Effects of melatonin and resveratrol on recognition memory and passive avoidance performance in a mouse model of Alzheimer’s disease

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ABSTRACT

Alzheimer’s disease (AD) is the foremost cause of dementia among other neurodegenerative diseases, leading to memory loss and cognitive deficits. AD has gained extensive attention in research for exploring possible interventions. One promising field is natural substances and compounds that could provide a wide range of neuroprotection against AD. This study aimed to investigate the possible effects of melatonin (MEL) and resveratrol (RES) in improving memory deficits in a sporadic mouse model of AD. Memory deficit was induced using AlCl3 and d-galactose for generating an AD mouse model. Mice were randomly distributed into five groups (n = 13): control, AD, AD + MEL (AD mice treated with 80 mg/kg of MEL), AD + RES (AD mice treated with 40 mg/kg of RES), and AD + Combination (AD mice that received 80 mg/kg of MEL and 40 mg/kg RES). A novel object recognition task (NORT) and passive avoidance task (PAT) were used for assessing memory. Moreover, acetylcholinesterase (AChE) level, brain-derived neurotrophic factor (BDNF), and cAMP-response element binding (CREB) protein expression were measured in the prefrontal cortex tissue. Our results showed that MEL significantly improved memory deficits in both the NORT and PAT of the AD model, while RES improved the PAT only in the AD model. Co-treatment with MEL and RES exerted beneficial additive effects on recognition memory impairment in the AD mouse model. Moreover, our results demonstrated that both MEL and RES enhanced the cholinergic system and BDNF and CREB signaling pathways in the prefrontal cortex in an AD mouse model.

1. Introduction

Alzheimer’s disease (AD) is the foremost cause of dementia among other neurodegenerative diseases, leading to memory loss and cognitive deficits. Globally, the number of AD patients was more than 43 million in 2015. These numbers are expected to reach 115 million by 2050 [1]. Clinically, AD is characterized by progressive and irreversible memory deterioration, cognitive decline, and behavioral alterations [2]. These clinical features are usually accompanied by specific pathologic changes and mainly manifest as extracellular β-amyloid (Aβ) plaque precipitation and neurofibrillary tangle (NFT) accumulation in the brain tissue. Additionally, neuroinflammation, oxidative stress, cholinergic dysfunction, and synaptic loss play a critical role in the pathogenesis of AD [3]. However, the exact mechanism of AD is still not fully understood, and further research is warranted.

The pineal product melatonin (MEL) (N-acetyl-5-methoxytryptamine) participates in several physiological functions, including regulation of body temperature and circadian rhythms [4–6], and it has been

Abbreviations: AD, Alzheimer’s disease; MEL, melatonin; RES, resveratrol; NORT, novel object recognition task; PAT, passive avoidance task; AChE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; CREB, cAMP-response element binding; Aβ, amyloid β; NFT, neurofibrillary tangle; AlCl3, aluminum chloride; KFMRC, King Fahd Medical Research Center; DW, distilled water; IP, intraperitoneally; OFT, open field task; TDM, total distance moved; PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; bp, base pairs; ANOVA, analysis of variance; DI, discrimination index; ns, non-significant; SEM, standard error of the mean.

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reported to be a remarkable antioxidant with anti-inflammatory actions in several studies [7–9]. MEL regulates the levels of antioxidant enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase 1, within the normal range in the prefrontal cortex of AD mice [10]. Additionally, MEL has been shown to prevent Aβ formation and NFT accumulation in other experimental AD models [11,12]. Moreover, the protective effects of MEL against cognitive impairment have been indicated in several experimental studies [13,14]. Another effective and widely studied candidate compound is resveratrol (RES) (3,4,5-trihydroxystilbene), which is a polyphenolic compound extracted mainly from grapes and other fruits, such as berries, pomegranates, and nuts [15]. In many previous in vivo and in vitro studies, RES exhibited a wide range of antioxidant, anti-inflammatory, and anti-mutagenic activities [13,16].

