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Chronic Melatonin Treatment Prevents Memory Impairment Induced by Chronic Sleep Deprivation

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Abstract Sleep deprivation (SD) has been associated with memory impairment through induction of oxidative stress. Melatonin, which promotes the metabolism of many reactive oxygen species (ROS), has antioxidant and neuroprotective properties. In this study, the effect of melatonin on memory impairment induced by 4 weeks of SD was investigated using rat animal model. Animals were sleep deprived using modified multiple platform model. Melatonin was administered via oral gavage (100 mg/kg/day). Spatial learning and memory were assessed using the radial arm water maze (RAWM). Changes in oxidative stress biomarkers in the hippocampus following treatments were measured using ELISA procedure. The result revealed that SD impaired both short- and long-term memory ($P<0.05$). Use of melatonin prevented memory impairment induced by SD. Furthermore, melatonin normalized SD-induced reduction in the hippocampus activity of catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD). In addition, melatonin enhanced the ratio of reduced to oxidized glutathione GSH/GSSG in sleep-deprived rats ($P<0.05$) without affecting thiobarbituric acid reactive substance (TBARS) levels ($P>0.05$). In conclusion, SD induced memory impairment, which was prevented by melatonin. This was correlated with normalizing hippocampus antioxidant mechanisms during chronic SD.

Keywords Melatonin · Sleep deprivation · Memory · Hippocampus · Maze

Introduction

Sleep is essential for physical and mental performance [1, 2]. Sleep consists of two alternating stages: non-rapid eye movement sleep and rapid eye movement or paradoxical sleep [3, 4]. Sleep deprivation can be acute, which involves continuous sleep deprivation in the range of several hours up to a week [5–10]. The other form of sleep deprivation is chronic form, which is characterized by daily sleep deprivation for several hours, typically 3–8 h/day, that lasts for weeks or months [11–16]. Several human and animal studies have shown that both acute [5–10] and chronic [11–16] sleep deprivation produced memory defects in a number of behavioral tasks. Additionally, sleep deprivation impaired long-term potentiation in the hippocampus, which is a common cellular correlate of learning and memory functions [10, 17–19]. Sleep deprivation also elevated hippocampal oxidative stress [13–16, 20–22], which was correlated with impairment of cognitive functions [13–16, 22].

Melatonin (*N*-acetyl-5-methoxytryptamine) is a naturally occurring compound that is found in animals, plants, and microbes. It is released from the pineal gland during dark periods and is suppressed upon exposure to day light [23]. Endogenous melatonin levels were reported in healthy young individuals to be about 100 pg/ml; these levels were increased to about 170 pg/ml upon exogenous supplementation at a dose of 0.3 mg [24]. Melatonin reduces the formation of free radicals either by direct scavenging of these anions (e.g.,

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superoxide anions, hydroxyl radical, hydrogen peroxide, nitric oxide, singlet oxygen…, etc.) or by increasing the antioxidant activity of glutathione-*S* transferase and glutathione reductase enzymes [25]. The antioxidant effect of melatonin has been previously observed during acute sleep deprivation-induced memory impairment [22, 26]. In this study, we investigated the possible preventive effect of melatonin on memory impairment induced by chronic sleep deprivation. Potential molecular changes in the oxidative stress biomarkers including the levels of GSH, GSSG, thiobarbituric acid reactive substance (TBARS), and the activities antioxidant enzymes (GPx, catalase, and superoxide dismutase (SOD)) associated with sleep deprivation and melatonin were also assessed.

Methods

Animals and Treatments

Adult male Wistar rats weighing 180–230 g were used in this study. The animals were purchased from the Animal Care Facility at Jordan University of Science and Technology/Jordan. The animals were housed in metal cages (six rats per cage) under hygienic conditions and maintained at 24 °C and 12-h light/dark cycle (light on at 8 am) with free access to food and water. All procedures were approved by the Animal Care and Use Committee (ACUC) of Jordan University of Science and Technology. Animals were randomly assigned into five groups (n=12–16 rats): control, wide platform (WPF), melatonin, chronic sleep deprivation (SD), and chronic sleep deprivation with melatonin (SD/melatonin). The melatonin and SD/melatonin groups were treated with melatonin at a dose of 100 mg/kg/day (Sigma Chemical CO., Saint Louis, MO, USA) via oral gavage for 4 weeks. We have used this dose, as it was shown to have protective effect against impairment of cognitive function in conditions other than SD [27–29]. The control, SD, and WPF groups were administered normal saline on a daily basis for 4 weeks via oral gavage. The SD and SD/melatonin groups were sleep deprived (8 h/day, 8:30 am to 4:30 pm) for 4 weeks. All manipulations including SD, melatonin, and normal saline administration were started on the same day and continued for 4 weeks. The radial arm water maze (RAWM) training was carried out immediately after 4 weeks of treatment. SD and/or melatonin treatments were continued throughout the RAWM testing days.

