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Short communication

Combined benefits of environmental enrichment and epigallocatechin gallate on memory impairment in a rat model of Alzheimer-Like disease

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Abstract

Objective: This study aimed to investigate the effects of combined environmental enrichment (EE) and (-)-Epigallocatechin-3-gallate (EGCG) on cognitive function and hippocampal factors in a rat model of streptozotocin (STZ)-induced Alzheimer-like disease.

Materials and methods: Adult rats were divided to five groups: Sham, Alzheimer (ALZ), ALZ+EGCG, ALZ+EE, and ALZ+EE+EGCG. Alzheimer's disease (AD) was induced by intracerebroventricular (ICV) injection of STZ (3 mg/kg). EGCG was administered orally (10 mg/kg) once daily for 21 consecutive days, and EE rats were housed in a large enriched cage (50×50×50 cm) containing various objects for 28 days. Spatial memory was assessed using the Morris water maze (MWM), and hippocampal gene expression of *Amyloid Precursor Protein (APP) and Brain Derived neurotrophic Factor (BDNF)* was measured by real-time PCR.

Results: STZ reduced learning performance during the training phase of the MWM by about 60% compared with the Sham group. Although all treated groups showed slight improvement compared with the STZ group, none significantly enhanced learning rate. In the probe test, STZ caused a severe memory deficit compared with baseline ($p<0.001$), while the combined treatment of EE and EGCG significantly improved memory and restored performance to the normal levels ($p>0.05$). STZ increased hippocampal APP expression compared with Sham ($p<0.01$), while combination treatment significantly reduced APP expression ($p<0.001$). Conversely, BDNF expression decreased in AD rats compared with Sham and was significantly upregulated by EE+EGCG combination ($p<0.01$).

Conclusion: These findings indicate that combined EE and EGCG treatment ameliorates memory deficits in AD rats, potentially through modulation of hippocampal APP and BDNF. Such interventions along with medical treatments may help to mitigate cognitive impairments in this clinical population.

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Introduction

Primarily as a result of the substantial rise in life expectancy, Alzheimer's disease (AD) has been identified as a global public health challenge. AD is a chronic degenerative and disabling brain disorder that is typically marked by gradual decline in memory scores and other cognitive and executive abilities, ultimately resulting in dementia and death. The typical neuropathological features of AD include intracellular neurofibrillary tangles and extracellular senile plaques accompanied by a heterogeneous variety of neurochemical changes, including neuroinflammation, disrupted oxidative stress balance, synaptic density and function, neurogenesis and neuronal loss (Self and Holtzman 2023). Amyloid Beta (Aβ) is the main constituent of extracellular senile plaques, and it is derived from a larger parent molecule named the amyloid precursor protein (APP) (Karran and De Strooper 2022).

Despite many decades of research, no definitive disease-modifying therapy or preventive strategy for AD has yet been established, largely because of its multifactorial etiology and complex pathogenesis. The available medications can offer only brief symptomatic relief with no neuroprotective efficacy. Since the neurochemical alterations are likely to precede the development of clinical symptoms, preventing or postponing the onset of AD can be effective (Self and Holtzman 2023). Therefore, it is of urgent importance to investigate the therapeutic potential of non-pharmacological approaches such as cognitive or physical stimulation, or dietary supplementation in combating this disabling disease.

A healthy lifestyle, which includes a healthy diet, regular physical activity, and engaging in social interactions that stimulate the brain, is believed to have cognitive benefits and may reduce the overall risk of AD (Williams et al. 2020; Alanko et al. 2022). Diet is now recognized to be a critical factor in sporadic AD (Ballarini et al. 2021), as many epidemiological studies confirm the benefits of dietary supplements with antioxidant activity, especially plant polyphenols, in lowering AD risk (Melrose 2023).

(-)-Epigallocatechin-3-gallate (EGCG) is the main catechin compound (50% of total polyphenols) found in green tea (*Camellia sinensis*) extract that has antioxidative, anti-cancer, anti-inflammatory, and anti- amyloidogenic activities (Payne et al. 2022). Studies have shown that EGCG decreases Aβ levels (Lee et al. 2009), and improves Aβ-induced mitochondrial dysfunction (Dragicevic et al. 2011) and memory impairment (Nan et al. 2021) of AD animal models. In the Tg2576 AD mouse model, EGCG decreased Aβ levels by increasing Aβ protein precursor (APP) cleavage (Obregon et al. 2006). Long-term dietary supplementation with EGCG (50 mg/kg) for six months reduced both soluble and insoluble Aβ levels and improved spatial learning performance in the radial-arm water maze (Rezai-Zadeh et al. 2008). Furthermore, oral administration of EGCG at low (5 mg/kg) and high (15 mg/kg) doses for 60 days attenuated Aβ accumulation and improved cognitive performance in senescence-accelerated prone 8 (SAMP8) mice (Chang et al. 2015).