Cumulative evidences have suggested neuroprotective properties of RES in neurodegenerative diseases, such as Parkinson’s disease, cerebral ischemia, and AD [4,15]. MEL has been shown to potentiate the neuroprotective effect of RES against cytotoxicity induced by Aβ1–42 [44].

Administration of β-galactose leads to increased production of reactive oxygen species and decreased levels of antioxidant enzymes [17]. Moreover, β-galactose can induce learning and memory impairment in different mouse strains [17,18]. Therefore, β-galactose treatment can mimic normal aging in the mouse. Aluminum chloride (AlCl3) is a neurotoxic agent that increases oxidative stress and activates the inflammatory response in rodents’ brains [18]. Furthermore, chronic administration of AlCl3 leads to NFT formation, Aβ overproduction, and cholinergic dysfunction [19]. Thus, the combined administration of AlCl3 and β-galactose has been used for inducing a non-transgenic sporadic AD mouse model that exhibits several pathological features of AD [17–20].

This study aims to investigate the prophylactic effects of MEL and RES in improving memory loss in an AD sporadic model.

2. Materials and methods

2.1. Animals

In total, 65 adult normal male Swiss mice (SWR/J) weighing 18–22 g were obtained from the animal facility of the King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Mice were housed in appropriate temperature (23 ± 2 °C) and humidity (65 %) conditions with a standard 12 h light/dark cycle and ad libitum access to water and standard food. All experimental mice were treated according to the guidelines of the animal unit committee of the KFMRC. All experiments were performed according to the guidelines of the biomedical ethics research committee (Reference No. 102-19) at King Abdulaziz University and followed the rules and regulations of the Animal Care and Use Committee at the KFMRC, which comply with the guidelines of “System of ethics of research on living creatures” prepared by the King Abdulaziz City for Science and Technology and were approved by the Royal Decree No. M/59 dated 24/08/2010.

2.2. Treatment preparation

Hydrated AlCl3 (Techno Pharmchem, Haryana, India) was dissolved in distilled water (DW). Extra pure β-galactose powder (LobaChemie Pvt Ltd, India) was dissolved in 0.9 % normal saline. β-galactose and AlCl3 were freshly prepared twice per week and were stored at 4 °C. Melatonin crystalline and resveratrol (Sigma-Aldrich, USA) were dissolved in 10 % ethanol and were then diluted with saline. MEL and RES were freshly prepared daily before administration and were stored in dark bottles to avoid possible oxidation by light. All treatments were administered to mice in a volume of 0.2 mL. Each dose was adjusted weekly according to the animal weight.

2.3. Treatment groups

Mice were randomly distributed into the following five groups (n = 13): Control group, received DW orally, 10 % ethanol in saline intraperitoneally (IP), and saline subcutaneously; AD group, mice were administered AlCl3 (20 mg/kg) orally, β-galactose (120 mg/kg) subcutaneously, and saline IP; AD + MEL group, AD model mice received MEL (80 mg/kg) via IP injection [9]; AD + RES group, AD model mice received RES (40 mg/kg) via IP injection [21,22]; AD + Combination group, AD model mice received MEL (80 mg/kg) and RES (40 mg/kg) via IP injections.

2.4. Experimental design

Long period of administration of AlCl3 and β-galactose has been used for inducing a non-transgenic sporadic AD mouse model that exhibits several behavioural and pathological features of AD [17]. In our study, AlCl3 and β-galactose lasted for eight weeks. MEL and RES treatments were administered during weeks -1 and 0, whereas AlCl3 and β-galactose were started on week 1. All treatments were administered once per day. From 8:00-9:00 am, mice were treated with MEL and RES and between 10:00 am and 12:00 pm, mice received AlCl3 and β-galactose. Behavioral tests were performed during the ninth week (Fig. 1).

2.5. Open field task (OFT)

To evaluate the locomotor activity of mice, an OFT was used for measuring the velocity and total distance moved (TDM). Mice were placed individually within an empty arena (square test box, 45 × 45 cm) and were allowed to move freely for 3 min. The EthoVision XT8A system (Noldus Information Technology, Wageningen, the Netherlands) was used for calculating all data.

