Heliyon 8 (2022) e11108

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Neuroprotective effect of low-dose paracetamol treatment against cognitive dysfunction in D-galactose-induced aging mice

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ARTICLE INFO

Keywords:
Paracetamol
D-galactose
Aging
Cognitive dysfunction
BDNF/TrkB signaling

ABSTRACT

Background: Aging is closely associated to several deleterious conditions and cognitive impairment. Administration of low-dose paracetamol (APAP) has previously been reported to improve cognitive performance in both human and animal studies. However, the altered cognitive effects of low-dose APAP treatment in the aging brain have not been elucidated.

Objectives: The purpose of this study was to determine whether low-dose APAP treatment improves cognitive dysfunction in a D-galactose (D-gal)-induced aging model.

Materials and methods: APAP (15 and 50 mg/kg p.o.) and vitamin E (Vit E 100 mg/kg p.o.) were administered once daily to D-gal-injected mice (200 mg/kg s.c.) for 6 weeks. The elevated plus maze (EPM), open field, novel object recognition (NOR), and Morris water maze (MWM) tests, respectively, were used to measure altered neurobehavioral functions, including anxiety-like behavior and exploratory locomotor activity, as well as learning and memory performance. The gene transcription of brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB) signaling in brain tissues was evaluated by real-time polymerase chain reaction.

Results: Compared to the control, D-gal significantly decreased exploratory locomotor activity and NOR and MWM performance but did not significantly change the activity in the EPM test. However, APAP50 and Vit E significantly reversed the effects of D-gal injection on exploratory locomotor activity. In addition, low-dose APAP (15 and 50 mg/kg) and Vit E significantly improved the reduction in NOR and MWM performance induced by D-gal. Real-time polymerase chain reaction analysis revealed that the mRNA expression of BDNF, neurotrophic tyrosine receptor kinase (NTRK), which is the gene coding TrkB receptor, and cAMP response element-binding protein (CREB) was significantly decreased in the frontal cortex and hippocampus of the D-gal mice. However, APAP50 and Vit E significantly increased BDNF and NTRK mRNA expression in both the frontal cortex and the hippocampus. A lower dose of APAP (15 mg/kg) significantly elevated the mRNA expression of NTRK, but only in the hippocampus. Moreover, APAP50 significantly increased CREB mRNA expression in the frontal cortex and hippocampus.

Conclusion: Low-dose APAP treatment has a neuroprotective effect on cognitive dysfunction in the D-gal aging model, and the underlying molecular mechanisms depend on the activation of BDNF/TrkB signaling.

1. Introduction

With a gradual increase in the population of the elderly worldwide, detrimental conditions related to aging have attracted the attention of numerous studies. Aging is associated with increased disability and illness [1]. Importantly, extensive medical studies revealed that brain senescence can result in serious neurological symptoms and diseases, including learning and memory deficit, anxiety, and depression [2, 3]. Several factors are also known to be closely associated with age-related cognitive impairment, including oxidative stress, inflammatory damage, imbalance of the neurotransmitter system, neuronal loss, and neurotrophin deficit [4, 5, 6]. With regard to neurotrophin deficit, the most

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https://doi.org/10.1016/j.heliyon.2022.e11108
Received 14 January 2022; Received in revised form 7 April 2022; Accepted 11 October 2022

typical finding in the aging brain and other neurological illnesses, such as Alzheimer's disease (AD) and depression, is a significant decline in brain-derived neurotrophic factor (BDNF) [7].

Brain-derived neurotrophic factor is well-recognized as an important mediator of the neuronal growth factor family, which plays a crucial role in promoting neuronal function and plasticity [8, 9, 10]. In the hippocampus and frontal cortex, the key brain regions involved in learning and memory processes, BDNF and its major receptor, tropomyosin receptor kinase B (TrkB), have been found to be highly distributed [11]. The phosphorylation and activation of transcription factors including cAMP-response element binding protein (CREB) by BDNF/TrkB signaling promotes the expression of genes involved in neuronal growth, survival, and long-lasting synaptic plasticity [12, 13]. Since BDNF is a downstream target of CREB that regulates neuroplasticity, restoring the key mediators or activating the BDNF/TrkB signaling pathway can be strategically positioned to improve memory deficits in the aging brain [14, 15].

Chronic systemic administration of D-galactose (D-gal) is a reliable model that is frequently employed to imitate the aging brain and age-related neurobehavior [16, 17, 18]. This animal model displays many reassembly features that can be observed in the aging brain, including increased oxidative stress, neuronal loss, and cognitive dysfunction [19, 20, 21]. Thus, the D-gal-induced aging model is a valid tool for understanding the aging brain and aids pharmacological studies in finding different therapeutic approaches for age-related brain diseases [22].

Paracetamol (APAP) is an over-the-counter pain reliever and antipyrolytic agent used worldwide. Numerous neurotransmitters and serotonergic, opioidergic, vanilloid, and cannabinoid receptors are suggested to be involved in the mechanism underlying the analgesic and hypothermic effects of APAP [23, 24, 25]. In addition to its analgesic and anti-pyloric effects, APAP has also been linked to cognitive-affective changes in both humans and animals; however, the exact effect of APAP treatment still needs to be further discussed. A number of earlier studies have documented the negative effects of APAP when used for a prolonged period of time at a therapeutic dose [26] or when the drug is present during an important stage of brain [27, 28]. In contrast, the beneficial effects of APAP on cognitive function have been previously established in several studies. It has been demonstrated that APAP could exert anxiolytic, antidepressant and anticompulsive effects [29, 30]. Furthermore, low-dose administration of APAP (15.1 mg/kg) improves learning and memory in animals [31, 32]. In a human investigation, healthy volunteers who received APAP showed improved spatial memory and decision-making abilities [33].