Several experiments have shown that it is possible to partially recover cognitive and brain alterations using medicine-free interventions, such as physical and cognitive training, in animal models (Pamidi et al. 2022; Andrade-Guerrero et al. 2023). Environmental enrichment (EE) is a housing condition that differs from the standard living spaces of laboratory animals, incorporating objects with varied forms, sizes, colors, and textures. These dynamic conditions are designed to enhance social interaction while providing greater cognitive, sensory, and motor stimulation (Baroncelli et al. 2010). It is well established that EE has cognitive-enhancing potential through the promotion

of synaptic plasticity, adult neurogenesis, and the expression of neurotrophic factors, among other mechanisms (Liew et al. 2022). EE as a non-pharmacological approach has beneficial effects on cognitive dysfunctions and neuropathological features of transgenic AD models (Hu et al. 2010). EE exerted neuroprotective effects in AD models by promoting the gene expression of neurotrophic factors and other signaling mediators contributed in cognitive function (Liew et al. 2022). Despite its beneficial effects, there are also some inconsistent data reporting that the effect of EE on AD or Down syndrome neuropathology, neurogenesis, or cognitive functions is heterogeneous and variable (Dierssen et al. 2003; Arendash et al. 2004; Petrosini et al. 2009).

The strategy of combining different compounds or treatments to achieve greater cognitive benefits has attracted interests for developing therapeutic goals in health and disease. Recently, we showed that combining EE with physical exercise or donepezil, a cholinesterase inhibitor agent, is more efficient than either treatment alone in promoting memory ability of healthy rats in Morris water maze test, or in ameliorating memory deficits in Aβ-induced Alzheimer-like rat model (Gholami et al. 2022; Saheb et al. 2023), suggesting synergistic or additive mechanisms .

Though the beneficial effects of EE and EGCG on health and disease have been widely documented, research on their combination to potentially achieve a superior impact on AD neurobehavioral alterations remains scarce. Thus, this study sought to explore the effects of combined EE and EGCG treatment on cognitive functions and hippocampal gene expression of two relevant factors in AD neuropathology, *APP* and *BDNF*, in a rat model of streptozotocin (STZ) -induced Alzheimer-like disease.

Materials and Methods

Animals

All animal experiments were carried out under the approval of the Laboratory Animal Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1400.081). Male rats purchased from the Animal Core Facility of the Faculty of Medicine, Mashhad University of Medical Sciences, Iran, were used in this study. Throughout the experiment, animals were maintained under controlled environmental conditions with a 12-hr light/12-hr dark cycle (06:00–18:00) and had unrestricted access to standard chow and drinking water. Experimental procedures were carefully designed to reduce animal discomfort by limiting the number of animals. Cage maintenance was performed once each week. An overview of the experimental design is provided in Figure 1.

Design

forty-two-month-old male Sprague-Dawley rats were assigned to one of the following five groups (n=8). 1) Sham-operated (Sham): Rats treated with the vehicle solution in an equivalent volume of STZ and via the same procedure as in the ALZ group. 2) Alzheimer (ALZ): Rats received bilateral intracerebroventricular (ICV) micro injection of STZ and were maintained in standard cage housing. 3) Alzheimer plus EGCG (ALZ+EGCG): Rats with Alzheimer received EGCG solution orally (10 mg/kg in 4 ml sterile distilled water) once a day for 21 consecutive days. 4) Alzheimer plus EE (ALZ+EE): Alzheimer rats were maintained in the larger cages measuring 50*50*50 cm for 28 consecutive days. These cages were supplied with different objects such as shelters, houses, nesting materials, ladders, tunnels, and colorful toys, which were weekly rearranged to promote the novelty. 5) Alzheimer plus EE plus EGCG (ALZ+EE+EGCG). Alzheimer rats were exposed to 28 days of EE combined with 21 days of EGCG administration. Following the completion of treatments, the rats were

decapitated for hippocampal dissection. The experimental design is depicted in Figure 1. The rats were kept in groups of four in standard cages and in groups of eight in enriched boxes.

