Epicatechin Plus Treadmill Exercise are Neuroprotective Against Moderate-stage Amyloid Precursor Protein/Presenilin 1 Mice

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ABSTRACT

Background: Epidemiological evidence suggests that exercise and dietary polyphenols are beneficial in reducing Alzheimer’s disease (AD) risk. Material and Methods: In the present study, 8 months old amyloid precursor protein/presenilin 1 (APP/PS1) mice (a moderate pathology phase) were given the green tea catechin (-)-epicatechin delivered orally in the drinking water (50 mg/kg daily), along with treadmill exercise for 4 months, in order to investigate whether the combination can ameliorate the cognitive loss and delay the progression of AD in APP/PS1 transgenic (Tg) mice. Results: At termination, untreated-Tg mice showed elevated soluble amyloid-β (Aβ₄₂) and Aβ₄₀ levels and deficits in spatial learning and memory, compared with their wild-type littermates. The combined intervention protected against cognitive deficits in the Morris water maze, lowered soluble Aβ₄₀ and Aβ₄₂ levels in the hippocampus as well as reducing brain oxidative stress. In addition, brain-derived neurotrophic factor proteins were elevated and Akt/GSK-3/cAMP response element-binding protein signaling was activated in the combination group. Conclusions: Dietary polyphenol plus exercise may exert beneficial effects on brain health and slow the progression of moderate- or mid-stages of AD.

Key words: Alzheimer’s disease, amyloid precursor protein/presenilin 1, amyloid-β, epicatechin, treadmill exercise

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder, characteristic of progressive cognitive dysfunction and senile plaques in the brain.¹ As the major component of senile plaques, amyloid-β (Aβ) peptide is regarded to be a crucial factor in the AD progression. According to Alzheimer's Association 2013, AD has a prevalence rate of 13% after 65 years of age, reaching 44% in people aged 75 years.² Until now, there is no pleasant therapy available to cure AD. The drugs that have been used in clinical trials such as the acetylcholinesterase

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inhibitors, only address the symptoms of AD. In addition, these drugs have shown unwanted side effects.\textsuperscript{[5]}

Lifestyle treatments have been studied in both animal and human models for AD.\textsuperscript{[6]} Epidemiological studies have revealed that an active lifestyle might be adequate to improve cognitive function and delay the onset of dementia in human and mice.\textsuperscript{[5]} Regular moderate physical exercise is currently advocated as a behavioral intervention to improve neural impairments.\textsuperscript{[6]} At present, the most common exercise modalities are the treadmill and the running wheel. Among these, treadmill exercise is closer to human physical training and a better correlation exists between exercise and any potential benefits.\textsuperscript{[7]}

Accumulating evidence demonstrated that AD pathology may be associated with increased oxidative stress and that antioxidant therapy is of great value. There is a general progressive imbalance between intracellular reactive oxygen species and antioxidant defenses in AD patients.\textsuperscript{[8]} A group of dietary polyphenols have been shown to possess antioxidant properties, which are beneficial for cognitive health.\textsuperscript{[9]} Polyphenols in the grape seed extract (GSE) could prevent the Aβ deposition and attenuates the inflammation in the brain of a transgenic (Tg) mouse mode.\textsuperscript{[10]} Epicatechin, as the major polyphenol of GSE, has been investigated in the AD model of 7 months old (TASTPM) thy1-APP²Tg and Thy-1 PS1.M146V mice, where it was shown to reduce Aβ pathology and Aβ levels after a 21-day oral delivery.\textsuperscript{[10]} In the amyloid precursor protein/presenilin 1 (APP/PSEN1) mice model, epicatechin significantly inhibited the deposits of amyloid in the brain and reduced the levels of Aβ in the blood, with no adverse event.\textsuperscript{[11]} Moreover, epicatechin appears to be well tolerated in relation to viability and systemic toxicity by APP/PSEN1 mice. Although these studies suggest a promising future for epicatechin application, there have been inconsistencies\textsuperscript{[12]} in findings and lack of a systematic approach to discover the underlying mechanism.