However, the anti-cognitive impairment and anti-brain aging effects of low-dose APAP treatment in D-gal aging models have not been explored. Thus, the present study aimed to investigate whether long-term APAP administration at two different low doses (15 and 50 mg/kg body weight) can attenuate cognitive deterioration in D-gal-induced aging mice. Neurobehavior, including locomotor activity, anxiety, learning, and memory, was monitored in all experimental animals, and the gene expression of key factor molecules related to the BDNF/TrkB signaling pathway (BDNF, NTRK, and CREB mRNAs) in the brain tissues was determined using real-time polymerase chain reaction (RT-PCR).

2. Material and methods

2.1. Animals

Forty–nine male ICR mice, weighing 20–25 g, were used in this study. Animals were purchased from Nomura Siam International Co. Ltd. (Pathumwan, Bangkok, Thailand). Mice were raised in a 12-hour light/dark cycle where the temperature and humidity were controlled, and they had unlimited access to a standard diet and water. All experimental protocols carried out in this study were reviewed and approved by the Animal Ethics Committee of Walailak University, Thailand (protocol number WUAICUC-63027).

2.2. Experimental designs

After 14 days of acclimatization, the animals were randomly divided into five groups, and the treatment regimen was performed for 6 weeks as follows:

1) Control group (n = 10): Mice were subcutaneously administrated with 0.9% normal saline solution followed by daily intragastric administration of distilled water.
2) D-gal group (n = 10): Mice were subcutaneously administrated with 200 mg/kg D-gal (Sigma-Aldrich, St. Louis, MO, USA) once daily to model the aging brain. Thirty minutes after half an hour of D-gal injection, the mice were intragastrically administered distilled water.
3) D-gal + APAP15 (n = 10): Mice that received D-gal injection at the aforementioned dose were intragastrically administered 15 mg/kg of APAP once daily.
4) D-gal + APAP50 (n = 10): Mice that received D-gal injection were intragastrically administered 50 mg/kg of APAP once daily.
5) D-gal + Vit E (n = 9): Mice injected with D-gal were intragastrically administered 100 mg/kg of Vit E once daily.

Analyses of behavior and biochemistry were carried out by the experimenters who were unaware of the study's purpose at the end of the treatment session.

2.3. Elevated plus maze test (EPM)

The EPM test was used to evaluate the animals' anxiety-like behavior. Briefly, the EPM consists of four arms – two open, two closed – that extend from a platform in a middle at a height of 30 cm above the ground. The test involved placing the mice in the maze's center, and the mice's activities were filmed using a digital video camera set above the maze for 5 min. Due to the fact that highly anxious animals were identified by a decrease in duration of stay in the open area and frequency of entry into an open area [34], the total length of time spent in the open area (open arms and central area) as well as the number of entries into the open area were extracted using BehaviorCloud video tracking software (https://www.behaviorcloud.com/, San Diego, CA, USA). To eliminate olfactory and intra-apparatus signals, the apparatus was thoroughly cleaned with 70% alcohol after each testing session.

2.4. Open field test

Mice were subjected to an open field (OP) test to estimate their locomotion and anxiety-like behavior. The OP test was carried out in accordance with the previous protocol [35] with minor modifications. Briefly, the task apparatus consisted of a black plastic open-field box measuring 40 × 40 × 40 cm. The center square of the box, which comprises 50% of the total area, was defined as the "inner area" of the OP. The animals were initially left in the middle of the box and allowed unrestricted movement for 5 min. A digital video camera mounted above the test apparatus captured animal activity, and data were extracted using BehaviorCloud video tracking software (https://www.behaviorcloud.com/, San Diego, CA, USA).

2.5. Novel object recognition test

The novel object recognition (NOR) test, which follows the previously stated protocol [36] with minimal adjustment, was used to test the recognition memory of all animals. Briefly, the testing equipment was a 40 × 40 × 40 cm black plastic open-field box and the procedure included three sessions: habituation, training, and testing. On the first day, a habituation session was performed by allowing the mice to explore an empty box for 10 min. The training and testing sessions were conducted on the second day. In the training session, the mice were placed in an

open arena where there were two identified objects (objects A and A′) and allowed to explore the objects for 10 min before returning them to the home cage. For the testing session, the mice were again placed in the box 1 h later with one of the previous objects (A) and a novel object (B) for 10 min. The mice were considered to be exploring when the animal's nose was toward the object (a distance of $\leq$2 cm), and rearing up against it. The exploring behaviors of mice were collected using a video camera positioned above the box and analyzed by investigators blinded to the group allocation. To remove olfactory cues, the test box and objects were cleaned with 70% alcohol after each session. The high discrimination index, which was used to define increased recognition memory, was calculated as follows:

Discrimination index = (time with novel object – time with familiar object) / total time exploring

2.6. Morris water maze test (MWM)

The MWM test was used to assess the mice's spatial learning and memory capacities. The MWM procedure was modified from a paradigm originally introduced in a previous study [37]. The test was done between 12.00 and 15.00 pm. The task apparatus was a 100 – cm – diameter, 50 – cm – deep circular pool that was filled to a depth of 30 cm with water that was 20–22 °C and rendered with non – toxic powder. The pool was separated into four quadrants and marked with a variety of symbols that the animals could easily see. The behavior of the animals during the test was recorded using a digital video camera located above the task apparatus. The protocol the MWM test was divided into three sessions including a visible platform test, a hidden platform test, and a probe trial. In visible platform test, a platform with a 10 – cm diameter and 30 – cm height was positioned 1 cm above the water's surface, and a black flag was placed on the platform to increase mouse visibility. For the four trials, the mice were carefully put in each of the four starting quadrants of the pool and given 60 s to locate the platform, with the platform being moved to a new spot for each succeeding trial. After 24 h of the visual platform test, the mice underwent 5 days of training where they were given four trials each day. During the training, the platform was submerged 2 cm below the water's surface and remained in the center of one quadrant. The mice were generally placed at the starting point in the pool and allowed to swim freely. The length of time to reach the hidden platform was referred to as escape latency. In the case of the mice that failed to find the hidden platform within 60 s, they were manually led to the platform and left there for 15 s. Following a 24-h hidden platform test, the platform was removed for the probing trial, which involved allowing each mouse swim around freely for 60 s in the pool. The amount of time each mouse spent in the target quadrant (the region where the platform was previously positioned) was examined using BehaviorCloud video tracking software (https://www.behaviorcloud.com/, San Diego, CA, USA). All of the animals were taken out of the pool, dried off, and put back in their cages at the completion of each trial day.