Induction of SD

SD was induced using multiple columns in water (modified platform) model [8, 10, 16, 17, 19, 30]. Rats were placed in a large tank (170 cm in length × 40 cm in width × 55 cm in depth) made of glass filled with 24 °C water. The tank contained 20 small platforms with a diameter of 5 cm, placed 10 cm apart from edge to edge, and arranged in tow rows. The tank was filled with water up to 2 cm below the platform surface. In this tank, animals (n=5–7 from the same group at each time) were capable to move and jump freely from one platform to another. When animals reached the paradoxical phase of sleep (REM) [31], muscle atonia set in and animals fell into the water and woke up, and they immediately climbed up to the platform and sat on it. The water in the tank was changed daily with a temperature of 24±1 °C. All rats had free access to food and water. To test the effect of possible stresses of the tank environment, WPFs with a diameter of 12 cm were used to allow the rats to sleep without interruption or falling into water.

The RAWM

The RAWM was used to test spatial learning and memory among all groups of animals. This model along with detailed procedure was previously described [11, 16, 32–34]. The RAWM is a black circular water-filled tub (water temperature 24±1 °C) with six V-shaped stainless steel plates arranged to form a swimming field on an open central area and six arms. All experiments were performed in a dimly light room and two different pictures in fixed positions on the wall to serve as visual cues for the rats. Animals had to find a hidden platform 2 cm under water at the far end of the goal arm. The goal arm was not changed for a particular rat during all learning trials and memory tests. Acquisition phase is accomplished by having the rats undergo 12 consecutive trials; the first six trials were separated by a 5-min resting time. This phase is followed 30 min later by a test for short memory and 5 h later by long-term memory tests. In each trial, the animal was started in a different starting arm (except the goal arm) in a particular day for a particular rat. In each trial, the rat was allowed 1 min to swim freely in the maze to find the hidden platform. Once the rat is on the platform, the rat was allowed 15 s to observe visual cues before the next trial. When a rat was unable to find the platform within the 1-min period allowed, it was guided toward the platform for a 15-s stay. During the 1-min period, each time that the rat entered an arm other than the goal arm, an error was counted. Entry occurred when the whole body of the rat (not including the tail) was inside the arm.

Hippocampus Dissection

Animals were killed by decapitation after 4 weeks of SD and/or melatonin treatment. Dissection was carried out as previously described in [35–38]. Briefly, the brain was removed immediately from the skull and placed on a filter paper containing 0.2-M ice-cold sucrose solution, over a glass petri dish filled with crushed ice. Dissected hippocampus sections were placed in test tubes then, immediately, transferred into liquid nitrogen and stored at −80° until analysis.