2.6. Novel object recognition task (NORT)

The NORT is one of the most common behavioral tests and is widely used for assessing short-term recognition memory deficits in rodents [23]. This experiment was preceded by one day of habituation for 15 min./mouse, and locomotor measurements were performed for 3 min./mouse and the next day. On the first day of the NORT, a familiarization trial was conducted in which two identical objects (e.g., two red cans) were placed inside the arena, and mice were allowed to explore the two objects for 3 min. (Supplementary Fig. 1). The test objects were chosen according to specific properties, i.e., cleanable and not easily moved. After 10 min., a test trial was performed in which one of the familiar objects was changed to a novel object (e.g., one red can was replaced with a yellow cube), and the object exploration was recorded for 3 min. (Supplementary Fig. 2). The objects and arena were cleaned after each trial to avoid odor cues. Parameters were automatically recorded and analyzed using a video tracking system (EthoVision XT8A). The frequency of sniffing in both zones was assessed in both the familiarization and test phases for ensuring that all mice had the opportunity to explore both objects. Animal memory was also assessed using the time spent in the familiar and novel zones during the test phase. The discrimination index (DI) was calculated according to the following equation: DI = (time spent on the novel object – time spent on the familiar object) / (time spent on the novel object + time spent on the familiar object).

2.7. Passive avoidance task (PAT)

The PAT is commonly used for measuring avoidance memory retention in mice [24]. A step-through PAT was performed as described by previous studies [24]. The passive avoidance procedure involves placing a mouse within a shuttle box (Columbus Instruments, connected to the PACS 30 software program). The apparatus consists of two
compartments separated by an automated door. On the first day of the
test, animals were placed within the apparatus for 10 min. for habitu-
ation with the door and gate opened. On the second day (acquisition
trial), mice were placed in the illuminated compartment. After 30 s of
acclimatization, the door was raised, and when the mouse moved from
the illuminated area (light intensity of 8) to the dark area, an electrical
shock (0.5 mA) was delivered for 10 s. On the third day (retention trial),
same procedures were performed in the acquisition trial but without
electric shock. In both the acquisition and retention trials, the transfer
latency time (sec) for each mouse was recorded.

2.8. Acetylcholinesterase (AChE) level

The level of AChE was measured in the prefrontal cortex tissue of
mice using mouse AChE enzyme-linked immunosorbent assay kit
(Elabscience, Houston, TX, USA) according to the manufacturer’s
protocol.

2.9. Gene expression

Expression levels of the genes encoding cAMP-responsive element-
binding protein (CREB1) and brain-derived neurotrophic factor (BDNF)
in the prefrontal cortex of mice were measured using real-time poly-
merase chain reaction (PCR).

2.9.1. RNA extraction and real-time PCR

Total pure RNA was extracted from the prefrontal cortex tissues
using PureLink™ RNA mini kit (Ambion, Austin, TX, USA) according to
the manufacturer’s protocol. Complementary DNA (cDNA) synthesis
was performed using SuperScript™ IV VILO™ master mix (Invitrogen)
kit according to the manufacturer’s protocol. Real-time PCR were
performed using SsoAdvanced™ Universal SYBR® Green Supermix
(Bio-Rad) according to the manufacturer’s protocol. Glyceraldehyde 3-
phosphate dehydrogenase (GAPDH) was used as a housekeeping gene.
The details of the primers used are summarized in Table 1.

2.10. Statistical analyses

All data are expressed as mean ± standard error of the mean and
were statistically analyzed using GraphPad Prism 8.3.8. The one-way
analysis of variance (ANOVA) followed by post hoc Tukey’s test was
used for comparing differences between the groups in all results except
for frequency of sniffing in which two-way ANOVA followed and post
hoc Bonferroni’s test were used. The differences between the groups
were considered statistically significant if P was < 0.05.

