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Original Article

Methamphetamine exposure during gestation and lactation periods impairs the learning and memory of offspring mice, which is reversed by melatonin: the role of oxidative stress and acetylcholinesterase

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Abstract

Background and purpose: Melatonin is a product of the pineal gland, which regulates the circadian cycle. Neurotoxicity is the most important side effect of methamphetamine (Met) abuse during pregnancy. This study aimed to explore the effect of Met exposure during gestation and lactation periods on the learning and memory of offspring mice. The protective effect of melatonin and the role of oxidative stress and acetylcholinesterase were also investigated.

Experimental approach: The pregnant mice were randomly divided into 2 groups. Saline or Met (5 mg/kg) was injected daily during pregnancy and lactation. After the lactation period, the offspring mice of each group were divided into 2 subgroups, and saline or melatonin (10 mg/kg) was orally (gavage) administered to the offspring mice from the post-delivery (PD) day 21 up to PD Day 60. The offspring mice were examined in the passive avoidance (PA) test. Finally, oxidative stress markers and acetylcholinesterase (AchE) activity were measured in the brains.

Findings/Results: As a result, Met decreased delay and light time while increasing the frequency of entry and time in the dark region of PA. However, melatonin alleviated the impairing effect of Met on PA performance. Meanwhile, the administration of Met increased malondialdehyde while decreasing superoxide dismutase and thiol content. Furthermore, AchE activity was significantly increased in Met-treated mice. Melatonin reversed the levels of antioxidants, lipid peroxidation, and AchE activity in the brain.

Conclusion and implications: Together, these results suggested that melatonin may be a potential therapeutic agent for alleviating Met-induced memory impairment by restoring redox hemostasis and AchE.

Keywords: Acetylcholinesterase; Methamphetamine; Melatonin; Oxidative stress.

INTRODUCTION

During the past decade, the use of amphetamines, including methamphetamine (Met), by pregnant women has increased. Increasing evidence indicates that Met abuse during pregnancy may be associated with cognitive impairments in infants that may be the underlying causes of future psychosocial issues (1). Met is a dopamine stimulant agent commonly used for recreational purposes (2,3).

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The half-life of Met is long and varies between 10 and 12 h. Met abuse leads to euphoria with motor stimulation, increased energy, active wakefulness, insomnia, and alertness and has some effects on the peripheral and central nervous systems (CNS), the immune system, and the gastrointestinal system (3,4). Due to its low molecular weight and high-fat solubility, Met easily passes through the placenta and blood-brain barrier, and it is also secreted in breast milk. Pregnant women tend to continue Met abuse even during breastfeeding, and their newborns are susceptible to a variety of complications after birth (5). Neurotoxicity effects of Met have been well documented (6,7). According to the evidence, Met damages the dopaminergic and serotonergic nerve endings. It also plays a significant role in the occurrence of memory impairments. In addition, Met has a role in other CNS diseases such as depression, anxiety, Parkinson's, and schizophrenia (8). Besides, Met impairs short and long-term memory by damaging the hippocampus and enhancing the extracellular levels of the monoamine neurotransmitters, especially dopamine in the synaptic cleft (9-12). Met abuse induces an oxidative stress status and mitochondrial dysfunction, which finally leads to neuronal apoptosis (13). Following Met administration, the activity of glial cells increases and thus leads to an increase in the production of free radicals (14). During the gestational period, CNS is extremely fragile against injuries induced by Met (15,16). By promoting oxidative stress and degenerative changes in different brain areas such as the hippocampus, Met abuse may be a risk factor for dementia and cognitive decline (16,17). There is some evidence suggesting that Met also affects the brain acetylcholine (Ach) system. Alterations in Ach receptors and Ach esterase (AchE) activity may contribute to the cognitive impairments observed following Met exposure during brain development (18-20).

Melatonin is the product of the pineal gland, which is known as the circadian cycle regulator (21). Melatonin is suggested to have some health-improving effects (22-24). It is also an antioxidant agent that reduces oxidative stress (25). Recent clinical and epidemiological studies have shown the benefits of melatonin in reducing the risk or slowing the progression of cognitive disorders (26). Melatonin increases the expression of neurotrophic factors and other signaling molecules involved in cognitive processing and exerts neuroprotective effects in Alzheimer's disease (AD) models (27). Melatonin has been shown to have antioxidant properties through scavenging free radicals and indirectly by stimulating the antioxidant enzymes such as superoxide dismutase (SOD), and catalase (28,29). It also exhibits neuroprotection by activating the anti-apoptotic proteins and reducing the pro-apoptotic proteins (30-33). Previous studies demonstrated that the cognitive-enhancing activities of melatonin are linked to its anti-inflammatory and antioxidant properties (29,33,34). Met-induced memory impairment was reported to be inhibited by melatonin in rats, and anti-inflammation effects of this neurohormone were also observed (34). However, whether melatonin counteracts memory impairment and cholinergic dysfunction following maternal and postnatal toxicities of Met remained unclear. Thus, this study aimed to determine the effect of Met on oxidative stress markers and AchE activity in newborn mice exposed to Met during prenatal periods. In addition, the protective effect of melatonin and the role of oxidative stress and AchE were also investigated.