Calorimetric Assays

Several markers of oxidative stress were assayed that include GSH, GSSG, GPx, and catalase. GSH (reduced form of glutathione) is a tri-peptide that plays an important role in protecting cells from damage caused by reactive oxygen species to cellular components. Once oxidized, GSH is converted into GSSG (glutathione disulfide). Change in the GSSG/GSH ratio is a biomarker that reflects oxidative stress status inside the body. Both GPx and catalase are enzymes that also play a role in normalization of oxygen free radicals. GPx reduces lipid hydroperoxides to alcohols and free hydrogen peroxide to water, whereas SOD catalyzes dismutation of the superoxide radical into H_2O_2. On the other hand, catalase mediates the conversion of H_2O_2 to water and oxygen. In order to assay the biomarkers, hippocampus tissues were homogenized manually using small pestle in lysis buffer containing the following: 8 g NaCl, 0.2 g KCl, 0.24 g KH_2PO_4, and 1.44 g Na_2HPO_4, 5 mM EDTA as preservative, and ready-to-use protease inhibitor cocktail (Sigma-Aldrich Corp, MI, USA), all dissolved in 1 L distilled water. Tissue homogenates were centrifuged to remove insoluble material (14,000×*g* for 5 min, 4 C). Thereafter, homogenates were divided into several aliquots; each of them was used for only a single assay. All molecular analyses were carried out over crushed ice. Total protein concentration was estimated using commercially available kit (Bio-Rad, Hercules, CA, USA). To quantify total GSH, tissues homogenates were deproteinized with 5 % of 5-sulfosalicylic acid (SSA), centrifuged (10,000×*g* for 10 min, 4 C) to remove the precipitated protein, and then assayed photometrically for glutathione according to the manufacturer's instructions (Glutathione Assay Kit, sensitivity 1 nmol/mL, Sigma-Aldrich Corp., MI, USA). Colors were read at 405 nm using Epoch BioTek microplate reader (BioTek, Winooski, VT, USA). For GSSG measurement, tissue lysate was first treated with 1 M 4-vinylpyridine; then, the procedure was carried as described above for GSH. To minimize variability between experimental runs, each single plate included equal samples from all experimental groups. GSH was calculated by subtracting total glutathione species value from GSSG value. Activity of glutathione peroxidase (GPx) was determined using GPx cellular activity assay kit (Sigma-Aldrich). Catalase and SOD activities were measured using commercially available kits according to the manufacturer's instructions (SOD: Sigma-Aldrich Corp; catalase: Cayman Chemicals Co, Ann Arbor, Michigan, USA). TBARSs were measured using TBARS assay kit (Cayman Chem, Ann Arbor, MI, USA). ELISA plates were read at kit's specified wavelengths using Epoch BioTek microplate reader (BioTek, Winooski, VT, USA).

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism (4.0) computer program. Values are expressed as mean±SEM. Two-way ANOVA was used to compare number of errors between study groups, followed by Bonferroni post test for multiple comparisons. Time (repeated measures factor) and treatment (between-subjects factor) groups were the independent variables. One-way ANOVA, followed by Bonferroni post hoc test, was used to compare hippocampus levels of oxidant and antioxidant enzyme activities. $P<0.05$ was considered statistically significant.

Results

The Effect of Chronic SD on Learning and Memory

Figure 1 demonstrates that all study groups were able to learn the location of the submerged platform, as determined by the marked reduction of errors in the learning phase (trials 1 to 12), with no significant difference among these groups in all training/learning trials (Fig. 1, control versus WPF: $P>0.05$, control versus SD: $P>0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P>0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P>0.05$, SD versus SD/melatonin: $P>0.05$, melatonin versus SD/melatonin: $P>0.05$). In the short-term memory test, which was done 30 min after the end of 12th trial, rats in the control, WPF, melatonin, and SD/melatonin groups made similar numbers of errors. In contrast, the SD group made significantly more errors than those of other experimental groups (Fig. 2a, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$). In addition, melatonin treatment in normal rats had no significant effect on memory performance at the RAWM. These results collectively indicate that chronic SD impaired short-term memory and that melatonin treatment prevented this impairment without affecting basal performance in normal animals

In the long-term memory test, which was done 5 h after training, the SD group made significantly more errors in finding the hidden platform compared to the other experimental groups (Fig. 2b). Similar numbers of errors were made in the control, WPF, melatonin, and SD/melatonin groups (control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus

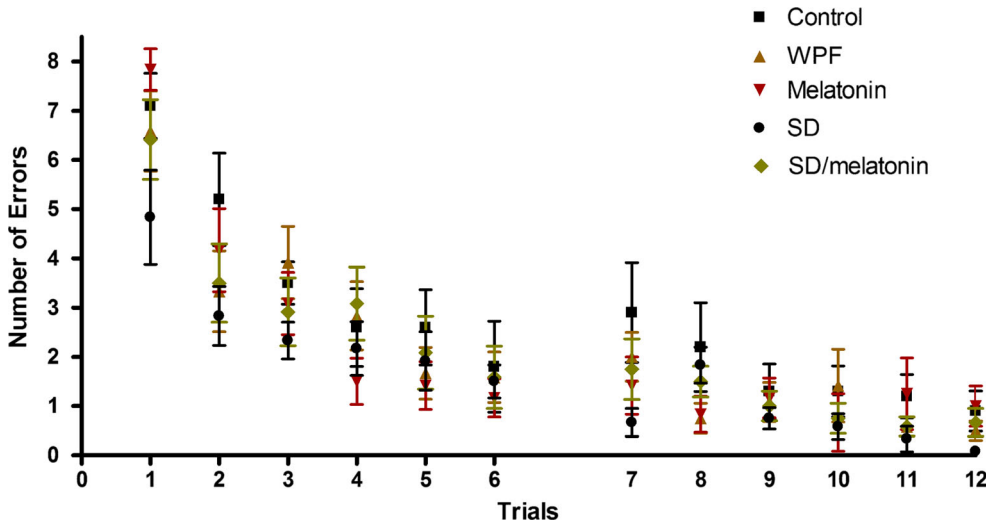

Fig. 1 Animal learning performance in the radial arm water maze. Learning performance among control (control), wide platform (WPF), melatonin, chronic sleep deprivation (SD), and chronic sleep deprivation with melatonin (SD/melatonin) groups. No change was observed in learning performance among all study groups, indicating that neither chronic sleep deprivation nor melatonin affected learning. Each point is the mean±SEM of 12–16 animals/group

melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$), indicating that melatonin treatment prevented SD-induced long-term memory impairment in the RAWM paradigm.

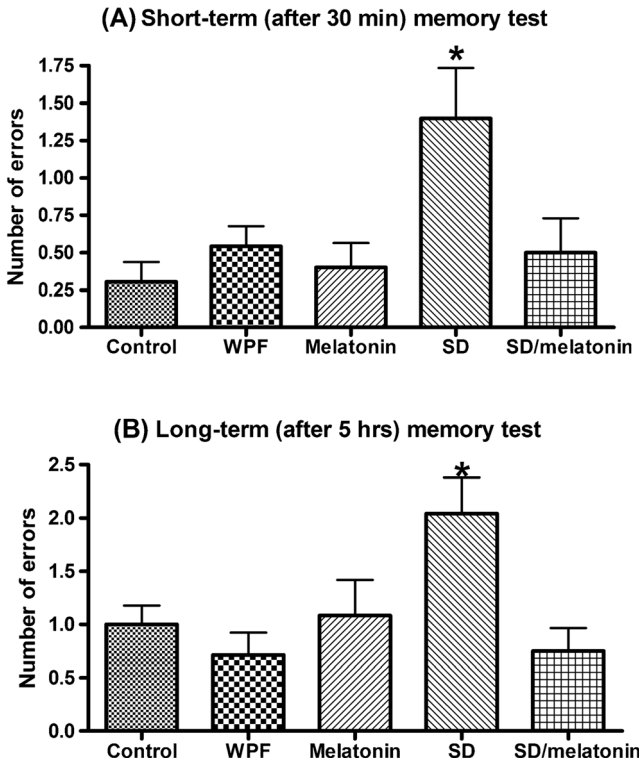

Fig. 2 Melatonin prevented hippocampal memory impairment induced by chronic sleep deprivation. Short-term memory (**a**) and long-term memory performance (**b**) among control, wide plates form (WPF), melatonin, chronic sleep deprivation (SD), and chronic sleep deprivation with melatonin (SD/melatonin). Each column is the mean± SEM of 12–16 rats. *Significant difference from all other groups using two-way ANOVA followed by Bonferroni post hoc test ($P<0.05$)

The Effect of Chronic SD and Melatonin on Hippocampus Oxidative Stress Biomarkers

Concerning levels of the reduced form of glutathione (GSH), no changes were observed in the levels of GSH among experimental groups (Fig. 3a; control versus WPF: $P>0.05$, control versus SD: $P>0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P>0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P>0.05$, SD versus SD/melatonin: $P>0.05$, melatonin versus SD/melatonin: $P>0.05$). For oxidized form of glutathione (GSSG), the SD group exhibited a significant increase in GSSG levels (Fig. 3b), whereas the melatonin and SD/melatonin groups showed a significant decrease in GSSG levels (Fig. 3b, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P<0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P<0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P<0.05$). The GSH/GSSG ratio was significantly lower in the SD group compared to all other groups (Fig. 3c). On the other hand, no significant differences were observed in the ratio of GSH/GSSG (Fig. 3c, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$) among control, WPF, melatonin, and SD/melatonin groups, indicating that melatonin normalized the GSH/GSSG ratio in the hippocampus.