The combined effects of diet and exercise can be more pronounced than single treatment as shown by epidemiological as well as experimental studies.\textsuperscript{[13]} Although it is now clear that exercise or epicatechin alone ameliorates AD pathology, it is obscure whether the combination would influence AD pathology to a greater extent. Moreover, most of the current studies focus on the preventive effect on the early stage AD mouse model. However, the onset of clinical signs and symptoms of the disease is insidious evolving over many years.\textsuperscript{[14]} Studies on the moderate-stage AD deserve more attention. Tg mice with a double genetic mutation in the APP and the PS1 developed amyloid plaques and cognitive impairment; therefore, serving as an ideal model for preclinical intervention studies of AD.\textsuperscript{[15]} Therefore, the present study was carried out to investigate possible preventative effects of treadmill exercise and epicatechin, alone or in combination on mid-stage APP/PSEN1 AD-like mice models.

**MATERIALS AND METHODS**

**Reagents, animals, and treatments**

Epicatechin (purity >98%, [Figure 1]) was purchased from Sigma-Aldrich (Shanghai, China). APP695/PSEN1-dE9 Tg (APP/PSEN1) mice and their wild-type littermates (nTg) were purchased from the Model Animal Research Center of Nanjing University. The animals were maintained at controlled environmental conditions in terms of constant temperature (22°C ± 2°C), humidity (60% ±10%), and a 12:12 h light/dark cycle. They were allowed chow and water ad libitum. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Eighth Edition, 2010). The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Hainan Medical University. Eight-month-old APP/PSEN1 mice were randomly divided into four groups (n = 8–10 per group): (1) Untreated-Tg group; (2) treadmill exercise-Tg group; (3) epicatechin-Tg group, mice that received epicatechin (50 mg/kg daily) in their drinking water; and (4) exercise plus epicatechin-Tg group. COMA group. nTg was designated as (5) nTg group. Animals received treatments at 8 months old, which lasted for 4 months, and the experiment was terminated at 12 months of age and mice were then sacrificed. One week before sacrifice, mice were subjected to Morris water maze (MWM) test, as shown in Figure 2.

**Treadmill exercise**

Mice in the exercise and COMA groups were made to run on the treadmill for 30 min a day, 13.2 m/min for 5 days/week, during 16 weeks.\textsuperscript{[16]} Prior to the treadmill exercise training, the mice received preadaptation training for 1 week. The workload of the preadaptation was 2 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the last 20 min.\textsuperscript{[17]}

**Morris water maze test**

The MWM test was carried out as previously described.\textsuperscript{[16]} The test was performed in a black circular pool with an inner surface. A round platform was placed below the water surface in the center of the target quadrant. Mice were exposed to two trials every day for four consecutive days. Their escape latencies were recorded. If the mice did not locate the platform within 120 s, it was placed on the platform for 15 s and the escape latency was recorded as 120 s. The platform was then removed for the probe test, and each rat was allowed to swim freely for 120 s, and the swimming trace was recorded.

**Determination of lipoperoxidation, catalase, superoxide dismutase, glutathione reductase, and glutathione oxidized form levels**

The cerebral hippocampus was dissected rapidly under standard conditions at 4°C and was homogenized in phosphate buffer (pH 7.4). The supernatant of tissue homogenates from hippocampus was used for the measurements of glutathione oxidized form (GSSG), glutathione reductase (GSH), superoxide dismutase (SOD), catalase (CAT), and lipoperoxidation (LPO) levels, according to the manufacturer’s protocols.

**Immunohistochemical studies of amyloid-β deposition**

Aβ plaques were analyzed by immunohistochemical staining as described previously.\textsuperscript{[18]} Briefly, sections from hippocampus tissues were dewaxed with xylene and graded ethanol series. The sections were incubated in 3% hydrogen peroxide to quench the activity of endogenous peroxidase. Sequentially, the slides were heated at 100°C to retrieve antigens. The slides were incubated

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**Figure 1:** The chemical structure of epicatechin

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S140

Pharmacognosy Magazine, Apr-Jun 2016, Vol 12, Issue 46 (Supplement 2)
with mouse anti-Aβ antibody (1:1,000, Sigma), followed by incubation with secondary antibody. Aβ immunoreactivity was evaluated with Image-Pro Plus (IPP) 6.0 Software. (Media Cybernetics, Inc., Rockville, MD, USA)

**Enzyme-linked immunosorbent assay for amyloid-β<sub>1–40</sub> and amyloid-β<sub>1–42</sub>**

Hippocampuses were collected and lysed in ice bath for 30 min. The supernatant was collected after centrifugation. The levels of Aβ<sub>1–40</sub> and Aβ<sub>1–42</sub> in the hippocampus were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kit as described previously, following the manufacturer’s instructions.

