition, they found that MEL supplement at 10 mg/Kg restored MEL level, and significantly reduced ALT, TNF-alpha, caspase-3 activity, apoptosis, and hepatic cell injury. Along with the previous study, we may speculate that MEL favorable effect on liver function in our study is due to its anti-inflammatory effect and its ability to reverse the histopathological changes induced by chronic PSD.

Moreover, we observed infiltration of the colonic mucosa and submucosa with mixed inflammatory cells that was associated with a significant decrease in goblet cell count in the SD group compared to the G and C groups. Sleep deprivation caused 88% goblet cell loss compared to controls. Melatonin supplementation decreased goblet cell loss to 18% compared to the control group and improved colonic mucosal architecture. In agreement with us, it was found that MEL administration significantly reduced PSD-induced ulcerative colitis in mice and prolonged their survival [45]. Melatonin treatment protected against PSD-induced exacerbation of inflammation and mucosal injury in mice model of colitis [30]. Melatonin reduced

inflammation and prevented the PSD-induced decrease of goblet cell count, villi/crypt (V/C) ratio, and the number of PCNA-positive cell counts in mice's small intestine [37]. Hence, they suggested the use of MEL as a probiotic agent to protect against PSD-induced intestinal mucosal injury.

Insufficient sleep increased the risk of neurodegenerative diseases such as Alzheimer's disease (AD) [46]. Histopathological sections of rat hippocampus and PFC of SD groups revealed disarranged pyramidal neurons with darkly stained pyknotic nuclei, pericellular haloes, and a significant decrease in cell count in both layers 3 and 5 PFC and hippocampal pyramidal cell layer. Examination of the dentate gyrus (DG) of the SD group showed dark shrunken granule cell bodies having pyknotic nuclei with pericellular haloes and widening of the subgranular zone (SGZ). Supporting the current result, it was found that 6 days of PSD decreased viable neuronal count, and increased degenerated cells with chromatolysis, pyknotic nuclei, and darkly stained cytoplasm in mice hippocampus, amygdala, and PFC [47]. Moreover, a relatively recent study reported that 21 days of SD caused a significant reduction in the basal and apical dendritic arborization of CA3 hippocampal neurons, impaired spatial memory, and caused anxiety-like behavior in rats [48]. The current study demonstrated the protective effect of MEL supplementation on PSD- induced histopathological changes in hippocampal as well as PFC neurons. In line with us, a previous study reported that MEL treatment (10 mg/Kg IP daily) during 72 hours of PSD increased the number of BrdU-positive cells in the subgranular zone of mice hippocampus that was associated with increased expression of epigenetic mediators [49]. Another study revealed that prophylactic MEL supplementation at 10 mg/Kg IP injection daily for 2 weeks and during 72 hours of PSD caused a 44% increase of neural precursors in SGZ of mice hippocampus [50]. It was reported that MEL treatment at 5 mg/Kg IP daily for 6 weeks ameliorated the experimental social isolation-induced degeneration and lost neurons of the hippocampus and frontal cortex in rats [51]. The present work showed that MEL supplementation improved the working and spatial memory function of rats in RAWM and protected hippocampus and PFC neurons from PSD-induced degeneration. Moreover, MEL significantly reduced stress, and improved glucose homeostasis and immune function of chronic PSD rats. It also improved liver function and structure as well as colon wall integrity. Taken together, we suggest the use of MEL as a prophylactic measure against the deleterious effects of chronic PSD.

## Conclusion

Posttraing REM sleep deprivation (PSD) in the window of (2-18 hours) caused significant impairment of memory retention as well as the ability of the rat to learn with repeating trials in the radial arm water maze (RAWM). The learning ability of rats in RAWM on day 1 testing was not affected by pretraining PSD for two weeks. In contrast to several previous studies, the present study revealed a significant reduction in body weight with chronic PSD. The results of the present study demonstrated the efficacy of MEL supplementation at a dose of 10 mg/Kg to reverse chronic PSD-induced rise of serum glucose, liver enzymes, cortisol, TNF-alpha, and IL-10. Moreover, MEL protected against memory impairment, neuronal degeneration of the hippocampus and PFC induced by PSD. It also improved liver histopathology and protected colon wall integrity. Melatonin administration failed to correct the PSD-induced reduction of body weight and caused a significant reduction in body weight compared to other treated groups. Melatonin had an antiatherogenic effect as it caused a significant reduction of serum TC, TGs, LDL-c, and increased HDL-c when compared to other treated groups. We suggested the use of MEL supplements to protect against the deleterious metabolic and cognitive effects of PSD. Future experimental as well as clinical studies are needed to elucidate other mechanisms, side effects, and proper doses of MEL, especially in astronauts and resident doctors exposed to chronic PSD.

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## Disclosure of conflict of interest

None.

## Abbreviations

MEL, Melatonin; REM, Paradoxical sleep; PSD, Paradoxical sleep deprivation; CSD, Chronic sleep deprivation; MS, Metabolic syndrome; ALT, Alanine transaminase; AST, Aspartate transaminase; TGs, Triglycerides; TC, Total cholesterol; LDL-c, Low density lipoproteins; HDL-c, High density lipoproteins; TNF-α, Tumor necrosis factor-alpha; IL-6, Interleukin-6; IL-10, Interleukin-10; INF-γ, Interferon-gamma; RAWM, Radial arm water maze; PFC, Prefrontal cortex.

**Address correspondence to:** Dr. Ghada S Mahmoud, Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt. Tel: +201069651669; Fax: +20882332278; E-mail: ghadaszam@gmail.com; ghadaszam@aun.edu.eg

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