Concerning antioxidative stress enzymes, chronic SD significantly decreased GPx levels compared to the control group ($P<0.05$; Fig. 4a). Relative to control, no significant

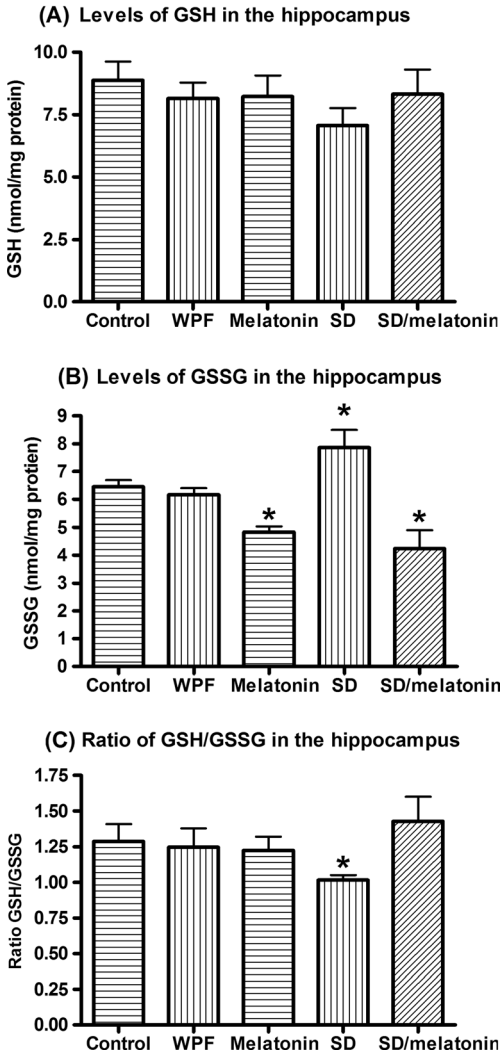

Fig. 3 Chronic melatonin treatment is associated with normalization of changes in oxidative stress biomarkers in the hippocampus of chronically sleep-deprived rats. No changes were observed in the levels of reduced-glutathione (GSH, **a**). Levels of oxidized glutathione (GSSG, **b**) were increased in SD group, whereas they were reduced in SD/melatonin group compared to other experimental groups. The ratio of GSH/GSSG (**c**) was reduced in SD group compared to control, WPF, melatonin, and SD/melatonin groups. Each column is the mean±SEM of 12 rats. *Significant difference from all other groups using one-way ANOVA followed by Bonferroni post hoc test ($P<0.05$)

differences were detected among other study groups (WPF, melatonin, and SD/melatonin), indicating that melatonin normalized GPx activity (Fig. 4a, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$). Additionally, SD significantly decreased catalase activity compared to control group ($P<0.05$; Fig. 4b). On the other hand, catalase activities in melatonin, WPF, and SD/melatonin groups were similar to that in the control group (Fig. 4b, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$), indicating that melatonin normalized hippocampal catalase activity, which is impaired by chronic SD. SD significantly decreased (SOD) activity compared to control group ($P<0.05$; Fig. 4c). On the other hand, no differences were observed among other groups (Fig. 4c, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$), indicating that melatonin restored SOD activity that was reduced by chronic SD. Finally, levels of TBARS were not changed among any of the study groups (Fig. 4d, control versus WPF: $P>0.05$, control versus SD: $P<0.05$, control versus melatonin: $P>0.05$, control versus SD/melatonin: $P>0.05$, WPF versus SD: $P<0.05$, WPF versus melatonin: $P>0.05$, WPF versus SD/melatonin: $P>0.05$, SD versus melatonin: $P<0.05$, SD versus SD/melatonin: $P<0.05$, melatonin versus SD/melatonin: $P>0.05$).

Discussion

Results of this study showed that chronic melatonin administration prevented chronic SD-induced short- and long-term memory impairment. Oxidative stress is associated with cognitive impairment during chronic SD. The antioxidant activity of melatonin was confirmed by normalization the activities of GPx, catalase, and SOD, which are major antioxidant enzymes of the hippocampus. Furthermore, melatonin enhanced GSSG levels and normalized GSH/GSSG ratio that were significantly altered in chronically sleep-deprived rats.

Consistent with our previous work [13–16], current results showed that chronic REM SD impaired hippocampus-dependent spatial memory. Additionally, rats that were sleep deprived by disc-over-water technique for 3 h/day for 14 days showed an impairment of spatial memory [12]. Furthermore, short-term memory was impaired following the RAWM task that was done after using modified multiple platform to induce acute SD [7, 8, 10].