3. Results

3.1. Effects of MEL and RES on locomotor activity

The OFT showed no significant difference between the AD and
Control groups for TDM (P = 0.0543). Additionally, no significant dif-
ference was observed in TDM values between the AD group and other
treatments groups: AD + MEL (P = 0.6661), AD + RES (P = 0.999), and
AD + Combination (P = 0.2473) (Fig. 2A). Moreover, statistical analysis
revealed no significant difference between the AD group and Control
group for the velocity (P = 0.1108). Furthermore, no significant dif-
ference in the velocity was observed between the treatment groups and
the AD group: AD + MEL (P = 0.9935), AD + RES (P > 0.999), and
AD + Combination (P = 0.1083) (Fig. 2B).

Table 1

Sequences of PCR primers.

| Gene   | Accession Number | Oligo                | Sequences                  | Amplicon length (base pairs, bp) |
|--------|------------------|----------------------|----------------------------|----------------------------------|
| GAPDH  | NM_001289726.1   | Forward primer       | GTGAACGGATTTGGCCGTATT      | 70                               |
|        |                  | Reverse primer       | CAATCTCCACTTTGCCACTGC      |                                  |
|        |                  |                      | ACCACAGGAGCACATGGC         |                                  |
| CREB1  | NM_001037726.1   | Forward primer       | FGCCCTGGCCGCTCCATTGA       | 120                              |
|        |                  | Reverse primer       | ACGGGGCAAGCTCTTATACTG      |                                  |
| BDNF   | NM_001048139.1   | Forward primer       | ATGGCCCTATAGAAGACTGTCTGGT  | 70                               |
|        |                  | Reverse primer       |                             |                                  |

BDNF: brain-derived neurotrophic factor; CREB1: cAMP-responsive element-binding protein; PCR: polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.
Fig. 2. Locomotor activity. A) No significant differences were observed in the TDM among all the groups. B) There were no significant differences in mouse velocity among all the groups. Data are presented as mean ± SEM. One-way ANOVA was used, followed by Tukey’s multiple comparisons test. Non-significant (ns) vs. Control group (P < 0.05) and ns vs. AD group (P < 0.05). AD: Alzheimer’s disease; MEL: melatonin; RES: resveratrol; TDM: total distance movement; SEM: standard error of the mean; ANOVA: analysis of variance.

Fig. 3. Novel object recognition task (NORT). A) Frequency of sniffing (%) in both the phases of the NORT. A) In the familiarization stage, there was no significant differences in the frequency of sniffing (%) of each object (familiar vs. novel) among all the groups. In the test phase, there were significant differences in the frequency of sniffing (%) of each object (familiar vs. novel) among the Control, AD, and AD + MLT groups. B) Discrimination index (DI). There was a significant reduction in the DI in the AD group than in the Control group. A significant increase in the DI was apparent in the AD + MEL, and AD + Combination groups than in the AD group. No significant increase in the DI was apparent in the AD + RES group. Data are presented as mean ± SEM. Two-way ANOVA followed by the Bonferroni multiple comparisons test (A), and one-way ANOVA followed by the Tukey multiple comparisons test (B) were used. Non-significant (ns) (#) vs. Control group, (ns) (*) vs. AD group. AD: Alzheimer’s disease; MEL: melatonin; RES: resveratrol; SEM: standard error of the mean; ANOVA: analysis of variance. (ns) P < 0.05, *P < 0.05, **P < 0.01, ***P < 0.001.
3.2. Effects of MEL and RES on NORT performance

Calculation of the sniffing frequency (%) of mice for both objects (familiar vs. novel) in the sample phase showed no significant differences among the groups (Fig. 3A). During the test phase, the frequency of sniffing for the familiar vs. novel objects was significantly different among the groups (Fig. 3A). In the Control group, the frequency of sniffing (%) significantly increased for the novel object than for the familiar object ($P < 0.0001$), whereas in the AD group, the frequency of sniffing (%) was significantly reduced for the novel object than for the familiar object ($P = 0.0016$). The AD + MEL group exhibited a significant increase in the frequency of sniffing (%) for the novel object than for the familiar object ($P = 0.0335$). However, the AD + RES and AD + Combination groups showed no significant difference in the frequency of sniffing (%) ($P = 0.9635$ and $0.1005$, respectively).

