

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

Zhiyuan Zhang[†], Hao Wu^{†1}, Houcai Huang²

School of Pharmacy, Hainan Medical College, Haikou, ¹School of Pharmacy, Anhui University of Chinese Medicine, Hefei, ²Key Laboratory of Delivery Systems of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing, China

[†]These authors contributed equally to this work

Submitted: 24-11-2015

Revised: 17-12-2015

Published: 11-05-2016

ABSTRACT

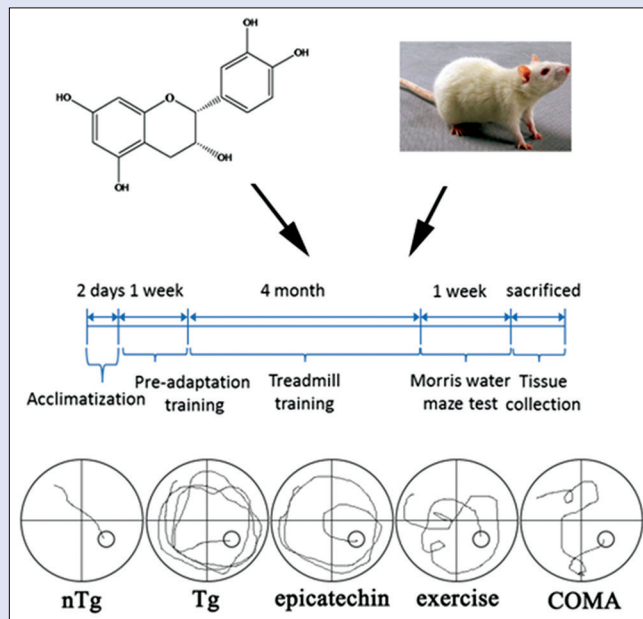
Background: Epidemiological evidence suggests that exercise and dietary polyphenols are beneficial in reducing Alzheimer's disease (AD) risk. **Materials and Methods:** In the present study, 8 months old amyloid precursor protein/presenilin 1 (APP/PS1) mice (a moderate pathology phase) were given the 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 ($A\beta_{1-40}$) and $A\beta_{1-42}$ 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 $A\beta_{1-40}$ and $A\beta_{1-42}$ 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

SUMMARY

- Amyloid precursor protein/presenilin 1 transgenic mice showed elevated soluble amyloid- β ($A\beta_{1-40}$) and $A\beta_{1-42}$ levels and deficits in spatial learning and memory, compared with their wild-type littermates
- Oral administration of epicatechin, combined with treadmill exercise for 4 months, could protect against cognitive deficits, and lowered soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ levels as well as reducing brain oxidative stress
- Brain-derived neurotrophic factor proteins were elevated, and Akt/GSK-3/cAMP response element binding protein signaling was activated in the combination group
- Dietary polyphenol plus exercise might exert beneficial effects on brain health and slow the progression of moderate- or mid-stages of Alzheimer's disease.



Abbreviations used: AD: Alzheimer's disease, Tg: APP/PS1 transgenic, BDNF: Brain-derived neurotrophic factor, $A\beta$: Amyloid- β , APP: Amyloid precursor protein, PS1: Presenilin 1, nTg: Wild-type littermates, IACUC: Institutional Animal Care and Use Committee, GSSG: Glutathione oxidized form, GSH: Glutathione reductase, SOD: Superoxide dismutase, CAT: Catalase, LPO: Lipoperoxidation, CREB: cAMP response element binding protein.

Correspondence:

Dr. Houcai Huang,
Jiangsu Provincial Academy of Chinese Medicine,
100 Shizi Road, Nanjing, Jiangsu 210028, China.
E-mail: zhangzhiyuan56d@163.com
DOI: 10.4103/0973-1296.182174

Access this article online

Website: www.phcog.com

Quick Response Code:



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 ($A\beta$) 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

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Zhang Z, Wu H, Huang H. Epicatechin plus treadmill exercise are neuroprotective against moderate-stage amyloid precursor protein/presenilin 1 mice. Phcog Mag 2016;12:S139-46.

inhibitors, only address the symptoms of AD. In addition, these drugs have shown unwanted side effects.^[3]