Intracerebroventricular (ICV) microinjection of STZ

Animals were anesthetized via intraperitoneal injection of ketamine-xylazine (100 and 10 mg/kg, respectively; intraperitoneally (IP); Vibac Laboratories, Carros, France) and positioned in a stereotaxic frame. The skull was drilled bilaterally to reach the lateral ventricles choosing the following coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to the sagittal suture, and 4.6 mm ventral to the skull surface (Figure 2). STZ was administered at a dose of 3 mg/kg in 4 µl of phosphate-buffered saline (PBS), or vehicle alone, bilaterally injected (2 µl per side) through a 27-gauge injection needle connected to a Hamilton syringe (10 µl) via a fine polyethylene tube. The solution was delivered manually in ~10 min, and the needle was left in place for an additional 60 sec to minimize dorsal diffusion.

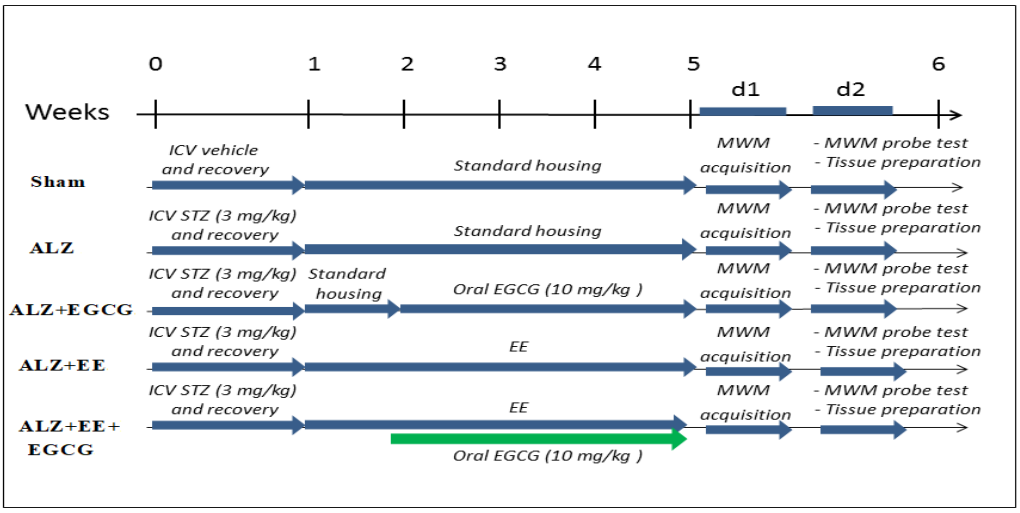

Figure 1. Experiment strategy for streptozotocin (STZ) injection, orally epigallocatechin-gallate (EGCG) administration and environmental enrichment (EE) exposure, and Morris water maze (MWM) task in Wistar rats.

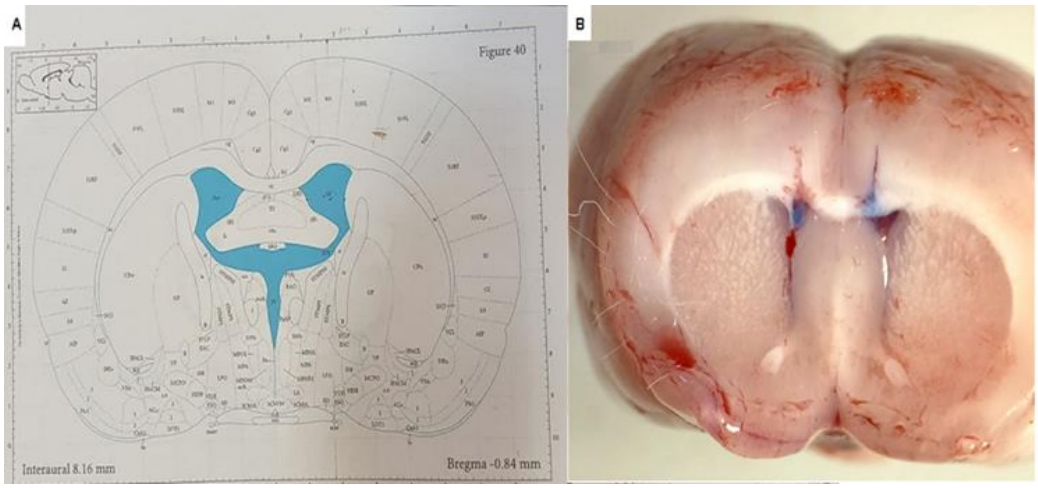

Figure 2. (A) Schematic illustration of the rat brain coordinates from Paxinos atlas. (B) The blue dye was injected stereotaxically into the lateral ventricles in the pilot experiment to assure the correct coordinates.