**Western blot analysis**

The Western blot analysis was performed as previously described. In brief, hippocampus samples were homogenized in lysis buffer provided with complete protease inhibitor. The primary antibodies used included the following: Rabbit polyclonal anti-GSK-3β, anti-phospho-GSK-3β (Abcam, USA), rabbit polyclonal anti-Akt, anti-phospho-Akt (Boster Bio-Engineering Ltd., Co., China), rabbit polyclonal anti-cAMP response element binding protein (CREB), anti-phospho-CREB (Proteintech, USA), rabbit anti-β-actin (Bioworld Technology, USA), and rabbit polyclonal anti-brain-derived neurotrophic factor (BDNF) (Santa Cruz Biotechnology, USA). Membranes were probed with HRP-coupled secondary antibodies (Cell Signaling Technology, USA). Membranes were visualized with chemoluminescence reagents. IPP software for densitometry analysis is applied for the quantification of protein expressions.

**Statistical analysis**

All values are expressed as mean ± standard error. One-way analysis of variance and post hoc Tukey’s multiple comparison tests were used to determine statistical significance between different groups. \( P < 0.05 \) was considered as significant.

**RESULTS**

**Exercise improved spatial learning loss in amyloid precursor protein/presenilin 1 mice**

The MWM was applied to evaluate spatial learning and memory in APP/PS1 mice. In the training sessions, there was a notable increment in the average time to find the platform for Tg mice across consecutive trials (escape latency), compared with their nTg (\( P < 0.01 \)), which means spatial learning deficits [Figure 3a]. In the probe trial, Tg mice also spent
Exercise and epicatechin reduced amyloid-β deposition and production in transgenic mice

Aβ accumulation plays a critical role in the cognitive deficits in AD. The occurrence of Aβ has been regarded as a mark of moderate- or mid-stage AD from about 6–12 months of age in APP/PS1 mice. Researches have shown that neural activity modulates Aβ production and deposition. To explore whether treadmill exercise associated neural activity could prevent Aβ deposition in Tg mice, brain sections were subjected to IHC staining. Immunohistochemistry results revealed the surge of Aβ plaques in the hippocampus of Tg mice compared with nTg mice (P < 0.01) [Figure 4a]. However, both single treatments failed to diminish Aβ plaque burden in the hippocampus (P > 0.05). COMA treatment showed a clear tendency to decline Aβ plaques, however, without a statistic difference [Figure 4b].

Next, we further examined whether treadmill exercise plus epicatechin, affect soluble Aβ levels in the hippocampus of all groups by ELISA. Levels of Aβ1-40 and Aβ1-42 in untreated-Tg mice increased significantly than those of nTg mice, neither of which was rescued by any single treatment. Intriguingly, the levels of soluble Aβ were evidently reduced in the COMA group than untreated-Tg mice (P < 0.05) [Figure 4c]. Collectively, these results show that 4 months of combination therapy could reduce soluble Aβ levels, rather than Aβ deposition for mid-stage APP/PS1 mice.

Exercise and epicatechin differentially regulated oxidative stress in transgenic mice

GSH, GSSG, LPO contents, SOD, and CAT activities were used as parameters to measure the oxidative stress in APP/PS1 mice. All the treatments reversed the augmentation of LPO content in Tg mice (P < 0.05). In addition, GSH levels of the Tg group were considerably lower than in the nTg mice, and the decline was reversed by epicatechin or COMA treatment (P < 0.05). Nevertheless, no differences in GSSG levels were observed across all the groups [Figure 5]. The GSH/GSSG ratio demonstrated that the epicatechin group had the best glutathione cycle status (P < 0.01), suggesting the potent pro-oxidant effect of epicatechin. The untreated-Tg group had faintly lower protein levels of SOD and CAT than nTg mice (P < 0.05). Likewise, these protein levels were considerably elevated (P < 0.05) in epicatechin or COMA group.