Lifestyle treatments have been studied in both animal and human models for AD.^[4] 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.^[5] Regular moderate physical exercise is currently advocated as a behavioral intervention to improve neural impairments.^[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.^[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.^[8] A group of dietary polyphenols have been shown to possess antioxidant properties, which are beneficial for cognitive health.^[9] Polyphenols in the grape seed extract (GSE) could prevent the A β deposition and attenuates the inflammation in the brain of a transgenic (Tg) mouse model.^[10] Epicatechin, as the major polyphenol of GSE, has been investigated in the AD model of 7 months old (TASTPM) thy1-APPswe and Thy-1 PS1.M146V mice, where it was shown to reduce A β pathology and A β levels after a 21-day oral delivery.^[9] In the amyloid precursor protein/presenilin 1 (APP/PS1) mice model, epicatechin significantly inhibited the deposits of amyloid in the brain and reduced the levels of A β in the blood, with no adverse event.^[11] Moreover, epicatechin appears to be well tolerated in relation to viability and systemic toxicity by APP/PS1 mice. Although these studies suggest a promising future for epicatechin application, there have been inconsistencies^[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.^[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 mice model. However, the onset of clinical signs and symptoms of the disease is insidious evolving over many years.^[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.^[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/PS1 AD-like mice models.

MATERIALS AND METHODS

Reagents, animals, and treatments

Epicatechin (purity >98%, [Figure 1]) was purchased from Sigma-Aldrich (Shanghai, China). APP695/PS1-dE9 Tg (APP/PS1) 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 \pm 2°C), humidity (60% \pm 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/PS1 animals 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.^[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.^[17]

Morris water maze test

The MWM test was carried out as previously described.^[18] 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

A β plaques were analyzed by immunohistochemical staining as described previously.^[19] 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

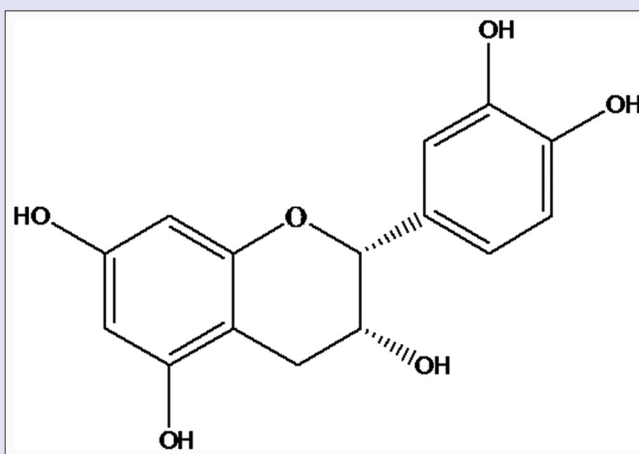


Figure 1: The chemical structure of epicatechin

with mouse anti-A β antibody (1:1,000, Sigma), followed by incubation with secondary antibody. A β immunoreactivity was evaluated with Image-Pro Plus (IPP) 6.0 Software. (Media Cybernetics, Inc., Rockville, MD, USA)

Enzyme-linked immunosorbent assay for amyloid- β_{1-40} and amyloid- β_{1-42}

Hippocampuses were collected and lysed in ice bath for 30 min. The supernatant was collected after centrifugation. The levels of A β_{1-40} and A β_{1-42} in the hippocampus were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kit as described previously,^[20] following the manufacturer's instructions.

Western blot analysis

The Western blot analysis was performed as previously described.^[21] 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-3 β , anti-phospho-GSK-3 β (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 \pm 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