MATERIALS AND METHODS

Animals, grouping, and treatments

Male and female BALB/c mice (body weight = 25 - 27 g, age = 80 - 90 days) were obtained from the animal breeding center in the School of Medicine, Mashhad University of Medical Sciences. The animals were kept in the animal house of the School of Medicine, Mashhad University of Medical Sciences, with free access to water and animal food in an appropriate environment. All the animal experiments were conducted in accordance with the approval of the Institutional Ethical Committee of Mashhad University of Medical Sciences (Ethical No. IR.MUMS.REC.1401.330).

For mating, the female and male mice (2 females:1 male) were placed in a common cage. Mating was confirmed by checking the vaginal

plaque. Finally, the pregnant mice (n = 34) were randomly divided into 2 groups including A. pregnant-saline (P/saline) in which saline was daily injected (intraperitoneally, i.p.) during pregnancy and lactation periods and B. pregnant-Met (P/Met) in which Met (5 mg/kg; i.p.) was injected during pregnancy and lactation periods. After the lactation period, the offspring mice of group A were divided into 2 sub-groups including P/saline-O/saline (n = 9) and P/saline-O/melatonin (n = 8) in which saline or melatonin (10 mg/kg; Sigma Aldrich Company, USA) was orally (gavage) administered for the offspring mice from the post deliver (PD) day 21 up to PD day 60. The offspring of group B were also divided into 2 subgroups, including P/Met-O/saline (n = 10) and P/Met-O/melatonin (n = 7) groups. The offspring mice of the latter 2 groups received saline or melatonin from PD day 21 up to PD day 60. The offspring mice (n = 7-10 in each group) were examined in a passive avoidance test. In this experiment, Met was donated by Iran's Drug Control Headquarters, and the purity of the drug was confirmed using high-performance liquid chromatography.

Passive avoidance test

A passive avoidance memory was performed using an apparatus divided into light and dark zones, with a gate between the 2 zones. The floor of the black segment had steel bars, and they were connected to a stimulator. Mice were subjected to 2 separate trials, training for the acquisition of fear and a retention test to investigate the fear memory. In the training, mice were initially placed in the light chamber of the apparatus, then the gate was opened. When the mice moved to the dark room, the middle partition was closed, and an inescapable foot shock (2 mA for 2 s) was delivered to the animals. After 3, 24, 48, and 72 h, the retention test was performed to measure memory consolidation. Mice were placed in the light chamber, and the step-through latency time to enter the dark room was recorded. The mice were also allowed to travel inside the apparatus for 5 min while the gate was opened. The time in light and dark zones and the number of entering into the dark zone were recorded.

Finally, under deep anesthesia by ketamine (100 mg/kg, i.p; Alfasan Company, Netherlands) and xylazine (10 mg/kg, i.p.; Alfasan Company, Netherlands) the animals were sacrificed, and their brains were rapidly dissected and weighed. For biochemical measurements, the hippocampal and cortical tissues were homogenized in a phosphate-buffered solution (Sigma Aldrich Company, USA) using a mechanical homogenizer (Heidlof, Germany), and a 10% (W/V) solution was provided for each sample. The homogenized tissues were centrifuged at 10000 × g for 10 min at 4 °C, and the supernatants were collected to be used for biochemical assessments (16,35).

Biochemical tests

Measurement of malondialdehyde level

The level of lipid peroxidation was determined by measuring the malondialdehyde (MDA) concentration. In this experiment, each sample (1 mL) was added to a mixture of trichloroacetic acid (Sigma Aldrich Company, USA, Catalog NO. T6399; 15% w/v), thiobarbituric acid (Sigma Aldrich Company, USA, Catalog NO. T5500; 0.37% w/v), and hydrochloride acid (Sigma Aldrich Company, USA, Catalog NO. 320331; 2% w/v). Then, the mixtures were incubated at 100°C for 40 min. After cooling, absorbance was measured by a spectrophotometer at 535 nm. The MDA concentration was calculated using Equation (1) previously described (36,37):

$$MDA\ concentration\ \left(\frac{mol}{L}\right) = Absorbance \div 1.65 \times 10^5 \quad (1)$$

Measurement of thiol content

The total thiol level in the brain tissue was measured as previously described (37). At first, 50 µL of each sample was mixed with ethylenediaminetetraacetic acid disodium salt (Na_2EDTA; Sigma Aldrich Company, USA, Catalog NO. E1644), and the absorbance was recorded at 412 nm (absorbance 1). Thereafter, 20 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Sigma Aldrich Company, USA, Catalog NO. 103291; 10 mmol/L) reagent was dispensed into the tubes, and the second absorbance was recorded at the same wavelength (absorbance 2). The total thiol level was calculated using Equation (2) (35,37):

$$Total\ thiol\ concentration\ \left(\frac{mmol}{L}\right) = (Absorbance\ 2 - absorbance\ 1 - blank) \times 1.07 \div (0.05 \times 13.6) \quad (2)$$