Exercise and epicatechin enhanced brain-derived neurotrophic factor expression in transgenic mice

BDNF signaling pathway are known to support neuronal survival, regulates neuroplasticity, and mediates memory fixation, we then assessed BDNF expressions in mice hippocampus. Western blot analysis revealed untreated-Tg mice had a lower level of BDNF than nTg mice (P < 0.05) [Figure 6], and BDNF protein levels in the hippocampus of exercise or epicatechin or COMA groups were considerably expanded than the untreated-Tg group. Although the COMA group showed the numerically highest BDNF protein level, there was no significant difference when comparing it with either the exercise or epicatechin group.

Akt/GSK-3/cAMP response element binding protein signaling pathway was involved in exercise and epicatechin-mediated neuroprotective effect

GSK-3 is closely related with Aβ deposition, oxidative stress, and memory impairment. GSK-3 is constitutively active in most cells and is inactivated by phosphorylation by plenty of signaling cascades,
including PI3K/Akt. Increasing evidence proves the disturbance of Akt/GSK-3/CREB signaling pathway in AD brains. Therefore, we assumed that Akt/GSK-3/CREB pathway might play a key part in exercise plus epicatechin-mediated neuroprotection against cognitive impairments in APP/PS1 Tg mice. Western blot analyses were applied to examine the expression of both GSK-3β and phosphor-GSK-3β (ser9). While no differences in the total GSK-3β or total Akt levels was observed across all the groups (P > 0.05), the phosphorylation of GSK-3β, CREB and Akt were significantly reduced in Tg mice than nTg mice. A notable reversal of the decreased phosphorylation of GSK-3β, CREB, and Akt was observed in exercise, epicatechin, or COMA groups. There is a significant difference of phosphor-GSK-3β and phosphor-CREB levels between COMA and epicatechin groups.

**DISCUSSION**

This study examined in APP/PS1 male mice whether the combined treatment of epicatechin and treadmill exercise afforded higher neuroprotection than single treatment. Four-month treatments began at a moderate pathology phase (8 months old) when animals already present cognitive loss and brain pathology. Overall, both epicatechin and treadmill exercise groups reverted the augmentation of LPO content and enhanced BDNF expression, compared with nTg mice. Differential neuroprotection was observed in other aspects. To be precise, spatial learning deficits were ameliorated by exercise and the decrement of GSH levels was reversed by epicatechin. Among all the treatments, the epicatechin group had the best glutathione cycle status. However, only the combination group reduced soluble Aβ levels, while both single treatments failed to diminish Aβ deposition. In addition, the cognitive function was improved in the combined therapy group.

The occurrence of Aβ has been regarded as a mark of moderate- or mid-stage AD from about 6–12 months of age in APP/PS1 mice. At the endpoint of this study (12 months of age), in the measure of cognitive affectations through the MWM test, there was a notable increment in escape latency for Tg mice, compared with their nTg, which means
Spatial learning deficits. Spatial memory impairment was also obvious in Tg mice. These findings were in accordance with previous studies reporting cognitive deficits in APP/PS1 mice. Cognitive deficits in these mice correlate with onset and progression of an AD-like pathology, associated with the aggregation of Aβ peptide, a primary cause of AD. As expected, immunohistochemistry results exhibited a surge of Aβ plaques in the hippocampus, paralleled with a significantly higher level of Aβ1-40 and Aβ1-42 in untreated-Tg mice than those of nTg mice.

There is an extensive evidence that exercise not only improves cognitive function in normal individuals but also delays AD progression. However, some epidemiological studies demonstrated inconsistent relationship between exercise and physical activity in patients with AD. It is worth mentioning that not all exercise shows similar effects on learning and memory, namely the impact of physical exercise was dependent on the type, duration, or intensity of physical activity. Although wheel running, a voluntary exercise, seemed to be more beneficial, a treadmill exercise without the electric stimulant is closer to human physical training.