2.7. Brain tissue collection

The mice were euthanized by intraperitoneal administration of 100 mg/kg of ketamine following behavioral testing. Mice were transcardially perfused with 200 mL of cooled phosphate buffer solution (PBS) at pH 7.4. Following decapitation, the whole mouse brain was quickly taken from the skull. The frontal cortical and hippocampus brain tissues were rapidly dissected on ice and stored separately at −80 °C until the start of the following experiment.

2.8. RT-PCR

Total RNA from the frontal cortex and hippocampus was purified using GENEzol reagent (Catalog no. GZR100, Geneaid, Taiwan), according to the manufacturer's instructions. Next, 10 μg of total RNA from each individual was used for first-strand cDNA synthesis using the iScript™ Reverse Transcription Supermix for RT-qPCR (Catalog no. 1708840, Bio-Rad, USA). cDNA (100 ng) was subsequently amplified with specific primers (Table 1) using 5× HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Catalog no. 08-24-00001, Solis BioDyne, Estonia). The following conditions were used to perform PCR reactions: an initial denaturation at 95 °C for 15 min, then 40 cycles of 95, 58, 72 °C for 30 s each, followed by a final extension at 72 °C for 15 min. Each analysis was carried out in triplicate. The $2^{-\Delta\Delta CT}$ method was used to normalize and compute the relative expression levels of all genes in the frontal cortex and hippocampus [38].

2.9. Statistical analyses

The escape latency from the hidden platform test in MWM was assessed using a two-way repeated measures analysis of variance (2-way RM ANOVA), whilst the other data were analyzed using a one-way ANOVA and the Bonferroni's post-hoc test. All analyses were performed using GraphPad Prism 9.1 (GraphPad Software Inc., La Jolla, CA, USA). Results are presented as the mean ± SEM, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. EPM test

The EPM test findings showed that there was no noticeable difference in the duration of time that the experimental groups spent in the open area (Figure 1 (a)). Furthermore, the number of open-area entries was not significantly different between the groups (Figure 1 (b)). The movement traces in the maze for all the experimental groups are shown in Figure 1 (c).

3.2. OP test

Using the OP test, locomotor activity and anxiety-like behavior were assessed. The results disclosed that, in comparison to the control group, mice given a single injection of D-gal showed a statistically significant reduction in their total travel distance ($P < 0.001$). Treatment with 50 mg/kg APAP significantly increased the distance traveled compared with the D-gal model ($P = 0.03$). A statistically significant increase in the distance traveled by animals was also observed in the D-gal + Vit E group ($P = 0.004$). There was no significant difference in the traveling distance between the mice in the D-gal and D-gal + APAP15 groups (Figure 2(a), (d)). We also assessed the speed in animals (Figure 2 (b)) and the time spent within the inner

Table 1. Primers used.

Name		Sequence 5′-3′	References	GenBank accession No.
GAPDH	Forward	5′-GTCTCCTGCGACTTCAG-3′	This study	GU214026.1
	Reverse	5′-TCATTGTCATACCAGGAAATGAGC-3′		
BDNF	Forward	5′-TGGCCCTGCGGAGGCTAAGT-3′	[39]	-
	Reverse	5′-AGGGTGCTTCCGAGCCTTCCT-3′		
NTRK2	Forward	5′-TGGACCACGCCAACTGACAT-3′	This study	XM_006517150.5
	Reverse	5′-GAATGTCTCGCCAACTTGAG-3′		
CREB1	Forward	5′-GGTCCGTCTAATGAAGAACA-3′	This study	XM_017314099.2
	Reverse	5′-GCTTTTAGCTCCTCAATCAA-3′		