EGCG administration

EGCG was purchased from Carbosynth Ltd, UK (CAS No: 989-51-5). The powder was diluted in sterile distilled water just before use to make the desired concentrations. EGCG was administered orally to the rats via intragastric gavage.

Spatial learning and memory in Morris water maze (MWM)

The Morris water maze (MWM) apparatus was the same as described previously (Gholami et al. 2022). The behavioral protocol consisted of two consecutive days. On the first day (acquisition phase) during which the spatial learning of the animals was examined, three blocks were conducted at 30-min intervals. Each block contained four trials. In each trial, the animal was released into the water from one of the four quadrants, randomly selected by the machine (with the animal's head facing the maze wall) and was given up to 60 sec to find the hidden platform using the spatial clues on the surrounding walls. If the animal failed to find and mount the platform within this time, it was manually guided to the platform and allowed to remain on it for 20-30 sec before being returned to its home cage until the next trial. Subsequent trials were repeated in the same way, with the animal being released from different quadrants. The elapsed time (escape latency) and the distance traveled to find the hidden platform across the three blocks were recorded as indices of spatial learning. 24 hr later, a single probe trial was conducted to assess spatial memory (retrieval phase). In this trial, the platform was removed, and the animal was allowed to swim freely for 60 sec. The time spent, distance traveled, and the number of crossings in the quadrant that contained the hidden platform during the acquisition phase (target quadrant) were collected and analyzed as spatial memory parameters. All groups were tested during the lights-on period between 10:00 and 12:00 a.m. to ensure consistent testing conditions across experiments.

Tissue dissection and real-time PCR

Following the completion of behavioral assessments (Figure 1), the animals were euthanized, and the hippocampi were immediately removed. The tissues were rapidly frozen in liquid nitrogen and stored at −80 °C until processing. Total RNA was extracted from hippocampal samples using the RNeasy Mini Kit (Parstous, Iran), and RNA purity and concentration were determined by measuring absorbance at 260 nm with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Germany). Subsequently, 1 µg of total RNA was used for cDNA synthesis with the Yekta-Tajhiz cDNA synthesis kit (Cat. Yt4500), following the manufacturer's instructions. Quantitative real-time PCR was performed using the CFX96 Real-Time System (Roche Applied Science, USA) and SYBR Green master mix (Amplicon, Denmark). All reactions were run in duplicate. Relative mRNA expression levels of BDNF and APP were calculated using the 2^−ΔΔCq method, with β-actin serving as the internal reference gene. The primer sequences used for quantitative real-time PCR (qRT-PCR) are listed in Table 1.

Statistical analysis

SPSS software was used for statistical analyses. Quantitative variables between independent groups were compared using one-way analysis of variance (one-way ANOVA), followed by Tukey's post-hoc multiple comparison test. Data across the three consecutive training blocks within each group in the MWM were analyzed using one-way repeated-measures ANOVA. Data are presented as mean ± SEM, and statistical significance was set at $p<0.05$.

Table 1. The primer sequences of RT-qPCR

Gene	Primer sequences
APP	F: 5'-GCGGCAACAGGAACAACTTT-3
	R: 5'-TGCCGTCGTGGGAAACAC-3'
BDNF	F: 5'-TTGTGTGGACCCTGAGTTCC-3
	R: 5'-CAGCCTTCATGCAACCGAAG-3'
GAPDH	F: 5'-GATGGTGAAGGTCGGTGTGA-3'
	R: 5'-TGAACTTGCCGTGGGTAGAG-3'

Notes: RT-qPCR, reverse transcription-quantitative polymerase chain reaction; *APP*: Amyloid Precursor Protein; *BDNF*: Brain-derived neurotrophic factor; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Results

Spatial learning and memory in MWM

Figures 3A and 3B illustrate the escape latency and swimming distance required to locate the hidden platform during the acquisition phase. Within-group repeated-measures analysis demonstrated progressive improvement across training blocks in all experimental groups, indicating acquisition of the task (data not shown). One-way ANOVA revealed that escape latency in block 2 and 3 was different between groups ($F_{(4,35)}$= 4.18, p= 0.007 and $F_{(4,35)}$= 3.63, p= 0.014 respectively). The mean distance traveled in all 3 blocks was also different between groups ($F_{(4,35)}$= 3.71, p= 0.013; $F_{(4,35)}$= 3.93, p= 0.01; and ($F_{(4,35)}$= 5.89, p= 0.001 respectively). For pairwise comparisons see the Figure caption. These data indicate that animals in the Alzheimer group required more time and traveled a greater distance to locate the platform compared to controls, and neither single treatments nor their combination were able to reverse the learning deficits during acquisition. Spatial memory parameters in the single probe are depicted in Figure 3C. One-way ANOVA revealed that the Alzheimer rats spent significantly less time in the target quadrant and traveled a shorter distance within this quadrant (Q3) than control group and combination of EE and EGCG could return the memory parameters to the control levels ($F_{(4,35)}$= 14.07, p= 0.000 and $F_{(4,35)}$= 16.16, p= 0.000 for time and distance respectively). For multiple comparisons refer to the Figure caption. The number of crossing over the target quadrant was not different between groups Figure 3C. Swimming speed was almost the same in all experimental groups Figure 3D.