In the NORT, a significant reduction was found in the DI of the AD group when compared with the Control group ($P = 0.0002$). Treated groups exhibited a highly significant increase in the DI than the AD group: $AD + MEL (P = 0.0012)$, and $AD + Combination (P = 0.0009)$. However, the $AD + RES$ group showed no significant increase in the DI ($P = 0.052$) than the AD group (Fig. 3B).

3.3. Effects of MEL and RES on PAT performance

In the PAT, the transfer latency for each mouse in both the acquisition and retention phases was evaluated. Statistical analysis using one-way ANOVA showed no significant difference in the transfer latency among all experimental groups in the acquisition test ($P = 0.9522$) (Fig. 4). However, in the retention test, a highly significant difference was observed in the transfer latency among all groups ($P < 0.0001$). The post hoc Tukey’s test showed a highly significant reduction in the latency time of the AD group than that of the Control group ($P < 0.0001$). The treatment groups exhibited significant increases in the transfer latency than the AD group: $AD + MEL (P = 0.0002)$, $AD + RES (P = 0.0457)$, and $AD + Combination (P = 0.0015)$.

3.4. Effects of MEL and RES on AChE levels in the prefrontal cortex of mice

The level of AChE in the prefrontal cortex tissue significantly decreased in the AD group than in the Control group ($P = 0.0336$, Fig. 5). A significant increase was seen in the AChE levels in the $AD + MEL (P = 0.0142)$, $AD + RES (P = 0.0001)$, and $AD + Combination (P = 0.0003)$ groups than in the AD group.

3.5. Effects of MEL and RES on BDNF and CREB1 protein expression in the prefrontal cortex of mice

A significant reduction was observed in the expression of $BDNF$ in the AD group than in the control group ($P = 0.0034$) (Fig. 6A). A significant increase was observed in the $BDNF$ expression in the $AD + RES$ group ($P = 0.0001$) and $AD + MEL$ group ($P = 0.0012$) than in the AD group. The expression of $CREB1$ significantly reduced in the AD group than in the control group ($P = 0.0003$) (Fig. 6B). The $AD + MEL$ group only showed a significant increase in $CREB1$ expression ($P = 0.016$) than the AD group.

4. Discussion

AD is a neurodegenerative disease and one of the leading causes of death with an annually increasing incidence. Several studies have attempted to identify possible prophylactic or therapeutic interventions for AD. Previous studies have shown that supplementation with MEL and RES have yielded promising results for reducing the progression of cognitive decline in different AD models [14,25-27]. However, this study aimed to investigate the possible prophylactic effects of MEL and RES treatments (alone and combined) against memory deficits in an $\beta$-galactose/AlCl$_3$-induced sporadic AD mouse model, focusing on the impacts of these treatments on recognition memory and passive avoidance performance.

4.1. $\beta$-galactose/AlCl$_3$-induced AD mouse model

Several animal models of AD have been used for investigating the effectiveness of different treatments for AD [28,29]. The
Several studies have indicated that MEL prevents the progression of AD and improves cognitive impairment associated with AD via several mechanisms [6,7,13,14]. In our study, NORT showed that MEL administration significantly improved recognition memory in AD mice (Fig. 3); similar results were reported in a previous study [36].

In the NORT, the frequency of sniffing and the DI for each group were measured. By calculating the frequency of sniffing for each object in the familiarization phase, we showed that each group had a similar opportunity for exploring both the objects. However, a significant difference was found in the frequency of sniffing between the familiarization and test phases in the control, AD, and AD + MEL groups (Fig. 3A). These results indicated that the control group could discriminate between familiar and novel objects by sniffing the novel object more than the familiar object. Conversely, the AD group could not discriminate between the familiar and novel objects. However, the MEL group exhibited a significant increase in the sniffing frequency of the novel object. Moreover, the NORT showed that MEL administration significantly improved the DI of mice when compared with the AD group (Fig. 3). This result is consistent with that of previous studies that showed that MEL improves recognition memory in an AD mouse model [36,37].