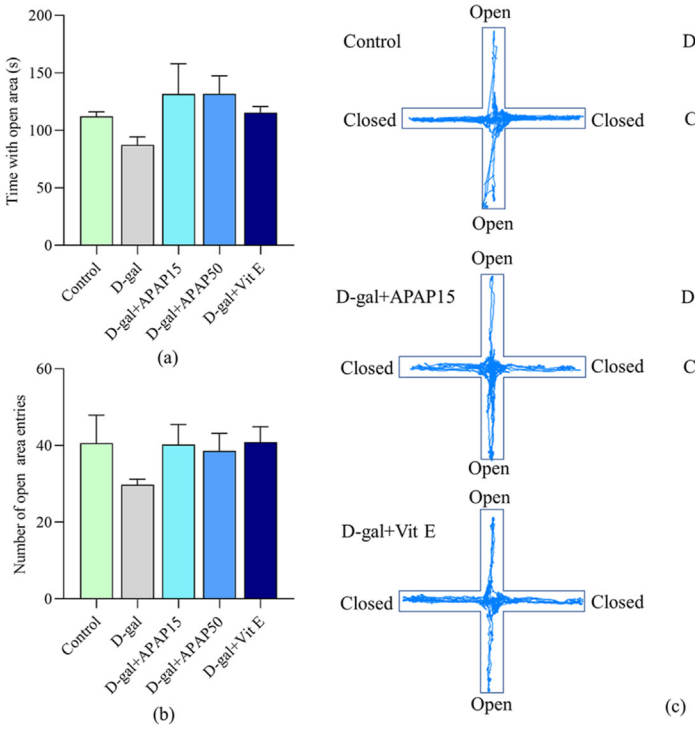

Figure 1. Effect of low-dose APAP treatment on anxiety-like behavior in D-gal-induced aging mice using EPM test after drug administration for 6 weeks. (a) Time spent in the open arena, (b) number of open arena entries, and (c) animal patch in the maze for all experimental groups. The bar graphs represent the mean ± SEM, n = 9–10. D-gal, D-galactose; D-gal + APAP15, D-galactose plus 15 mg/kg of paracetamol; D-gal + APAP50, D-galactose plus 50 mg/kg of paracetamol; D-gal + Vit E, D-galactose plus 100 mg/kg of vitamin E; EPM, elevated plus maze.

zone of the task (Figure 2 (c)). However, differences in these parameters were not detected among the experimental groups. The path of movement in the OP task, detected by the animal video tracking software, is shown in Figure 2 (d).

3.3. NOR test

Recognition memory was measured in all mice using the NOR test. As shown (Figure 3 (a)), the mice were allowed to explore a novel object with a retention time of 1 h. The results obtained from NOR showed that D-gal model mice displayed a marked reduction in recognition memory, whose discrimination index was lower than that of the control group ($P < 0.001$). However, administration of APAP (15 and 50 mg/kg) significantly increased the discrimination index compared with the D-gal group ($P < 0.001$ and $P = 0.002$, respectively). Compared with D-gal, treatment with Vit E showed an effect similar to that with APAP ($P = 0.002$) (Figure 3 (b)), indicating that APAP and Vit E can improve recognition in D-gal-treated mice.

3.4. MWM test

Spatial learning and memory were evaluated in all animals using the Morris water maze test. In the visible platform test, mice in each group were able to search for a visible platform. The results revealed that the escape latency over the four trials of the test was not significantly different among the experimental groups (Figure 4 (a)).

In the hidden platform test, the mean escape latency was analyzed across 5 days of the session. Our results showed that chronic treatment with D-gal alone delayed the finding of the hidden platform on the 3rd, 4th, and 5th days of the training session compared with mice in the control group ($P = 0.001$, $P = 0.009$, and $P < 0.001$, respectively). Compared with the D-gal-treated mice, 6 weeks of treatment with APAP (at a dose of 50 mg/kg) significantly decreased the escape latency time on the 3rd, 4th, and 5th days ($P = 0.026$ for the 3rd day, $P = 0.024$ for the 4th day, and $P < 0.001$ for the 5th day). When compared to those in the D-gal group, the administration of 15 mg/kg APAP and 100 mg/kg Vit E demonstrated a marked decline in escape latency on the last day of the training session ($P < 0.001$; Figure 4 (b)).

For the probe trial, the experimental groups spent significantly different amounts of time in the target quadrant according to statistical analysis (Figure 4 (c), (d)). The length of time the D-gal group spent in the target zone was noticeably less than that of the control mice ($P = 0.042$). Conversely, the time spent in the target zone by mice that had prolonged administration of APAP (15 and 50 mg/kg) was markedly higher than that observed in the D-gal group ($P = 0.012$ and $P = 0.023$). In addition, a significantly higher time spent in the target zone was observed in D-gal

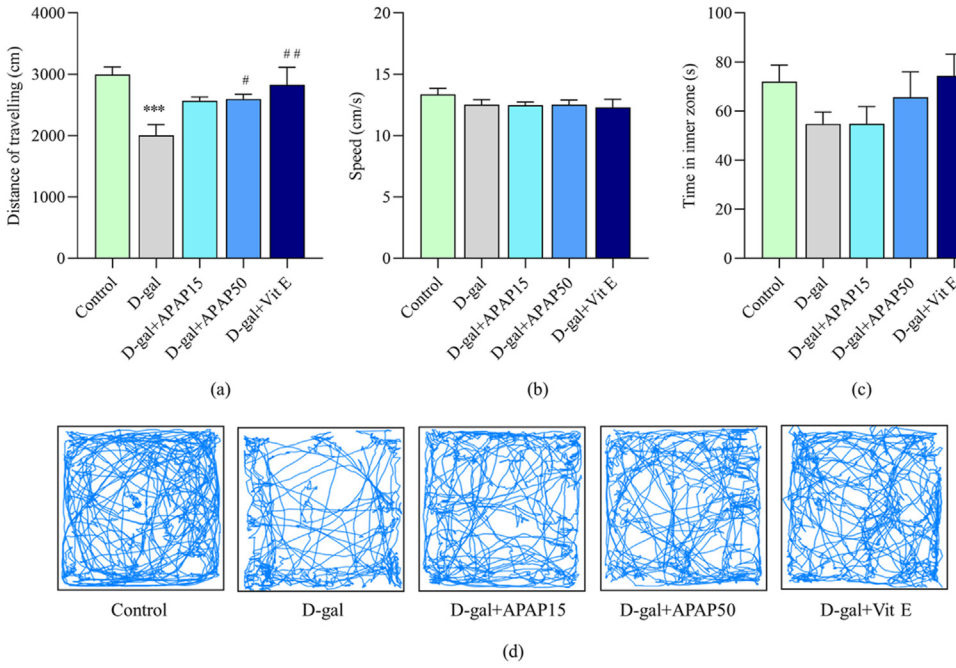

Figure 2. Effect of low-dose APAP treatment on locomotor activity in D-gal-induced aging mice using OP test after drug administration for 6 weeks. (a) Distance traveled by the animal, (b) speed of the animal during testing, (c) time spent in the inner zone by the animals, and (d) the path of movement in the OP task for all experimental groups. The bar graphs represent the mean ± SEM, n = 9–10. Significant differences tested by one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test; ***P < 0.001 compared to the control group. #P < 0.05 and ##P < 0.01 compared to the D-gal group; D-gal, D-galactose; D-gal + APAP15, D-galactose plus 15 mg/kg of paracetamol; D-gal + APAP50, D-galactose plus 50 mg/kg of paracetamol; D-gal + Vit E, D-galactose plus 100 mg/kg of vitamin E; OP, open field.