APP and *BDNF mRNA* expression within the hippocampus

Figures 4A and 4B show the results of APP and BDNF mRNA expression in the hippocampus of rats. One-way ANOVA showed that hippocampal APP levels were significantly different between groups ($F_{(4,20)}$= 8.5, p= 0.000). Tukey's post-hoc comparison test revealed that APP gene levels were increased in Alz group compared to sham animals (p<0.01) and it was significantly decreased in Alz+EE+EGCG group (p<0.000). APP levels in Alz+EE+EGCG animals were also less than those in Alz+EE group (p<0.000). Hippocampal BDNF levels were also different between groups ($F_{(4,20)}$= 5.59, p= 0.003). Multiple comparisons using Tukey's post-hoc test revealed that BDNF gene expression in Alz+EE+EGCG group is higher than those in Alz, Alz+EGCG, and Alz+EE groups (p<0.001, p<0.05, and p<0.05 respectively).The mean difference between Alz and sham group did not reach statistical significance (p=0.284).

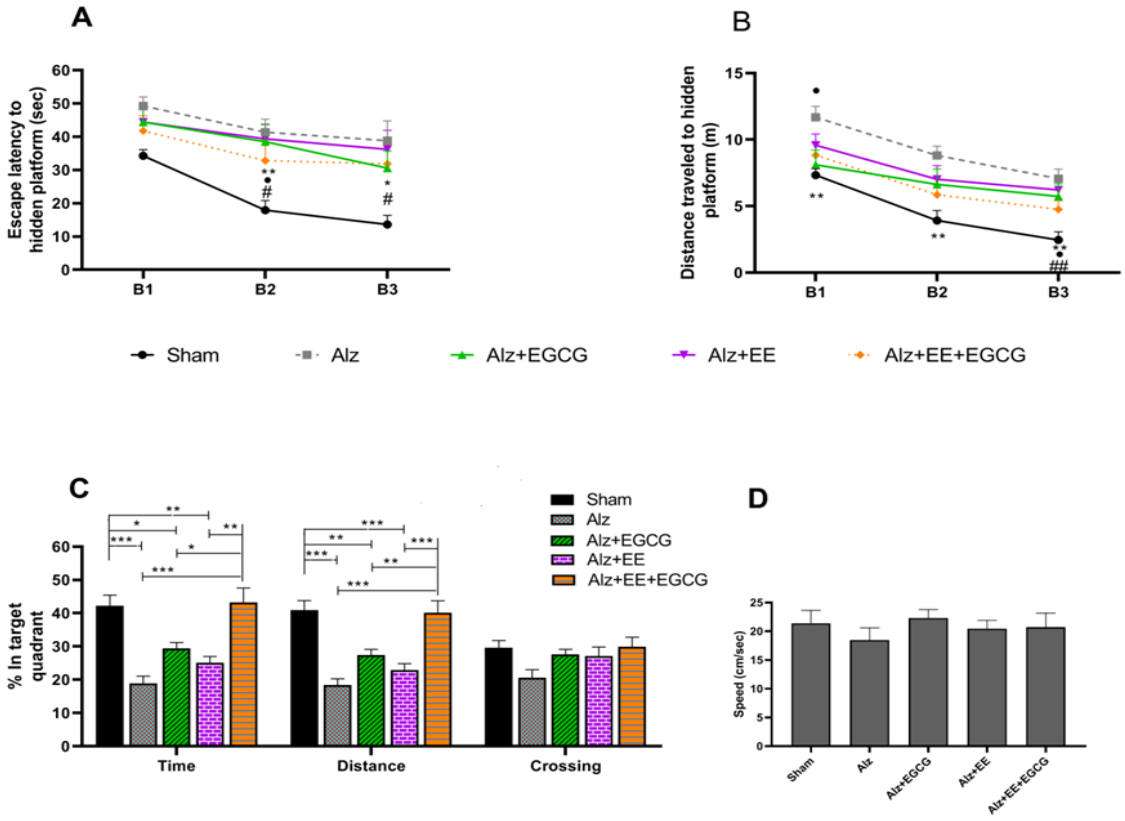

Figure. 3 A-D. Performance of the experimental groups in the Morris water maze (MWM) test (n = 8). Learning performance was assessed by escape latency (A) and swimming distance (B), expressed as the mean of four consecutive training trials. Memory retention was evaluated during the probe trial by measuring the percentage of time spent in the target quadrant, the percentage of distance traveled within the target quadrant, and the percentage of platform crossings (C). Significant differences were observed