However, when compared with the AD group, there was a significant improvement in the PAT performance in the MEL group than in the RES group. This finding is consistent with that of a previous study that showed a significant improvement in the avoidance memory of AD mice treated with MEL [38].

The prefrontal cortex and hippocampus are the most profoundly affected regions in the AD brain [39,40]. Previous studies have reported that the prefrontal cortex plays a major role in recognition memory, whereas the hippocampus is mainly associated with avoidance memory [41,42]. Based on these findings, we suggest that MEL exhibits its neuroprotective effect against β-galactose/AlCl₃ neurodegeneration in both the hippocampus and prefrontal cortex of mice.

4.2. Effects of MEL alone on behavioral performance

Several studies have indicated that MEL prevents the progression of AD and improves cognitive impairment associated with AD via several mechanisms [6,7,13,14]. In our study, NORT showed that MEL administration significantly improved recognition memory in AD mice (Fig. 3); similar results were reported in a previous study [36].

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4.3. Effects of RES alone on behavioral performance

The neuroprotective effect of RES has been reported in many in vivo and in vitro studies on AD [43,44]. Several studies have indicated that RES has beneficial cognitive effects on AD animal models [45,46]. In our study, a protective, non-toxic, and high dose of RES was administered as previously used [21,22].

To the best of our knowledge, no previous studies have assessed the effect of RES on NORT performance. Our NORT findings showed that RES administration did not reverse recognition memory impairment in the AD group. Previous studies have reported that the prefrontal cortex plays a major role in recognition memory [47,48]. Hence, it is conceivable that RES affected only the hippocampus and had no effect on the prefrontal cortex.

However, in the PAT, our results indicated that RES improved passive avoidance memory in the mouse AD model, which is consistent with the finding of prior studies reporting that RES treatment prevented passive avoidance memory deficits in a sporadic streptozotocin-induced AD rat model [49]. Additionally, Wang et al. [50] found that RES ameliorated passive avoidance memory impairment in an Aβ1–42-induced AD mouse model, which is consistent with our findings.

Fig. 6. Expression of BDNF and CREB1 in the prefrontal cortex of mice. A) Significant reduction was observed in the expression of BDNF in the AD group than in the Control group. A significant increase was observed in the BDNF expression in the AD + MLT and AD + RES groups than in the AD group. B) Significant reduction was observed in the expression of CREB1 in the AD group than in the Control group. A significant increase was observed in the CREB1 expression in the AD + MLT group than the AD group. Data are represented as mean ± SEM. One-way ANOVA was performed, followed by the Tukey’s multiple comparisons test. (*) vs. Control group, Non-significant (ns)(*) vs. AD group. AD: Alzheimer’s disease; MEL: melatonin; RES: resveratrol; SEM: standard error of the mean; ANOVA: analysis of variance. (ns) P < 0.05, **P < 0.01, ***P < 0.001.
4.4. Effects of combined MEL and RES treatment on behavioral performance

Few studies have investigated the potential effects of combining MEL and RES because of their similar neuroprotective properties. Kwon et al. reported that both MEL and RES exhibited antioxidant, anti-inflammatory, and anti-apoptotic activities [4]. This in vitro study indicated that the combined MEL and RES administration produced neuroprotective actions against cytotoxicity induced by Aβ42 [4]. Additionally, one animal study reported that MEL and RES had a synergistic effects against memory deficits in an animal model of vascular dementia [13]. Interestingly, no previous study has investigated MEL and RES combinatorial effects against memory impairment in an AD mouse model. Our study showed that MEL potentiates the effects of RES mainly on recognition memory as indicated by the NORT.