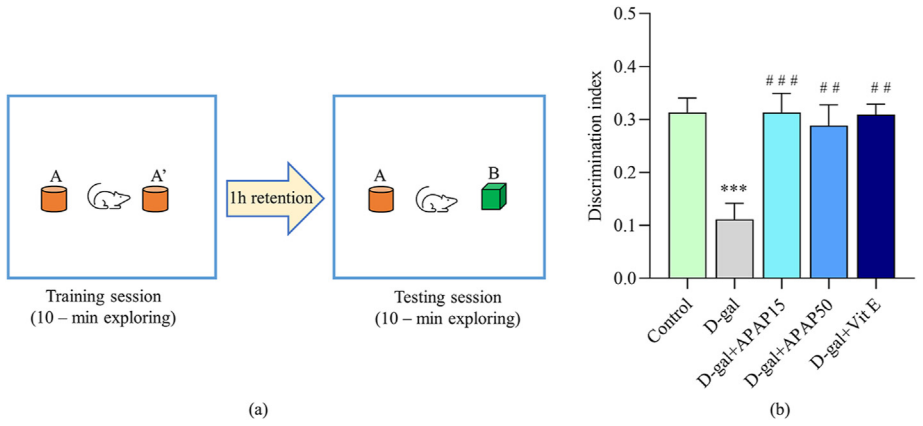

Figure 3. Effect of low-dose APAP treatment on recognition memory in D-gal-induced aging mice using NOR test after drug administration for 6 weeks. (a) an illustration of NOR test procedure, and (b) discrimination index among the experimental group. The bar graph represents the mean ± SEM, n = 9–10. Significant differences tested by one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test; ***P < 0.001 compared to the control group. ##P < 0.01 and ###P < 0.001 compared to the D-gal group; D-gal, D-galactose; D-gal + APAP15, D-galactose plus 15 mg/kg of paracetamol; D-gal + APAP50, D-galactose plus 50 mg/kg of paracetamol; D-gal + Vit E, D-galactose plus 100 mg/kg of vitamin E; NOR, novel object recognition.

mice treated with 100 mg/kg Vit E as compared with the D-gal group (P = 0.0013).

3.5. RT-PCR analysis

The expression of genes related to the BDNF/TrkB signaling pathway, including BDNF, NTRK, and CREB, in the frontal cortex and hippocampus were measured using RT-PCR, and the results are summarized in Figure 5. In the frontal cortex, a significantly lower BDNF mRNA level was observed in the D-gal-treated mice than in the control group (P = 0.002; Figure 5 (a)). The results also showed that treatment with neither low-dose APAP (15 or 50 mg/kg) nor Vit E significantly reversed the decreased BDNF gene expression induced by chronic D-gal administration (P > 0.999, P = 0.094 and P = 0.225, respectively). In line with the observation in the frontal cortex, the relative BDNF level was significantly decreased in the hippocampus of the mice received D-gal alone as compared to that in the control (P < 0.001; Figure 5 (a)). The data showed no significant change in BDNF mRNA levels in the D-gal vs. D-gal + APAP15 groups (P = 0.143). In contrast, the mRNA levels of BDNF were significantly increased in the hippocampus of the mice received APAP50 (P < 0.001) and Vit E (P = 0.008).

In line with BDNF gene expression, a significant decrease in NTRK mRNA, a specific gene encoding the cognate TrkB receptor, was detected in the frontal cortex of the D-gal group when compared with the control group (P = 0.007; Figure 5 (b)). In comparison with the D-gal group, the results showed no significant difference in NTRK mRNA levels in mice treated with 15 mg/kg of APAP (P = 0.255). However, the mRNA expression of NTRK significantly increased in the APAP50 group (P < 0.001). The results obtained from the D-gal + Vit E group also showed an effect similar to that observed with APAP50 (P = 0.008). The mRNA expression of NTRK in the hippocampus of D-gal mice was similar to that observed in the frontal cortex. Chronic D-gal administration significantly decreased the mRNA expression of NTRK compared to that in control mice (P < 0.001; Figure 5 (b)). However, low-dose treatment with APAP (15 and 50 mg/kg) reversed the lower mRNA expression of NTRK induced by D-gal administration (P = 0.01 and P = 0.004, respectively). Treatment with 100 mg/kg of Vit E also showed a similar effect to that observed in the APAP-treated group (P = 0.001).

The mRNA expression of CREB in the frontal cortex of D-gal mice was significantly lower than that in the control group (P = 0.005; Figure 5 (c)). There was no change in the CREB mRNA level in the frontal cortex of the D-gal + APAP15 group compared to the D-gal group (P > 0.999). Conversely, significantly higher mRNA expression of CREB was detected