among the experimental groups for learning and memory indices, whereas swimming speed remained comparable across groups (D). Data are expressed as mean ± SEM. *p<0.05, **p<0.01, and ***p<0.001 versus the Alz group; •p<0.05 versus the Alz+EGCG group; and #p<0.05 and ##p<0.01 versus the Alz+EE group.

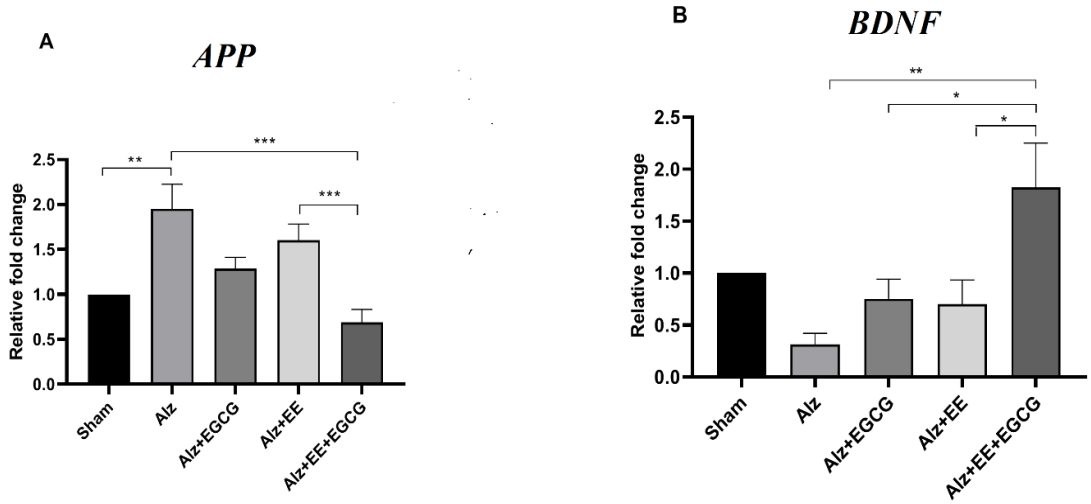

Figure 4. A and B. The hippocampal mRNA expression of *APP* and *BDNF* in experimental groups (n=5). The levels of both factors are significantly different between groups. *p<0.05, **p<0.01, and ***p<0.001. Data are shown as mean ± S.E.M.

Discussion

The present study suggest that STZ caused deficits in spatial learning and memory as indicated by increased platform finding time, increased distance traveled to locate the platform, and diminished time spent in the target quadrant in the MWM. Moreover, decreased *BDNF* and increased *APP* gene expression were observed in the hippocampus of rats treated with STZ. These results confirm the induction of an Alzheimer-like model by ICV injection of STZ in rats. On the other hand, the results of the EE+EGCG combination treatment confirm the improvement in cognitive deficits of AD animals compared to EE and EGCG treatment alone. Similarly, decreased APP and increased BDNF gene expression were observed in the EE+EGCG combination treatment group compared to the other groups. These data suggest that the EE+EGCG combination treatment improves memory deficits in the STZ-induced AD model rats.