Herein, treadmill exercise remains the effective modality of exercise in rodent studies. Previous studies have shown that exercise exerts preventive effect on both learning and memory deficits in several early-stage AD-like animal models, such as TgCRND8 mice, NSE/APPsw mice, APP/PS1 mice, AD-like mice by intracerebroventricular (ICV) injection of Aβ25–35, and 3xTg-AD mice, but protection against cognitive loss in moderate- or late-stage AD mice was controversial. Our study showed that 4 months of exercise alone only exerted a slight effect on spatial memory loss of moderate- or late-stage Tg mice whereas the combined therapy (treadmill exercise plus epicatechin) enhanced cognitive function. These data demonstrated that the effect of exercise is closely related with the timing of the treatment.

Likewise, the timing of treatment is also crucial for the effect of exercise on Aβ levels. There is evidence that when treadmill exercise started at 3 months of age, an obvious decline of soluble Aβ levels and Aβ deposition was observed in brains. At 17 months of age, when Aβ plaque has probably emerged in APP/PS1 mice, treadmill exercise beginning at this stage only brought down soluble Aβ levels, without prevention on Aβ deposition in the hippocampus. Consistently, in the current study, 4 months of combination therapy, which was initiated at 8 months of age, could reduce soluble Aβ levels in mid-stage APP/PS1 mice, but was not enough to reduce the Aβ plaque loading in the hippocampus of APP/PS1 mice. Interestingly, our findings described that long-term exercise could not lessen Aβ plaque, but decrease the levels of soluble Aβ1-40 and Aβ1-42 in aged Tg mice, indicating that the decline of soluble Aβ levels is responsible for the amelioration of impaired cognition function. Abnormal modifications in tau interfere with its interaction with microtubules leading to self-aggregation into neurofibrillary tangles. There were discrepancies about the effect of epicatechin on cognition function. Epicatechin has been reported to reduce Aβ levels in 7 months old TASTPM mice. One study of chronic treatment of epicatechin diet at 3 months of age for 9 months reported that epicatechin significantly reduced total Aβ in brain and serum by 39 and 40%, respectively. In the same study, long-term treatment of epicatechin failed to alter learning and memory behaviors in APP/PS1 mice. In the present study, 4 months of treatment of epicatechin diet at 8 months of age exerted no obvious effect on Aβ deposition or production.

There is an accumulating evidence supports that oxidative stress is closely associated with AD pathology. Increased lipid and protein oxidative damage was shown in lymphocyte mitochondria of AD patients. In AD patients, SOD and CAT activities were lower in both the central nervous system and peripheral tissues. The brain is vulnerable to oxidative stress due to the high density of oxidizable substrates, and relatively low antioxidant defense. The Tg mice overexpressed with APP mutant, and a deficiency in Mn-SOD had higher oxidative stress and augmented Aβ plaques. Epicatechin is a potent scavenger of lipid peroxidation products. A single oral dose of epicatechin (30 mg/kg) can block the oxidative damage of the hippocampus induce by Aβ25–35 injection. Epicatechin can channel the reactive oxygen to itself, undergo oxidation, and subsequently protect the reactive cytokines from oxidation by interacting with them. In our study, APP/PS1 mice had significantly lower SOD, CAT activities, GSH level, and higher levels of LPO in brains of the Tg group, than those of the nTg mice. The impaired antioxidant enzymes in Tg mice were restored with epicatechin treatment alone. Moreover, epicatechin group had the best glutathione cycle status, better than the COMA group. There is a reason which might explain such unpredictable phenomena. Treadmill exercise at moderate intensity augments the muscle aerobic capacity and transiently elevates serum corticosterone levels similar to mild stress, which might offset the anti-oxidative effect of epicatechin in the combination group.

BDNF is known to manipulate synaptic plasticity, neural transmission, and neuronal survival. Enhanced BDNF in the hippocampus could improve both short- and long-term memories. Moreover, BDNF is closely associated with exercise-induced enhancement of brain plasticity. Treadmill exercise facilitated neurogenesis and BDNF levels in the hippocampus of AD rats induced by ICV injection of Aβ25–35. The decrement of BDNF protein levels in untreated-Tg mice was greatly attenuated in both exercise, epicatechin and COMA groups. Even though COMA group showed the numerically highest BDNF protein level, there was no significant difference between COMA and exercise or between COMA and epicatechin.