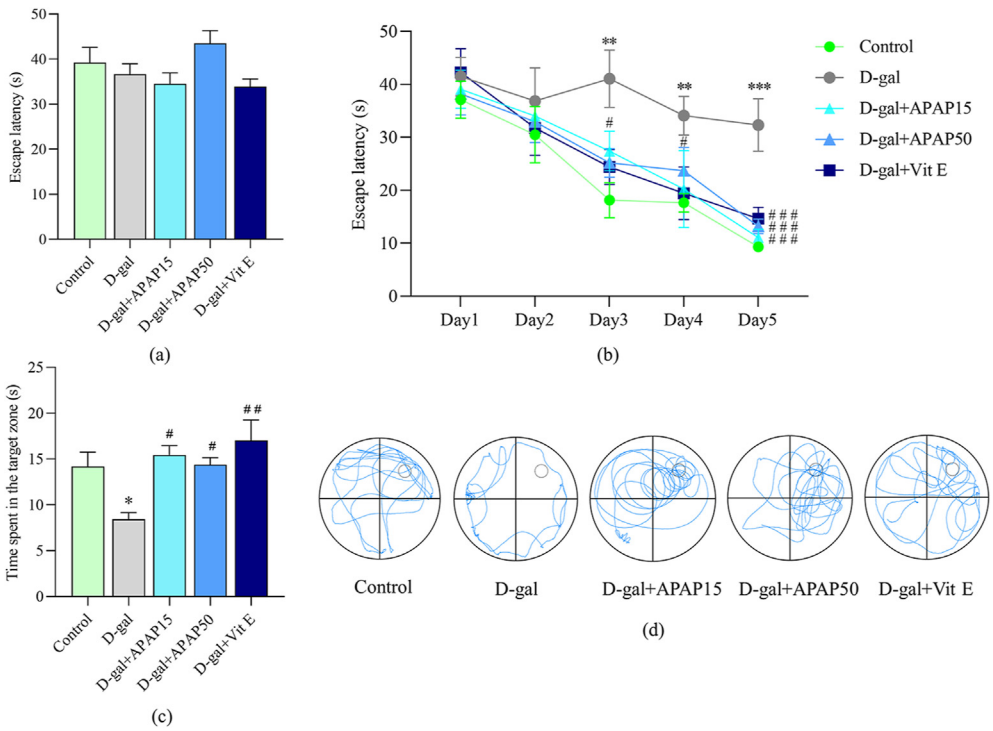

Figure 4. Effect of low-dose APAP treatment on spatial learning and memory in D-gal-induced aging mice using MWM test after drug administration for 6 weeks. (a) Escape latency time in the visible platform test, (b) escape latency time in the hidden platform test, (c) time spent in the target zone in probe trial, and (d) swimming path for all experimental groups in the probe trial. The bar graphs represent the mean ± SEM, n = 9–10. Significant differences tested by 2-way RM ANOVA for the escape latency time in the hidden platform test and one-way ANOVA followed by Bonferroni's post-hoc test for escape latency time in the visible platform test and probe trial; *P < 0.05 compared to the control group. #P < 0.05 and ##P < 0.01 compared to the D-gal group; D-gal, D-galactose; D-gal + APAP15, D-galactose plus 15 mg/kg of paracetamol; D-gal + APAP50, D-galactose plus 50 mg/kg of paracetamol; D-gal + Vit E, D-galactose plus 100 mg/kg of vitamin E; MWM, Morris water maze.

in the D-gal + APAP50 group than in the D-gal group (P = 0.014), but we did not observe a significant change in the mRNA expression of CREB in the D-gal + Vit E group as compared to the D-gal groups (P = 0.119). The results analyzed in the hippocampus were similar to those in the frontal cortex. Chronic administration of D-gal significantly decreased the mRNA expression of CREB compared to that in the control (P = 0.001; Figure 5 (c)). As compared to the D-gal group, there was no significant difference in the mRNA expression of CREB the D-gal + APAP15 and D-gal + Vit E groups (P > 0.999 and P = 0.138, respectively). However, a significant increase in the mRNA expression of CREB was observed in the D-gal + APAP50 (P = 0.028).

4. Discussion

The present study demonstrated that prolonged systemic administration of D-gal (200 mg/kg for 6 weeks) causes lower exploratory locomotor activity, learning, and memory impairment and may induce anxiety-like behavior. Moreover, deleterious neurobehavior was observed in parallel with a decrease in the transcription of BDNF, NTRK, and CREB in the frontal cortex and hippocampus. Treatment with low doses of APAP (15 and 50 mg/kg) and Vit E modulated these detrimental effects in the D-gal senescence model.

During aging, impairments in cognitive performance, such as learning and memory, and alterations in emotion and locomotor activity normally occur. The possible cause of progressive loss of functional capacities might be partially due to the manifestation of neuronal loss or synaptic plasticity decline in specific brain areas [18]. In this study, we developed an aging brain model with chronic D-gal injection, which has been a well-established model for anti-aging pharmacological therapy in several studies [16, 17, 18]. We decided to employ the D-gal aging model because it is beneficial for studying solely aging development without any confounding variables such as diabetes, hypertension, and malignancies, which can be observed in the natural aging model [22].

The aging brain is widely recognized to contribute to anxiety, and several lines of evidence have revealed increased anxiety-like behavior in the D-gal-induced aging brain [40, 41]. In the present study, however, we did not detect a significant change in the indicators of increased anxiety in D-gal-induced mice. However, a trend toward increased anxiety indices, including a decreased number of entries and time spent in open areas, was observed in D-gal mice. Previous studies have shown that injection of D-gal at a dose of 500 mg/kg for 6 weeks induces anxiety-like behavior in mice [40, 41]. Together with these results, it might be suggested that dose- and regimen-dependent D-gal administration contributes to anxiety-like behavior. A higher dose can easily induce deteriorating results and/or a longer duration of treatment may result in obvious exhibition. A trend toward decreased anxiety (increased number of entries and time spent in the open area) was observed in mice treated with low doses of APAP (15 and 50 mg/kg). In 2018, Chen et al. reported that treatment with 50 mg/kg APAP reduced nerve injury-associated anxiety, probably by reducing neuropathic pain [42]. Another study found that APAP mediates anxiolytic-like effects via cannabinoid 1 (CB1) receptors [30]. Therefore, the results of the present study support the idea that APAP has anxiolytic effects, and low doses of APAP may exert anxiolytic properties in the D-gal-induced senescence model.