SD leads to impairment of memory functions, which was associated with increasing hippocampus oxidative stress [13–16, 20–22, 39]. Several studies showed that antioxidative stress enzymes/molecules (GPx, catalase, SOD, GSSG, etc.) are necessary for cognitive functions [14, 40, 41]. Oxidative stress occurs when there is an imbalance between reactive oxygen species and the antioxidant opposing forces [42, 43].

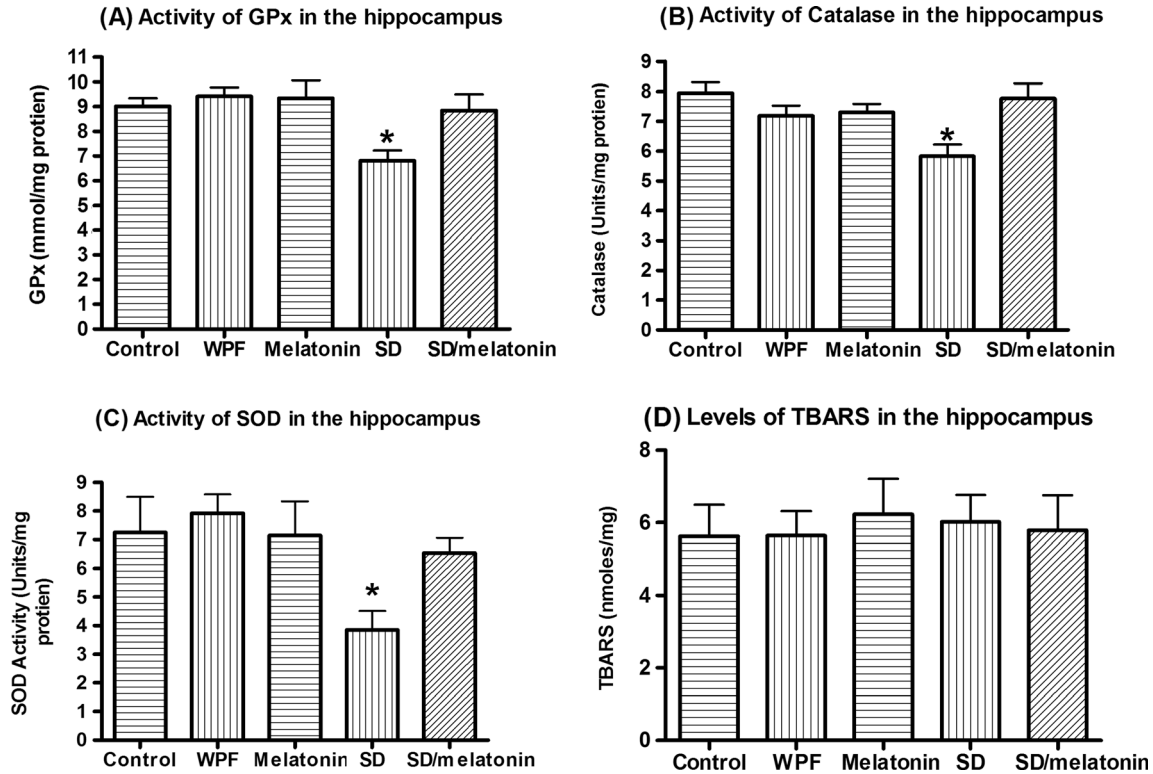

Fig. 4 Chronic melatonin treatment is associated with normalization of reduction in activities of antioxidant enzymes in the hippocampus of chronically sleep-deprived rats. Activity of glutathione peroxidase (GPx, **a**), catalase (**b**), and superoxide dismutase (SOD, **c**) among control, wide plate form (WPF), melatonin, chronic sleep deprivation (SD), and chronic sleep deprivation with melatonin (SD/melatonin). **d** Levels of thiobarbituric acid reactive substances (TBARSs) were not changed among experimental groups. Each point is the mean±SEM of 12 rats. One unit of glutathione peroxidase will cause the formation of 1.0 mmol of NADP+ from NADPH per minute at pH 8.0 at 25 °C in a coupled reaction in the presence of reduced glutathione, glutathione reductase, and tert-butyl hydroperoxide. *Significant difference from all other groups using one-way ANOVA followed by Bonferroni post hoc test ($P<0.05$)