GSK-3 plays a negative role in cognitive function. It has been shown to promote amyloid pathology. As the most studied protein kinase, Akt is capable of phosphorylating and inactivating GSK-3. Aβ exposures will downregulate the level of phosphor-Akt and phosphor-GSK-3. Furthermore, Akt can also be activated by BDNF CREB, a transcription factor that regulates a variety of brain genes, including BDNF, plays crucial role in neuronal plasticity. Aβ exposure could enhance the GSK-3-mediated inhibition of CREB phosphorylation, then afterward decreasing BDNF expression. The phosphorylation of GSK-3β, CREB, and Akt were significantly reduced in Tg mice than nTg mice. In exercise, epicatechin or COMA groups, Akt/GSK-3β/CREB signaling pathway was activated.
at 8 months of age when APP/PS1 mice are in moderate-stage pathology, and the combination treatment exhibited therapeutic effects by reversing many analyzed parameters to nTg levels, including decreasing soluble Aβ levels, improving cognitive function, and activating of Akt/GSK-3β/CREB signaling pathway. However, in order to apply these methods in humans, further studies are needed to determine the underlying mechanisms.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Kato J, Khan A. Reducing Aβ load and tau phosphorylation: Emerging perspective for treating Alzheimer’s disease. Eur J Pharmacol 2015;784:57-61.

2. Liu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer’s disease: Occurrence, determinants, and strategies toward intervention. Dialogues Clin Neurosci 2009;11:111-28.

3. Alva G, Cummings JL. Relative tolerability of Alzheimer’s disease treatments. Psychiatriy (Edgmont) 2008;5:27-38.

4. Ke HC, Huang HJ, Liang KC, Hsieh-Li HM. Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. Brain Res 2011;1403:1-11.

5. Nichol KE, Poon WY, Paradikov AI, Cribs DH, Glabe CG, Cotman CW. Exercise alters the immune profile in Tg2576 Alzheimer mouse toward a response coincident with improved cognitive performance and decreased amyloid. J Neuroinflammation 2008;5:13.

6. Seo TB, Cho HS, Shin MS, Kim CJ, Je SY, Baek SS. Treadmill exercise improves behavioral outcomes and spatial learning memory through up-regulation of reelin signaling pathway in autistic rats. J Exerc Rehabil 2013;9:220-9.

7. Garland T Jr., Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: Human and rodent perspectives. J Exp Biol 2011 15:214(Pt 2):206-29.

8. Garcia-Mesa Y, Cole J, Corral R, Crisóstom R, Comellas F, Nebra R, et al. Oxidative stress is a central target for physical exercise neuroprotection against pathological brain aging. J Gerontol A Biol Sci Med Sci 2016;71:40-9.

9. Cox C, Choudhry P, Feacey E, Perkinton MS, Richardson JC, Howlett DR, et al. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: Human and rodent perspectives. J Exp Biol 2011 15:214(Pt 2):206-29.

10. Wang YJ, Thomas P, Zhong JH, Bi J, Li Z, Yang W, et al. Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer’s disease mouse. Neurotox Res 2009;15:3-14.

11. Zeng YQ, Wang YJ, Zhou XF. Effects of (-) epicatechin on the pathology of APP/PS1 transgenic mice. Front Neurol 2014;5:69.

12. George RC, Lew J, Graves DJ. Interaction of cinamaldehyde and epicatechin with tau: Implications of beneficial effects in modulating Alzheimer’s disease pathogenesis. J Alzheimers Dis 2013;36:21-40.

13. Gomez-Pinilla F. Collaborative effects of diet and exercise on cognitive enhancement. Nutr Rev 2011;69:49-60.

14. Chen M, Sultana R, Mecocci P, Mangialasche F, Cecchetti R, Baglioni M, Butterfield DA. Increased physical activity and nanoencapsulated curcumin suppress β-amyloid-induced cognitive impairments in rats: Involvement of BDNF and Akt/GSK-3β signaling pathway. Neurobiol Learn Mem 2013;106:134-44.

15. Jimenez S, Torres M, Vizuete M, Sanchez-Vare R, Sanchez-Mejias E, Trujillo-Estrada L, et al. Age-dependent accumulation of soluble amyloid beta (Abeta) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP α) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt/GSK-3β pathway in Alzheimer mouse model. J Biol Chem 2011;286:18414-25.