Assessment of superoxide dismutase activity

The superoxide dismutase (SOD) activity in the brain tissue was measured according to the previous study (35). In this experiment, 60 µL of each sample was dispensed into each well of a 96-well plate containing 60 µL of phosphate buffer saline (PBS; Sigma Aldrich Company, USA, Catalog NO. P4417). Flowingly, 15 µL of pyrogallol solution (Tokyo Chemical Industry Company, Japan, Catalog NO. P0570; 0.1 mg/mL) and 6 µL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT; Sigma Aldrich Company, USA, Catalog No. 475989; 0.5 mg/mL) were added. The plate was then incubated for 5 min at room temperature. Finally, dimethyl sulfoxide (Scharlab Company**,** Barcelona, Spain, Catalog No. SU01551000) was added to solubilize the produced color. The optical absorbance was measured at 570 nm. The brain tissue SOD activity was expressed as U/g of tissue.

Assessment of AchE activity

AChE activity in the brain was estimated according to the previous study (35). In this method, each sample (40 µL) was dispensed into the tubes containing cold PBS (3 mL). One-hundred µL of DTNB (0.01 mol/L) was transferred to each tube, then vortexed and kept for 10 min at room temperature. Then, 20 µL of acetylthiocholine iodide (Tokyo Chemical Industry Company, Japan, Catalog No. A0116; 0.07 mol/L) was added to start the reaction, and the absorbance was read. After 10 min, the second absorbance was read, and the change in absorbance was calculated. AChE activity was estimated by using Equation (3) and expressed as nmol/min/g tissue (35):

$$R = 5.74(10^{-4})\ change\ in\ absorbance\ per\ min \div Concentration\ of\ tissue \quad (3)$$

Statistical analysis

All data were presented as the mean ± standard error of the mean. One-way ANOVA followed by Tukey post hoc test was used to determine significant differences among the groups. $P < 0.05$ was considered significant.

RESULTS

Passive avoidance test

The results showed that the delay time for entering the dark compartment was lower in the P/Met-O/saline group than the P/saline-O/saline and P/saline-O/melatonin groups at all times after the shock (Fig. 1A-D). The treatment of animals with melatonin significantly increased the delay time for entering the dark compartment in the P/Met-O/melatonin group compared to the P/Met-O/saline group at 3, 24, 48, and 72 h after receiving the shock (Fig. 1A-D). Interestingly, the delay time for entering the dark compartment in the P/Met-O/melatonin group was lower than the P/saline-O/saline group at all times after the shock. In addition, the delay time in the P/saline-O/melatonin group was significantly higher than that in the P/saline-O/saline group at 3, 24, and 48 h (Fig. 1A-D).

The results also showed that the time spent in the dark compartment was longer in the P/Met-O/saline group than that in both P/saline-O/saline and P/saline-O/melatonin groups at all times after the shock (Fig. 1E-H). Treatment of offspring mice born from the Met-intoxicated mothers with melatonin decreased the time spent in the dark compartment in the P/Met-O/melatonin group compared to the P/Met-O/saline group at 3, 24, 48, and 72 h after receiving the shock (Fig. 1E-H). In addition, the time spent in the dark compartment in the P/saline-O/melatonin group was lower than that in the P/saline-O/saline group at 72 h (Fig. 1H). Notably, the time spent in the dark compartment in the P/Met-O/melatonin group was higher than that in the P/saline-O/saline group at 3 (Fig. 1E) and 48 h (Fig. 1G) after the shock.

Melatonin also increased the total time spent in the light compartment in the P/saline-O/melatonin group compared to the P/saline-O/saline group at 72 h post-shock time. Hence, Met negatively affected the performance of the mice in PA test, and the light time in P/Met-O/saline group was significantly lower than that in both the P/saline-O/saline and P/saline-O/melatonin groups at all post-shock times (Fig. 2A-D).