AD is initially characterized by various signs and symptoms, including progressive memory damage, cognitive deficits, and behavioral alterations. Several factors have been implicated in the pathogenesis of AD, such as genetic predisposition, advancing age, traumatic brain injury, and exposure to environmental toxins (Abubakar et al. 2022; Self and Holtzman 2023). The key pathological characteristics of AD are the deposition, accumulation, and oligomerization of Aβ plaques, especially in the hippocampus region. The accumulation of Aβ plaques causes a loss of cellular integrity. The Aβ plaques are exposed to oxidative damage that leads to oligomerization of the plaques and neuroinflammation. Subsequently, neuroinflammation disrupts neurogenesis, which in turn leads to synaptic atrophy and memory impairment. The precursor protein of Aβ is APP (Karran and De Strooper 2022). In experimental AD models, APP expression was increased and BDNF expression decreased in the hippocampus region (Biasibetti et al. 2017). ICV injection of STZ leads to multiple metabolic disturbances within the brain. STZ increases acetylcholinesterase levels, causing a decrease in acetylcholine levels, especially in the hippocampus, and impairs new memory formation (Biasibetti et al. 2017). STZ enters the brain via glucose transporter type 2 (GLUT-2). It then induced insulin signaling defect through disruption of the glycogen synthase kinase-3 β (GSK-3β) and phosphoinositide 3-kinase (PI3-K) pathways, leading to tau phosphorylation and formation of intracellular neurofibrillary tangles. STZ also induces oxidative stress in the animal brain by increasing malondialdehyde and decreasing glutathione. These changes all lead to memory impairment (Duelli et al. 1994; Grieb 2016). Due to the similarities in behavioral and cognitive deficits between STZ-induced AD-like pathology and human AD, ICV-STZ administration can be considered a non-transgenic preclinical model of AD (Grieb 2016). Accordingly, ICV administration of STZ in rats has been extensively employed as an experimental model for studying AD-related pathological and metabolic abnormalities and for evaluating potential therapeutic and neuroprotective agents.

Both the EE paradigm and EGCG treatment have individually been shown to enhance cognitive performance in healthy subjects as well as in models of cognitive impairment. EE is a non-invasive intervention for improving cognitive defects in experimental rodent models. In the present study, animals were exposed to the EE for a long time (28 days), and it was cleaned and tidied weekly. EE has been reported to improve memory and cognitive defects in both short- (14 days) and long-term (28 days) periods (Singhal et al. 2019). The novelty of objects due to the weekly tidiness of the EE stimulated the animals to continuously explore and interact with objects and the environment in the long term. Regular change and novelty of objects improved cognitive and memory defects (Pardon et al. 2009; Ramírez-Rodríguez et

al. 2022). Cellular and molecular mechanisms involved in cognitive recovery by EE include improved synaptic plasticity, increased synaptic density, dendritic branches, and cell proliferation within the hippocampal regions involved in cognitive functions (Ohline and Abraham 2019). EE increases the several neurotrophic and signaling factors, including BDNF and CREB (Wang et al. 2019). EE has increased the activity of mGluR5 and p70s6k phosphorylation by stimulating the signaling cascades associated with ERK and mTOR (Hullinger et al. 2015; Cortese et al. 2018). Therefore, EE via these mediators could exert its potential improving effects on memory formation.

Our findings revealed that oral administration of EGCG for 21 days partially improved memory deficits in STZ-induced AD model rats. EGCG improved MWM performance in senescence-accelerated mouse prone 8 (SAMP8) and amyloid precursor protein/presenilin-1 (APP/PS1) mouse models. (Guo et al. 2017; Ettcheto et al. 2020). EGCG in Down syndrome experimental models has been shown to have therapeutic benefits for cognitive disabilities (Stagni et al. 2017). These findings indicate the positive therapeutic effects of EGCG for memory impairments and were consistent with the results of our research. EGCG is the most important polyphenolic bioflavonoid of green tea for all its beneficial effects. EGCG exerts these effects by reducing the accumulation of Aβ and oxidative stress level in the brain (Youn et al. 2022), improving neurogenesis and the signaling of several neurotrophic factors including BDNF, reduction in the processing of Aβ precursor protein (APP) and inhibition of apoptosis (Youn et al. 2022). Therefore, in the present study, EGCG, with its neuroprotective effects, improved memory impairment of AD model rats.

The primary objective of the present study was to investigate whether co-treatment with EGCG and EE is more effective in alleviating cognitive impairments in AD rats than either treatment alone. The results showed that although both exercise alone and EE exposure alone each partially attenuated memory deficits in AD rats, their combined application produced a significantly greater improvement. The combined treatment produced an additive effect rather than a synergistic one, as each intervention yielded only partial benefits individually, whereas their combination led to a significant improvement. It was reported that the combined intervention of EE and EGCG was more efficient than either one alone to improve age-associated cognitive deficiency of older Ts65Dn mice (Catuara-Solarz et al. 2015), proposing a synergistic mechanism. The same combination therapy has been examined in Down syndrome research. A study by Catuara-Solarz et al showed that EE exposure plus EGCG administration ameliorated cortico-hippocampal-dependent cognitive impairments in young trisomic mice. These improvements were accompanied by restoration of dendritic spine density and re-establishment of the balance between inhibitory and excitatory markers within the hippocampal synapses of mice (Catuara-Solarz et al. 2016). A phase II clinical trial in young adults with Down syndrome demonstrated that 12 months of combined EGCG treatment and cognitive training produced greater improvements in cognitive function, adaptive behavior, and neurophysiological recovery than cognitive training with placebo (de la Torre et al. 2016). These findings propose that treatments targeting several components of cellular and molecular mechanisms involved in cognitive dysfunction when combined with a physiologic cognitive stimulant intervention such as EE may be the most potent disease-modifying therapies.