16. Trinchese F, Liu S, Battaglia F, Walter S, Mathews PM, Arancio O. Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. Ann Neurol 2004;55:801-14.

17. Liu HL, Zhao G, Cai K, Zhao HH, Shi LD. Treadmill exercise prevents decline in spatial learning and memory in APP/PS1 transgenic mice through improvement of hippocampal long-term potentiation. Behav Brain Res 2011;218:308-14.

18. Akbaraly TN, Portet F, Fustinoni S, Dartigues JF, Artero S, Rouaud O, et al. Leisure activities and the risk of dementia in the elderly: Results from the three-city study. Neurology 2009;73:863-41.

19. Lin TW, Chen SJ, Huang TY, Chang CY, Chuang JJ, Wu FS, et al. Different types of exercise induce differential effects on neuronal adaptations and memory performance. Neurobiol Learn Mem 2012;97:140-7.

20. Zhao G, Liu HL, Zhang H, Tong XJ. Treadmill exercise enhances synaptic plasticity, but does not alter β-amyloid deposition in hippocampi of aged APP/PS1 transgenic mice. Neuroscience 2015;288:357-66.

21. Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer’s disease. J Neurosci 2005;25:4217-21.

22. Kim BK, Shin MS, Kim CJ, Baek SB, Ko YC, Kim YP. Treadmill exercise improves short-term memory by enhancing neurogenesis in amyloid beta-induced Alzheimer disease rats. J Exerc Rehabil 2014;10:2-8.

23. Garcia-Mesa Y, Lopez-Ramos JC, Gimenez-Lloret L, Revilla S, Guerra R, Gault A, et al. Physical exercise protects against Alzheimer’s disease in 3×Tg-AD mice. J Alzheimers Dis 2011;24:421-54.

24. Sultana R, Meccoci P, Mangialasche F, Cechetti R, Baglioni M, Butterfield DA. Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer’s disease: Insights into the role of oxidative stress in Alzheimer’s disease and initial investigations into a potential biomarker for this demening disorder. J Alzheimers Dis 2011;24:77-84.

25. Chen G, Tang J, Ni Z, Chen Q, Li Z, Yang W, et al. Antiatherosclerotic effects of resveratrol in ovalbumin-induced asthma model mice involved in the upregulation of PTEN. Biol Pharm Bull 2015;38:507-13.

26. Tung BT, Rodriguez-Bies E, Talero E, Gamaro-Estévez E, Motilva V, Navas P, et al. Anti-inflammatory effect of resveratrol in old mice liver. Exp Gerontol 2015;84:1-7.

27. Zhu Q, Zheng ZP, Cheng KW, Wu JJ, Zhang S, Tang YS, et al. Natural polyphenols as direct trapping agents of lipid peroxidation-derived arachinone and 4-hydroxy-trans-2-nonenal. Chem Res Toxicol 2009;22:1721-7.

28. Cuevas E, Limón D, Pérez-Severiano F, Díaz A, Ortega L, Zenteno E, et al. Antioxidant effects of epicatechin on the hippocampal toxicity caused by amyloid-β in rats. Eur J Pharmacol 2009;616:122-7.
39. Kim SE, Ko IG, Shin MS, Kim CJ, Jin BK, Hong HP, et al. Treadmill exercise and wheel exercise enhance expressions of neutrophic factors in the hippocampus of lipopolysaccharide-injected rats. Neurosci Lett 2013;538:54-9.

40. Cho HS, Shin MS, Song W, Jun TW, Lim BV, Kim YP, et al. Treadmill exercise alleviates short-term memory impairment in 6-hydroxydopamine-induced Parkinson's rats. J Exerc Rehabil 2013;9:354-61.

41. Liu HL, Zhao G, Zhang H, Shi LD. Long-term treadmill exercise inhibits the progression of Alzheimer's disease-like neuropathology in the hippocampus of APP/PS1 transgenic mice. Behav Brain Res 2013;256:261-72.

42. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995;378:785-9.

43. DaRocha-Souto B, Coma M, Pérez-Nievas BG, Scotton TC, Siao M, Sánchez-Ferrer P, et al. Activation of glycogen synthase kinase-3 beta mediates β-amyloid induced neuritic damage in Alzheimer's disease. Neurobiol Dis 2012;45:425-37.

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