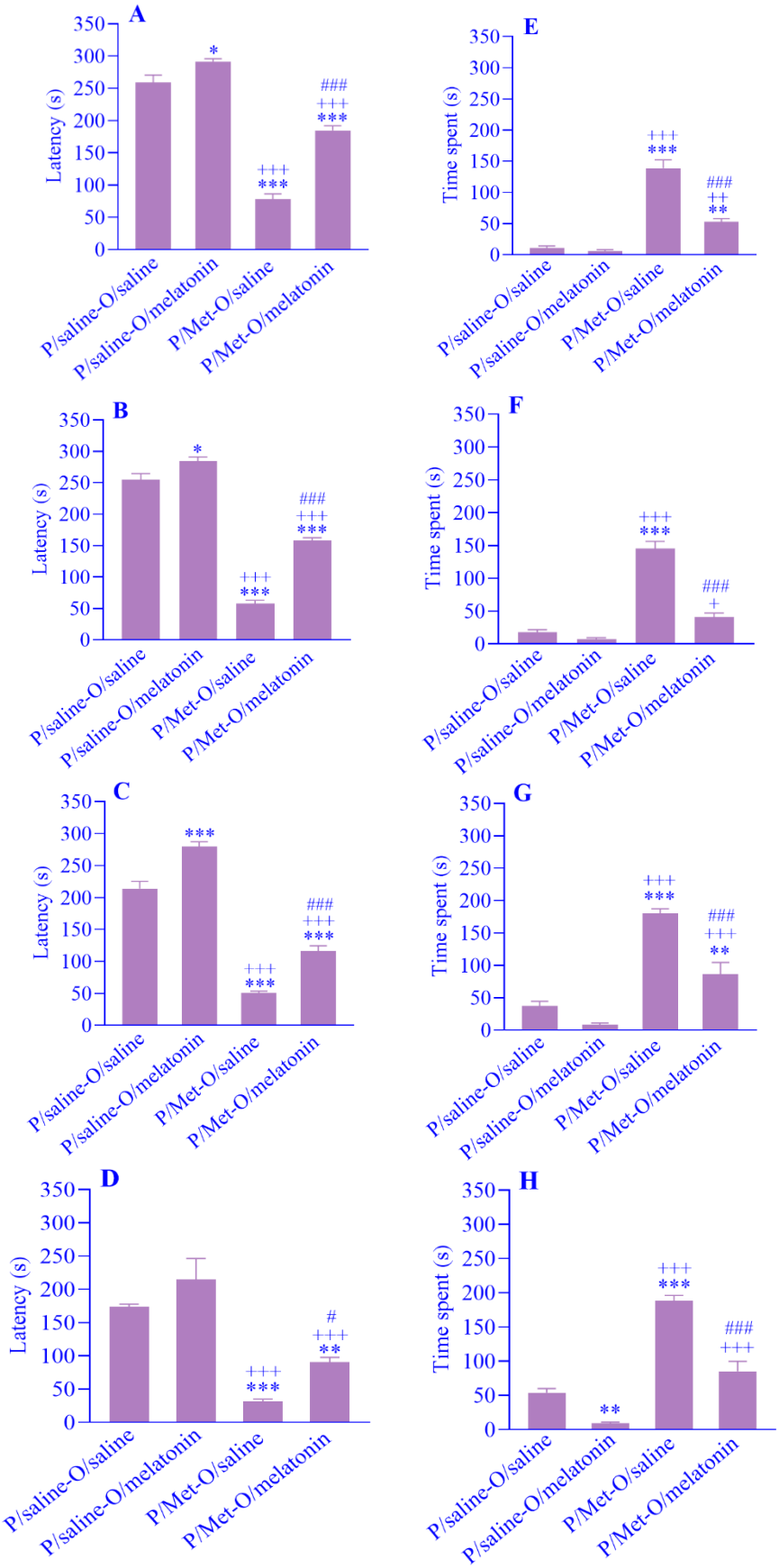

Fig. 1. The comparison of latency for entering the dark compartment and the total time spent in the dark compartment in the passive avoidance test among all groups. (A and E) 3, (B and F) 24, (C and G) 48, and (D and H) 72 h after receiving the shock. Data were expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ indicate significant differences compared to P/saline-O/saline group; $^{+}P < 0.05$, $^{++}P < 0.01$, and $^{+++}P < 0.001$ versus P/saline-O/melatonin group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring.