Based on our previous findings, it can be hypothesized that concurrent application of exercise and EE may enhance neurogenesis through potentially overlapping mechanisms, which could

contribute to the greater improvement in cognitive performance observed with the combined intervention (Saheb et al. 2023). These effects were mediated by the simultaneous activation of multiple mechanisms, including increased synaptic plasticity and cell proliferation in the hippocampus, increased synaptic density and dendritic branches (Ohline and Abraham 2019), increased levels of neurotrophic factors, especially BDNF (Santoso et al. 2020), reduced Aβ accumulation, balancing the antioxidant system in the brain, and inhibition of apoptosis (Lee et al. 2009; Liew et al. 2022).

The hippocampus is the main region for memory formation. Impaired hippocampal function can lead to AD (Biasibetti et al. 2017). The main marker of AD is the accumulation of Aβ plaques. The precursor protein of Aβ plaques is APP. In AD, to prevent oxidative damage, the amount of APP protein synthesis and subsequently Aβ plaques increased. Then Aβ plaques accumulation in the cell causes cell damage and rupture and so beta-amyl Aβ plaques is exposed to oxidative damage and oligomerization. This oligomerization causes neuroinflammation, and impaired memory and cognitive functions (Masters and Selkoe 2012; Karran and De Strooper 2022). Impaired memory and cognitive functions in AD is associated with the disruption of multiple neural pathways, including the BDNF/TrkB and PLCγ-Ca2+ pathways in the brain (Abubakar et al. 2022). In the present study, combination of EE and EGCG reduced APP and increased BDNF gene expressions in the hippocampus of AD rats. BDNF plays a critical role in CNS function by promoting neuronal proliferation, survival, differentiation, neurogenesis, and long-term memory formation in the hippocampus (Gonzalez et al. 2019). In this study, treatment with EE and EGCG alone did not lead to a significant change of hippocampal BDNF and APP expression of AD rats. Combined treatment with EE and EGCG significantly increased BDNF and decreased APP gene expression in the hippocampus of AD rats. EGCG in combination with ferulic acid reversed cognitive impairment in APP/PSI transgenic mice by decreasing Aβ deposition in the brain parenchyma and cerebral vessels and reducing the abundance of Aβ proteins compared to a single treatment group (Mori et al. 2019). A superior amelioration of oxidative stress, neuroinflammation, and synaptotoxicity was also detected in this co-treatment model (Mori et al. 2019). Therefore, the additive therapeutic effect of EE and EGCG combination for memory impairments of AD rats in our observations could be partially attributed to the amplified impact of this combination therapy on hippocampal *BDNF* and *APP* gene expression.

Taken together, the results of the present study demonstrated that combination therapy with EE and EGCG is more effective than either treatment alone in alleviating cognitive damage in AD model rats. We demonstrated that the cognitive improvements induced by EE-EGCG treatment were associated with hippocampal modulation, characterized by increased BDNF expression and reduced APP gene expression in AD rats. These findings suggest that EGCG consumption, in combination with a cognitive training intervention, has the potential to recover cognitive deficits in AD, which would be an optimal complementary approach to medical treatments in alleviating cognitive impairments in this clinical population.

Acknowledgment

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Conflicts of interest

The authors declare that they have no competing financial interests or personal relationships that may have appeared to influence this project.

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Ethical Considerations

Animal experiments were carried out under the ethical standards established by Mashhad University of Medical Sciences and in accordance with institutional regulations governing the care and use of laboratory animals. Ethical approval was granted by the University's Animal Ethics Committee (Mashhad, Iran).

Code of Ethics

The ethical clearance code for this study is IR. MUMS.MEDICAL. REC.1400.081.

Authors' Contributions

SSN and ES conceived and designed this study; BH contributed to the manuscript editing. FF and NH assisted in performing the experiments; FF processed the data and VH drafted the manuscript and was responsible for all the process. The final manuscript was reviewed and approved by all authors.

Abbreviations

AD: Alzheimer's disease
ALZ: Alzheimer
APP: Amyloid precursor protein
BDNF: Brain-derived neurotrophic factor
EGCG: (-)-Epigallocatechin-3-gallate
EE: Environmental enrichment
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
ICV: Intracerebroventricular
MWM: Morris water maze
qRT-PCR: Quantitative real-time polymerase chain reaction
STZ: Streptozotocin

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