The OP is the test that is widely used to examine locomotor activity in rodents, and decreased activity in the central area of the apparatus is likewise indicative of anxiety [43, 44]. In the present study, chronic D-gal administration induced lower activity in the OP test, as a reduction in traveling distance was observed. These results are in line with those of previous studies that identified a reduction in exploratory locomotor activity in D-gal-treated rodents [45, 46]. Treatment with 50 mg/kg of APAP and Vit E reversed the reduction in exploratory locomotor activity induced by D-gal, suggesting the potential of APAP to impair locomotor activity in the aging model. However, a lower dose of APAP (15 mg/kg) did not reverse the decreased exploratory locomotor activity observed in this study. This result suggests that APAP has a dose-dependent improving effect on reducing locomotor with aging. Alternatively, the time spent in the inner zone of the OP is an indicator of decreased anxiety. We could not detect a significant difference in time spent in the inner zone of OP among the experimental groups, but the trend of increased anxiety in D-gal was seen in the EPM test, which might be due to different conditions between these two tests.

Several previous studies have demonstrated impairment of learning and memory in the D-gal-induced aging brain model [22, 41, 47].

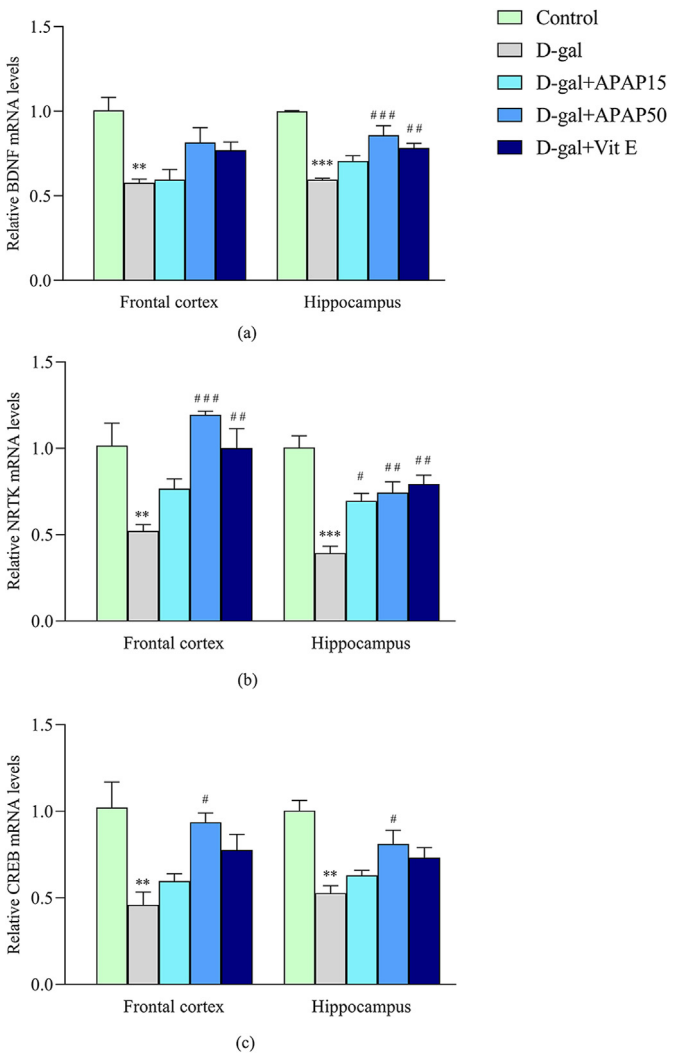

Figure 5. Effect of low-dose APAP treatment on gene expression in the frontal cortex and hippocampus in D-gal-induced aging mice using RT-PCR technique after drug administration for 6 weeks. (a) Relative BDNF mRNA level, (b) relative NTRK mRNA level, and (c) relative CREB mRNA level. The bar graphs represent the mean ± SEM, n = 5. Significant differences tested by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test; **P < 0.01 and ***P < 0.001 compared to the control group. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, and $^{\#\#\#}$P < 0.001 compared to the D-gal group; D-gal, D-galactose; D-gal + APAP15, D-galactose plus 15 mg/kg of paracetamol; D-gal + APAP50, D-galactose plus 50 mg/kg of paracetamol; D-gal + Vit E, D-galactose plus 100 mg/kg of vitamin E.

Consistent with these studies, the results obtained in the present study demonstrated that chronic D-gal administration induces a reduction in performance in NOR and MWM tasks. D-gal-treated mice showed a reduction in the recognition index, indicating impairment of novelty-induced exploratory behavior or working memory. Moreover, mice in the D-gal group also displayed an increased escape latency and decreased time spent with the target zone. Altogether, these results indicate an impairment of spatial learning and memory in the D-gal group. The results obtained from the visible platform test revealed no differences in escape latency between the experimental groups. This indicated that the learning and memory deficits in D-gal-treated mice were not due to impaired visual capacity. In contrast, low-dose APAP (15 and 50 mg/kg) treatment following D-gal injection increased NOR and MWM performance. The improvement of NOR and MWM performance was also observed in D-gal mice treated with Vit E. The effects and mechanisms of action of APAP in altering cognitive function remain unclear. Some studies have reported that APAP has deleterious effects on learning and memory performance. For example, a previous study demonstrated that neonatal APAP treatment (single or repeated injection of 30 mg/kg APAP) impaired spatial memory in adulthood [28]. Another study reported that maternal APAP (5–50 mg/kg) exposure induced an impairment in spatial memory performance in rats [27]. In addition, our earlier report demonstrated an alteration in synaptic plasticity and elevated oxidative stress in the frontal cortex and hippocampus following chronic APAP (200 mg/kg) treatment in rats [26]. Conversely, the earlier studies have reported the nootropic activity and neuroprotective effects of low-dose APAP (15.1 mg/kg) [31, 32]. The discrepancies in the reported findings may result from differences in the timing of APAP treatment (during brain development vs. matured brain), as well as different models (natural vs. neurotoxic induction) used in those studies. Based on the results obtained in our study, low-dose APAP treatment improved learning and memory deficits in the D-gal-induced aging brain through neuroprotective effects.