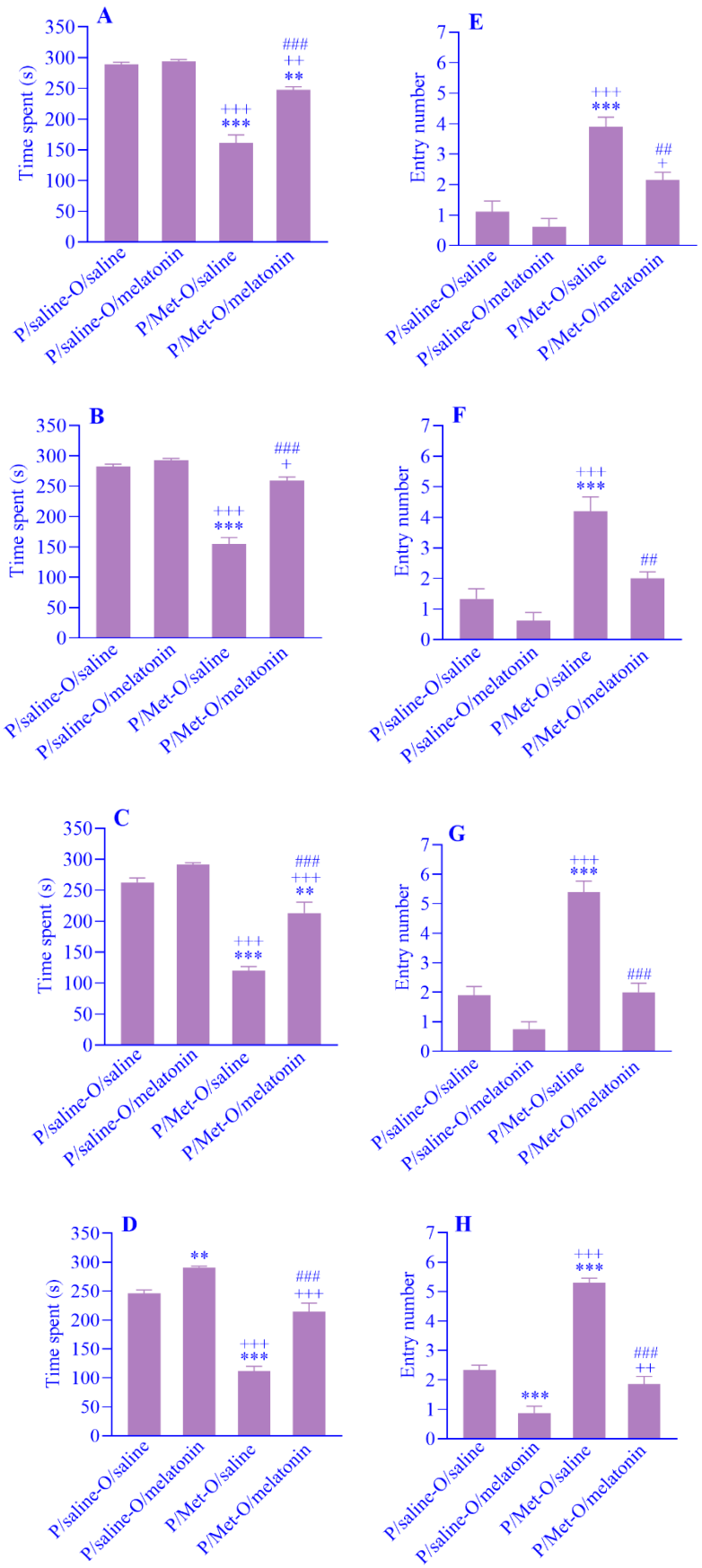

Fig. 2. The comparison of total time spent in the light compartment and the number of entries into the dark compartment in the passive avoidance test among all groups. (A and E) 3, (B and F) 24, (C and G) 48, and (D and H) 72 h after receiving the shock. Data were expressed as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared to P/saline-O/saline group; $^{+}P < 0.05$, $^{++}P < 0.01$, and $^{+++}P < 0.001$ versus P/saline-O/melatonin group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring.

Melatonin also reversed the effects of Met, and the time spent in the light compartment in the P/Met-O/melatonin group was longer than in the P/Met-O/saline group at 3, 24, 48, and 72 h after receiving the shock. At the 3 and 48 h post-shock times, the time spent in light compartment in P/Met-O/melatonin was shorter than that in P/saline-O/saline (Fig. 2A-D).

The number of entries into the dark compartment at 3, 24, 48, and 72 h after receiving the electric shock in the P/Met-O/saline group significantly increased compared to the P/saline-O/saline and P/saline-O/melatonin groups (Fig. 2E-H). The results indicated a significant decrease in the number of entries into the dark compartment in the P/Met-O/melatonin group at 3, 24, 48, and 72 h after receiving the shock compared to the P/Met-O/saline group. Melatonin also significantly decreased the number of entries into the dark compartment in the P/saline-O/melatonin group compared to the P/saline-O/saline group only 72 h post shock time (Fig. 2H).

Biochemical assessments

MDA concentration

MDA concentration in both hippocampus and cortex of P/Met-O/saline group enhanced in comparison to P/saline-O/saline and P/saline-O/melatonin groups, significantly. However, MDA concentration in both the hippocampus and cortex of the P/Met-O/melatonin group significantly decreased compared to the P/Met-O/saline group (Fig. 3A and B).

Thiol concentration

As Fig. 4 illustrates, there was a significant reduction of thiol concentration in both the hippocampus and cortex in the P/Met-O/saline group compared to the P/saline-O/saline group. Also, thiol concentration significantly decreased in the hippocampus in the P/Met-O/saline group in comparison to P/saline-O/melatonin group (Fig. 4A), but it was observed no significant difference in cortex (Fig. 4B). The thiol concentration in both hippocampus and cortex of P/Met-O/melatonin group was significantly higher than the P/Met-O/saline group (Fig. 4).