The SOD enzyme catalyzes the dismutation of superoxide into hydrogen peroxide, which is then neutralized by catalase or by GPx [44, 45]. The glutathione system could work in either enzymatic way using the GPx and/or non-enzymatic by action of GSH, which scavenges free radicals [46]. GPx assists the transformation of GSH (the reduced form) into GSSG (oxidized form). These mechanisms are critical to attenuate brain oxidative stress and related neuronal damage, cognitive dysfunction, and the subsequent impaired spatial learning and memory [47–49]. Oxidative stress is associated with cognitive impairments in several health conditions such as Alzheimer's disease [50–54], traumatic brain injury [55], and aging [56]. Findings of the current and previous studies showed that chronic SD decreased antioxidant mechanisms, namely, GSH/GSSG ratio, GPx, catalase, and SOD and impaired both short- and long-term memory [13–16, 20, 22]. These findings provide strong evidence supporting the role of oxidative stress in brain cellular damage and cognitive impairment in several disease states and conditions including SD.

In this study, we showed that administration of melatonin to rats prevents short- and long-term memory impairment induced by chronic SD. Previous studies have documented the protective effect of melatonin on learning and memory deficits induced by a number of pathological or physiological conditions such as Alzheimer's disease [57, 58], aging [59], diabetes [60], and head trauma [61]. Additionally, melatonin was shown to prevent cognitive deficits in memory due to administration/ingestion of chemicals and medications such as aromatic thinner solvents [62], pesticides [63], D-galactose [59], and dexamethasone [64].

Melatonin is a powerful antioxidant that exhibits relevant antiaging properties [65–67]. The levels of melatonin are reduced with aging [68]. For example, endogenous peak levels of melatonin were significantly lower in young adult patients as compared to older patients [24]. This reduction was also correlated with an increase in the oxidative damage during aging [68]. Therefore, older patients are particularly expected to benefit from the antioxidant and the memory-protective properties of agents like melatonin.

The mechanism by which melatonin prevents SD-induced memory impairment is unclear. We believe that melatonin prevents memory impairment through normalizing antioxidant enzyme activities in the hippocampus of sleep-deprived rats. Current results showed that molecules/ enzymes involved in the antioxidant defense systems such as GSH/GSSG ratio, GPx, catalase, and SOD were restored by melatonin treatment

during SD. In fact, melatonin has the ability to neutralize free radicals [69] and to prevent tissue damage associated with oxidative stress by utilizing different mechanisms that include scavenging the free radicals [70–72], increasing mRNA levels and activities of several important antioxidant enzymes, including SOD and glutathione [73], and preventing free radical formation at the mitochondrial level by reducing the leakage of electrons from the electron transport chain [74]. Thus, current results highlight the importance of using antioxidants such as melatonin in relieving some of the deleterious effects of SD on individual health.

A previous study showed that melatonin treatment prevented acute SD-induced cognitive impairment [26]. This was associated with normalization of increased oxidative stress biomarkers, namely, SOD and malondialdehyde in the hippocampus and cortex [26]. The current study showed that melatonin treatment prevented chronic SD-induced cognitive impairment, which was associated with restoration of decreases in oxidative stress biomarkers in the hippocampus including GSH/GSSG, GPx, catalase, and SOD.

Notably, studying the effect of melatonin/chronic SD on brain regions other than the hippocampus is recommended in future studies. Previous studies on acute SD showed that 24 h of SD resulted in increased levels of oxidative stress biomarkers including glyoxalase and glutathione reductase in the hippocampus, cortex, and amygdala [39]. In another study, it was shown that 96 h of paradoxical SD was associated with a fall in oxidative stress in cerebral cortex and brain stem, whereas oxidative stress was elevated in the hippocampus, thalamus, and hypothalamus [5]. Furthermore, 6 h of acute total SD was associated with increased oxidative stress in the rat cortex, brainstem basal forebrain hippocampus, and cerebellum [75]. Finally, prolonged SD for 5–11 days was associated with decreased SOD activity in both the hippocampus and the brainstem [20].

Current results showed no change in the levels of TBARS in association with chronic melatonin and/or chronic SD. These results indicate that lipid peroxidation is not affected by chronic SD. In contrast, previous studies using acute, but not chronic, SD models showed increased TBARS or lipid peroxidation levels in the hippocampus, hypothalamus, thalamus, and cortex during acute SD [5, 26].

In conclusion, melatonin prevents short- and long- term memory impairments induced by chronic SD, which was associated with attenuating oxidative stress in the hippocampus.

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