4.5. Effects of MEL and RES on AChE levels in the prefrontal cortex of mice

Reductions in cholinergic markers are associated with cognitive deficits in senescence and AD [51]. Therefore, targeting the cholinergic system is a crucial AD treatment strategy. AChE is a prime enzyme that hydrolyzes acetylcholine (Ach), which is a cholinergic neurotransmitter with a key role in memory and learning [17]. Cholinergic dysfunction, including alteration in Ach and AChE levels, is involved in the pathogenesis of AD [52]. Several previous studies reported that AChE is significantly reduced in AD animal models [17,20,53]. These results are consistent with our results, which showed a considerable reduction in the AChE level, where MEL reversed the reduction of AChE as reported in previous studies [54,55]. Previous clinical and animal studies indicated that the neuroprotective function of RES is probably via the enhancement of the cholinergic system [51,56]. Our findings showed that RES and the combination treatment (MEL & RES) have beneficial effects on the cholinergic system through enhancement of the AChE level in the AD mouse model.

4.6. Effects of MEL and RES on BDNF and CREB1 expression levels in the prefrontal cortex of mice

BDNF expression has been associated with AD pathology [57]. It has been indicted that BDNF levels decreases in the early-stage AD [58]. BDNF has important roles in learning and memory [57]. BDNF is expressed in the hippocampus and the cortex [59]. Previous studies showed that BDNF expression is reduced in the cortex of AD patients [60].

Our study indicated that BDNF expressions were significantly reduced in the cortex of the AD model as previously reported [61]. However, MEL considerably increased the expression of BDNF in the cortex as previously reported [45,61,62]. The RES group also showed a remarkable increase in the BDNF expression in the prefrontal cortex, which is consistent with the findings of a previous study, which indicated the positive effects of RES on the expression of the BDNF gene in the hippocampus [63,64] and prefrontal cortex of an animal [65]. However, co-treatment with MEL and RES did not exhibit significant improvement in the BDNF expression.

CREB is a nuclear transcription factor that regulates the expression of some essential genes involved in neuronal functions [66]. The transcription of BDNF is regulated by CREB phosphorylation [67]. The downregulation in the CREB phosphorylation process leads to the reduction in BDNF levels [68]. Moreover, CREB has a key role in the formation and retention of memory [69]. However, there is a great possibility that the levels of CREB can be reduced with age [69] as well as in some neurodegenerative diseases such as AD [70]. In our study, CREB1 expression levels were markedly decreased in the prefrontal cortex of the AD mice model, which is consistent with the findings of a previous study [61]. MEL treatment significantly improved the reduction of CREB1 expression, which is similar to the findings of previous reports [71,72]. RES and combination treatments did not exhibit a significant increase in the CREB1 expression.

5. Limitation

In this study, we explored the effects of RES and MEL alone and in combination as well. Interestingly, combining RES and MEL did not show any additive or synergetic effect, which could be due to several factors. The fact that a higher dose of both RES (40 mg/kg) and MEL (80 mg/kg) was used resulted in a maximum effect on improving memory and cognitive function in AD model. RES and MEL also share common antioxidant, free radical scavenger and anti-inflammatory properties, which could partially explain the lack of additive effect. Also, both compounds could possibly show a drug interaction “competitors” on the same receptor sites, which affects the overall additive or possible synergetic effect of using these compounds in combination [6,13,73,74]. Other studies used RES and MEL have used much lower doses to analyze and compare these compounds [13,75]. Furthermore, biochemical, genetic, and histological analyses are required to provide more information about the underlying mechanisms of action of MLT and RES.

6. Conclusion

In summary our study provides evidence that MEL has a potent prophylactic effect against memory deficits in AD. Additionally, MEL can improve the beneficial cognitive effects of RES on recognition memory. We believe that the neuroprotective effect of MEL on learning and memory impairment in the AD model may rely on up-regulating CREB/BDNF signaling and cholinergic transmission in the prefrontal cortex (Fig. 7). However, RES had no effect on recognition memory and only improved cholinergic transmission in the prefrontal cortex.

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Authors’ contributions
SL and BSA conducted behavioral experiments. SL analysed the data and wrote the first draft of manuscript. BSA, FSA, and MK supervised the work and revised the manuscript.

Ethics approval
All experiments were performed according to the guidelines of the biomedical ethics research committee (Reference No. 102-19) at King Abdulaziz University.

Availability of data and materials
All relevant data files are available.

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Declaration of Competing Interest
The authors report no declarations of interest.

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Appendix A. Supplementary data
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