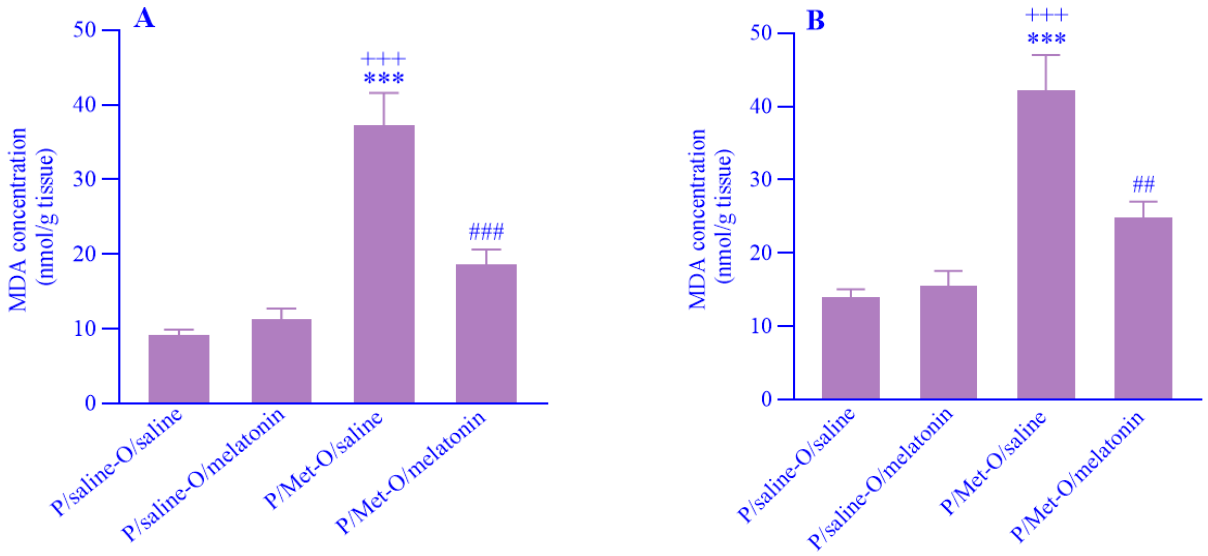

Fig. 3. The comparison of MDA concentration in the (A) hippocampus and (B) cortex among all groups. Data were expressed as mean ± SEM. $^{***}P < 0.001$ indicates significant difference compared to P/saline-O/saline group; $^{+++}P < 0.001$ versus P/saline-O/melatonin group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring; MDA, malondialdehyde.

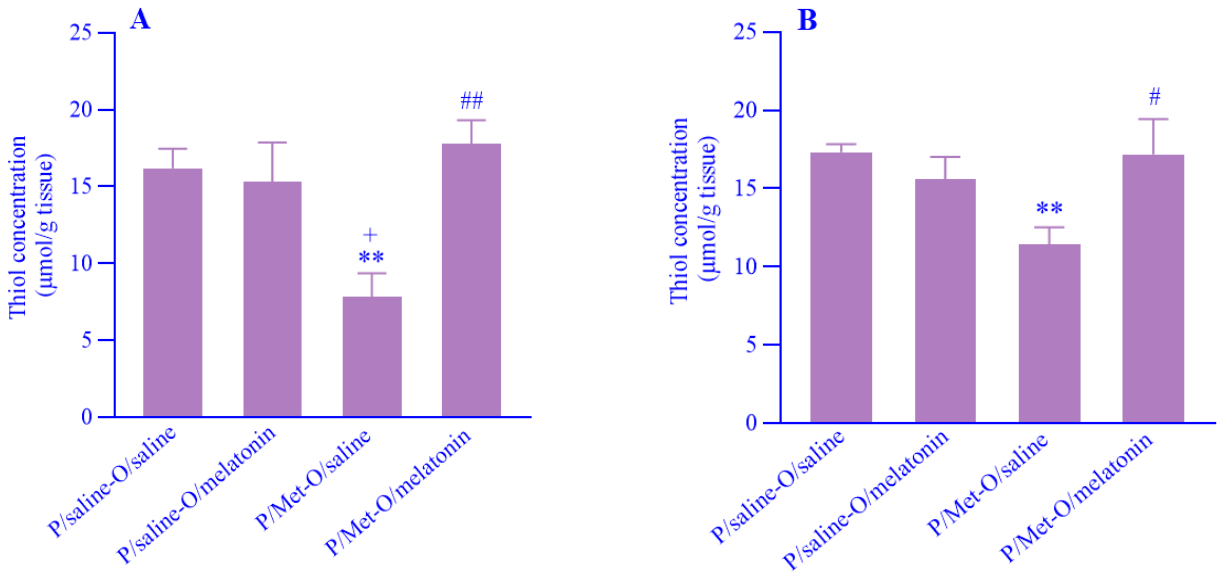

Fig. 4. The comparison of thiol concentration in the (A) hippocampus and (B) cortex among all groups. Data were expressed as mean ± SEM. $^{**}P < 0.01$ indicates significant difference compared to P/saline-O/saline group; $^{+}P < 0.05$ versus P/saline-O/melatonin group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring.