In the present study, cognitive impairment in D-gal mice was accompanied by downregulated transcription of BDNF/TrkB signaling molecules, including BDNF, NTRK and CREB, in both the frontal cortex and hippocampus. However, we also discovered that the decrease of the aforementioned mediators in the D-gal aging model was variably modified by treatment with low doses of APAP (15 and 50 mg/kg) and vitamin E.

Activation of BDNF/TrkB/CREB signaling is recognized as a vital mechanism for supporting functional and morphological synaptic plasticity, which are vital factors for learning and memory [48, 49]. Impairment of BDNF/TrkB/CREB signaling is considered a biological change that causes cognitive disability in the aging brain [7, 50] and the D-gal-induced aging model [17, 40, 51]. Our results are in agreement with the previous studies, in which a reduction of genes of BDNF/TrkB/CREB signaling was observed in D-gal mice. Although the evidence to explain how D-gal induces a decrement of BDNF/TrkB/CREB signaling is restricted, several studies have shown intimate links between elevated oxidative stress and cognitive dysfunction in D-gal aging [21, 22, 52]. Elevated oxidative stress is proposed to manipulate the BDNF expression through several mechanisms [53], including a decrease in DNA-binding activities of the activator protein-1 [54] and dysfunction of N-methyl-D-aspartate (NMDA) channel due to energy depletion [55, 56]. Regarding these accumulative data, we suggest that the reduction of mRNA expression of BDNF, NTRK, CREB in D-gal mice might be due to an elevation of oxidative stress and the downregulation of these genes may explain the reduction in cognitive function in these animals.

It has been suggested that the metabolite of APAP, AM404, can interact with the cannabinoid system to mediate analgesic and anxiolytic-like effects [30, 57] and this interaction can further modulate BDNF levels [28]. APAP could also facilitate spatial memory by mediating endogenous COX-2 and 5-HT neuronal activities [31]. Increase in the gene transcription of BDNF can be promoted by activating 5-HT receptors coupled with cAMP production and CREB activation [58]. Therefore, the improved cognitive effects of low-dose APAP treatment in the D-gal aging model suggests the involvement of restoring BDNF/TrkB signaling, and these effects might be due to the manipulation of neurotransmitters and the analgesic system in the key brain regions.

Additionally, our findings demonstrated that low doses of APAP exhibited advantageous effects comparable to those of vitamin E, the potential antioxidant to lower the risk for cognitive impairment in AD and aging [59, 60]. Vit E can modify the expression of many genes involved in hormone metabolism, apoptosis, growth factors, neurotransmission, amyloid-β (Aβ) metabolism, all of which are implicated in the onset and progression of AD [61]. While the antioxidant and anti-inflammatory activities of APAP have previously been reported in neuronal cells cultured with Aβ [62] and menadione [63]. Another study revealed that low-dose APAP (15.1 mg/kg) treatment exerts neuroprotective effects against neurotoxin-induced amnesia owing to its antioxidant properties [32]. Taken together, our findings also suggest that

low-dose APAP treatment may exert a neuroprotective effect due to its anti-inflammatory and antioxidant properties in the D-gal aging brain model.

The maintenance of neuronal transmission and plasticity, the vital process for learning and memory, relies on dynamic regulation of the functional proteins [64]. In the present study, changes in gene expression of BDNF, NTRK, and CREB were partially implicated in the protection of APAP and Vit E in learning and memory deficits induced by D-gal, while an alteration of those functional proteins was not monitored. It is possible that post-transcriptional and/or post-translational modifications of these mediators contribute to the protective effect of APAP and Vit E in D-gal-induced cognitive impairment. Furthermore, since both 15 and 50 mg/kg APAP treatments ameliorated object recognition and spatial memory deficits in the NOR and MWW tests, but these APAP showed different effects on BDNF, NTRK, and CREB mRNA expressions. These findings suggest CREB-independent neural and molecular mechanisms involved in the impairment and protection of object recognition and spatial memory. In addition, it is implied that there might be other mediators involved in the protection and impairment of learning and memory (i.e., phosphatidyl inositol 3-kinase [PI3K], protein kinase B [Akt], nuclear factor erythroid 2-related factor 2 [Nrf2], and mitogen-activated protein kinases [MAPK]) [65, 66]. Further study to investigate the protein expression of those mediators is needed to better understand the mechanisms involved in the protection of APAP in the learning and memory impairment in D-gal aging mice.

5. Conclusion

This study demonstrates that low doses of APAP treatment reversed diminishing exploratory locomotor activity and learning and memory impairments in the D-gal aging brain model by enhancing the transcription of BDNF, NTRK, and CREB in the key brain regions responsible for cognitive performance. Collectively, this study provides supporting evidence that low-dose APAP is a potential agent for therapeutic strategies to delay the aging process. Further studies are required to investigate the expression of functional proteins to help better understanding the mechanism underlying the protective effect of low doses of APAP in the D-gal-induced aging model.

Declarations

Author contribution statement

Laddawan Lalert: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Supang Maneesri le-Grand, Soontaraporn Huntula: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tachpon Techaranga: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Chuchard Punsawad: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Dr. Laddawan Lalert was supported by Walailak University [WU-IRG-63-083].

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to acknowledge laboratory workers at the Animal Experiment Building, Walailak University, Thailand, for facilitating all processes performed in mice.

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