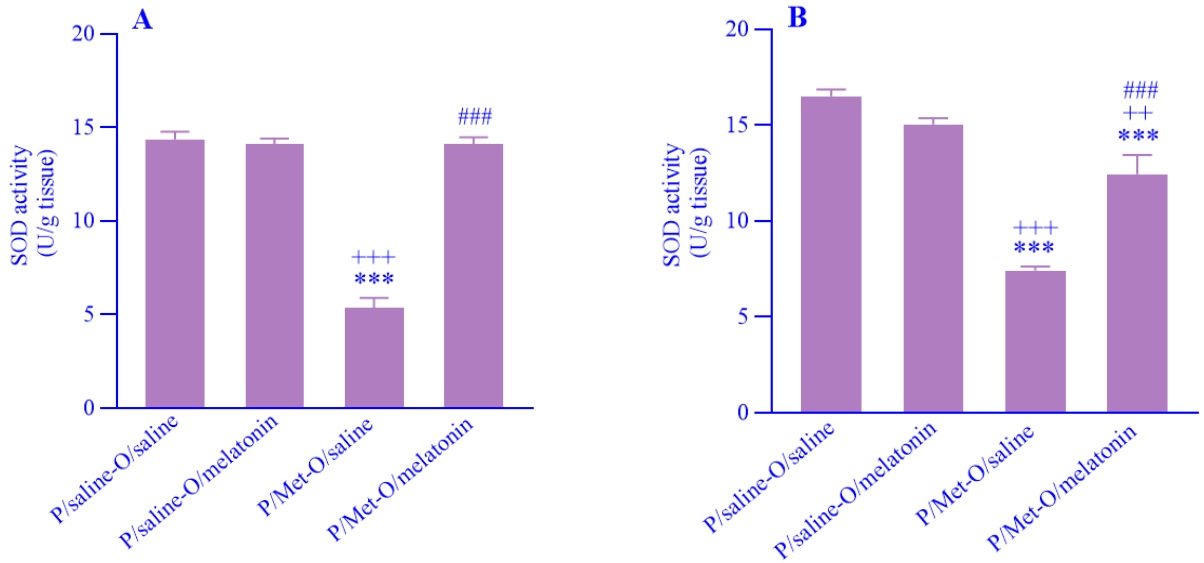

Fig. 5. The comparison of SOD activity in the (A) hippocampus and (B) cortex among all groups. Data were expressed as mean ± SEM. $^{***}P < 0.001$ indicates significant difference compared to P/saline-O/saline group; $^{++}P < 0.01$ and $^{+++}P < 0.001$ versus P/saline-O/melatonin group; $^{\#\#\#}P < 0.001$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring, SOD, superoxide dismutase.

SOD activity

Assessment of SOD activity in the hippocampus and cortex indicated that SOD activity in P/Met-O/saline group was significantly lower than the P/saline-O/saline and P/saline-O/melatonin groups (Fig. 5). However, the activity of SOD in both hippocampus and cortex of P/Met-O/melatonin group significantly increased compared to P/Met-O/saline (Fig. 5A and 5B). In the cortex of P/Met-O/melatonin group, the SOD activity was significantly lower than both P/saline-O/saline and P/saline-O/melatonin groups, but there was no significant difference in the hippocampus among the groups (Fig. 5). Moreover, there was no significant difference in the cortical and hippocampal SOD activity between P/saline-O/saline and P/saline-O/melatonin groups (Fig. 5).

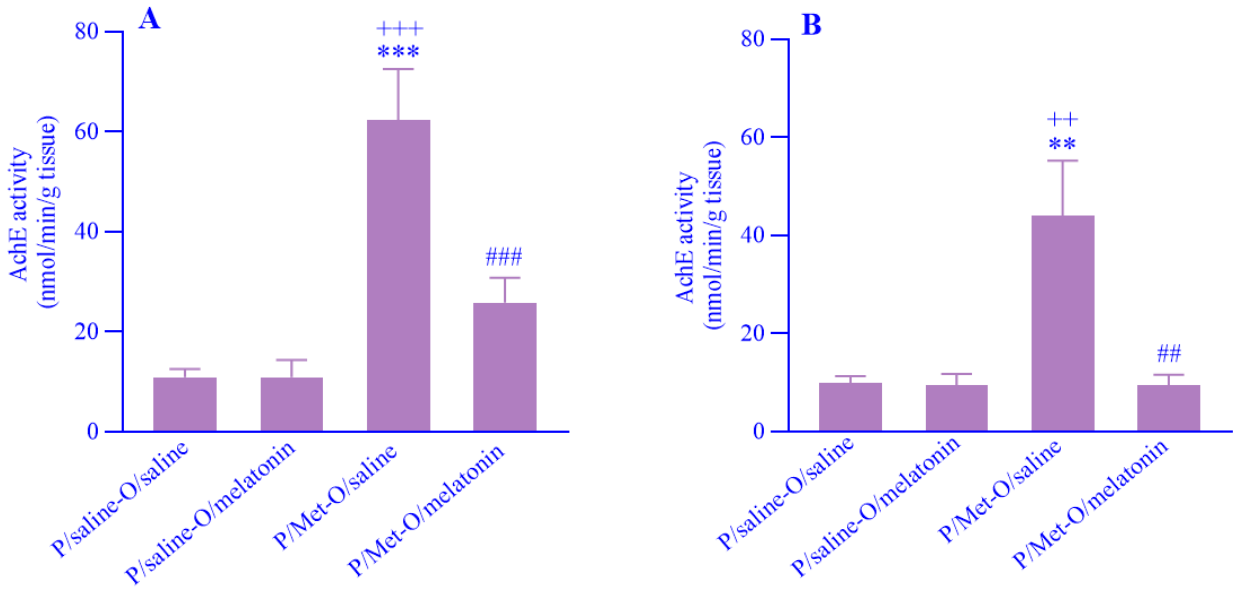

Fig. 6. The comparison of AchE activity in the (A) hippocampus and (B) cortex among all groups. Data were expressed as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared to P/saline-O/saline group; $^{++}P < 0.01$ and $^{+++}P < 0.001$ versus P/saline-O/melatonin group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus P/Met-O/saline groups. P, Pregnant mothers; Met, methamphetamine; O, offspring, AchE, acetylcholinesterase.

AchE activity

The results showed that there was no significant difference in hippocampal and cortical AchE activity between P/saline-O/saline and P/saline-O/melatonin groups (Fig. 6). Met significantly increased AchE activity in both hippocampus and cortex of P/Met-O/saline group compared to both P/saline-O/saline and P/saline-O/melatonin groups. However, melatonin reversed the effects of Met on AchE activity in the hippocampus and cortex reflected by lower AchE activity in P/Met-O/melatonin group in comparison to P/Met-O/saline group (Fig. 6).

DISCUSSION

This study showed that melatonin supplementation improved learning and memory impairments, abnormal AchE activity, and oxidant/anti-oxidant status in Met-treated mice during gestation and lactation periods. According to previous studies, Met causes neurocognitive deficits by affecting hippocampal neurotransmitters, and by altering synaptic transmission in the striatum and hippocampus, it also causes short and long-term memory loss by reducing hippocampal plasticity (38). In the present study, cognitive and behavioral changes were monitored by the PA test. The results showed a decreased delay time to enter the dark and total time spent in the light compartment while increasing the frequency of entry and time spent in the dark region of PA in the offspring of the mice exposed to Met during pregnancy and lactation. Thus, results from the behavioral observations demonstrated that Met treatment induces cognitive and behavioral impairment in mice, which were in line with previous studies (15,16,40). This study further showed that melatonin supplementation protected the animals from the behavioral deficits induced by Met. The current findings supported previous evidence indicating the memory-enhancing effects of melatonin (26). One study revealed that melatonin improved learning and memory performance in shuttle-box and water maze tests in a mouse model of D-galactose-induced amnesia (41). On the other hand, in the current study, the offspring mice of Met-exposed mothers were then treated with melatonin after breastfeeding, and the results showed that the time latency to enter the dark compartment was increased significantly at 3, 24, 48, and 72 h after receiving the electric shock compared to P/Met-O/saline group. The results were in line with the results of a previous study (41).

It was shown that Met causes excess dopamine accumulation in the synaptic cleft space, which leads to the creation of free radicals and the occurrence of oxidative stress

(17,39,42). The brain is very susceptible to oxidative stress due to its higher oxygen consumption and poor antioxidant system. Hippocampal and cortical regions are particularly susceptible to oxidative damage. Oxidative stress is one of the risk factors responsible for the neuronal damage mediating cognitive or behavioral deficits (35). The antioxidant system including SOD enzyme and thiol groups can remove free radicals generated inside the cells and organelles including mitochondria. MDA results from lipid peroxidation of polyunsaturated fatty acids and is a marker for oxidative stress (35,36). Furthermore, a significant decrease in brain SOD activity and glutathione content is an indicator of brain oxidative stress (35). The results showed a decrease in brain SOD activity and thiol content after Met exposure that may be a response to free radicals' overproduction. Thus, a positive correlation was observed between oxidative stress and the activation of the antioxidant defense mechanism. This finding was in line with previous studies (17,43). Hence, oxidative stress assumes a significant role in amnesia induced by Met. Previous studies indicated the antioxidant potential of melatonin (29,44). In this study, melatonin improved antioxidant systems in Met-treated animals indicated by restoring levels of SOD and thiols.

Activities of the cholinergic neurotransmitter, acetylcholine, are regulated by cholinesterase. The cholinergic system in the central nervous system plays crucial roles in the cognition and memory process. Increased AchE activity causes the breakdown of acetylcholine, leading to cholinergic dysfunction followed by cognitive deficits (45). An increase in brain AchE activity was observed in the present study. Our biochemical findings indicated that melatonin improved cholinergic transmission by lowering the AchE activity in the Met-exposed mice. These observations are in line with previous reports where the neuroprotective role of melatonin was studied in colchicine-treated rats (29,46).

Collectively, the learning and memory-improving effects of melatonin in offspring mice whose parents were exposed to Met were attributed to its antioxidant effects in the present study. However, the precise signaling pathways were not examined in the present study, and molecular experiments need to be investigated in the future. In addition, the effects of melatonin on AchE activity as a marker of cholinergic function were challenged in the present study. However, further precise studies are suggested to reveal the exact mechanisms.

CONCLUSION

The results of the study showed that melatonin administration alleviated the Met-induced memory and learning dysfunction. Melatonin seems to exert its positive effects by facilitating cholinergic function and inhibiting oxidative stress, which in turn attenuates neurodegeneration in the brain and thus preserves memory and cognitive functions.

Acknowledgments

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Conflicts of interest statement

All authors declared no conflict of interest in this study.

Author's contributions

M. Hosseini conceived and designed research; F. Ghorbani, N. Osatd-Rahimi, A. Rajabian, and F. Mansouritorghabeh conducted experiments; M. Hosseini analyzed the data; M. Hosseini and A. Rajabian wrote and revised the manuscript. All authors read and approved the finalized version of the manuscript.

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