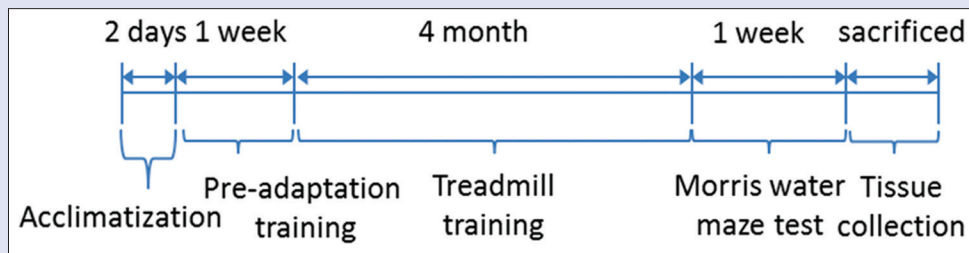


Figure 2: Experimental time schedule

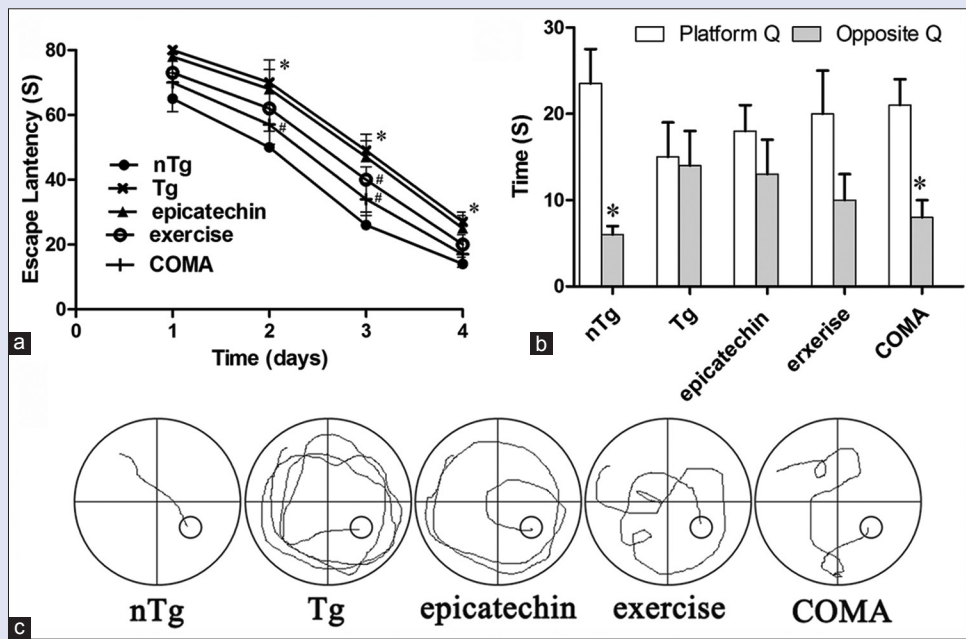


Figure 3: Cognitive performance of transgenic and wild-type littermate mice, as evaluated by the Morris water maze test. Values are expressed as mean \pm standard error $n = 8$. (a) Performance in the reference memory protocol, based on escape latency. * $P < 0.05$ compared with wild-type littermates; $^{\#}P < 0.05$ compared with transgenic; (b) time spent (seconds) in the target quadrant compared to the opposite quadrant. * $P < 0.05$ between the times spent in quadrants in each group; (c) Representative search strategy trails on the 4th day. The traces show the swim path of all groups

less time in the target quadrant ($P < 0.05$), inferring spatial memory loss. The long escape latency was greatly reversed in treadmill exercise or COMA groups during the training ($P < 0.05$). However, epicatechin “*per se*” showed no effect on escape latency ($P > 0.05$). Moreover, the combined therapy demonstrated differences in time spent in target quadrant, compared with Tg group whereas exercise alone only exerted a slight effect on spatial memory loss [Figure 3b]. Swim tracing analysis showed that nTg mice swam in the direction of the platform whereas untreated-Tg mice took longer swimming paths. In the COMA groups, Tg mice reached the hidden platform with small loops. However, in the exercise or epicatechin-treated groups, Tg mice swam randomly within the entire pool, signifying that these mice did not remember the location of the hidden platform [Figure 3c]. The results indicated that treadmill exercise only improve the spatial learning deficits rather than the memory impairment, but the combination therapy was able to improve both spatial learning and memory activity of Tg mice.

Exercise and epicatechin reduced amyloid- β deposition and production in transgenic mice

A β accumulation plays a critical role in the cognitive deficits in AD. The occurrence of A β has been regarded as a mark of moderate- or mid-stage AD from about 6–12 months of age in APP/PS1 mice.^[22] Researches have shown that neural activity modulates A β production and deposition.^[23] To explore whether treadmill exercise associated neural activity could prevent A β deposition in Tg mice, brain sections were subjected to IHC staining. Immunohistochemistry results revealed the surge of A β plaques in the hippocampus of Tg mice compared with nTg mice ($P < 0.01$) [Figure 4a]. However, both single treatments failed to diminish A β plaque burden in the hippocampus ($P > 0.05$). COMA treatment showed a clear tendency to decline A β plaques, however, without a statistic difference [Figure 4b].

Next, we further examined whether treadmill exercise plus epicatechin, affect soluble A β levels in the hippocampus of all groups by ELISA. Levels of A β_{1-40} and A β_{1-42} 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 A β 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 A β levels, rather than A β 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 reverted the augmentation of LPO content in Tg mice ($P_s < 0.05$). 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 ($P_s < 0.05$). 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.^[24] 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 A β deposition, oxidative stress, and memory impairment. GSK-3 is constitutively active in most cells and is inactivated by phosphorylation by plenty of signaling cascades,

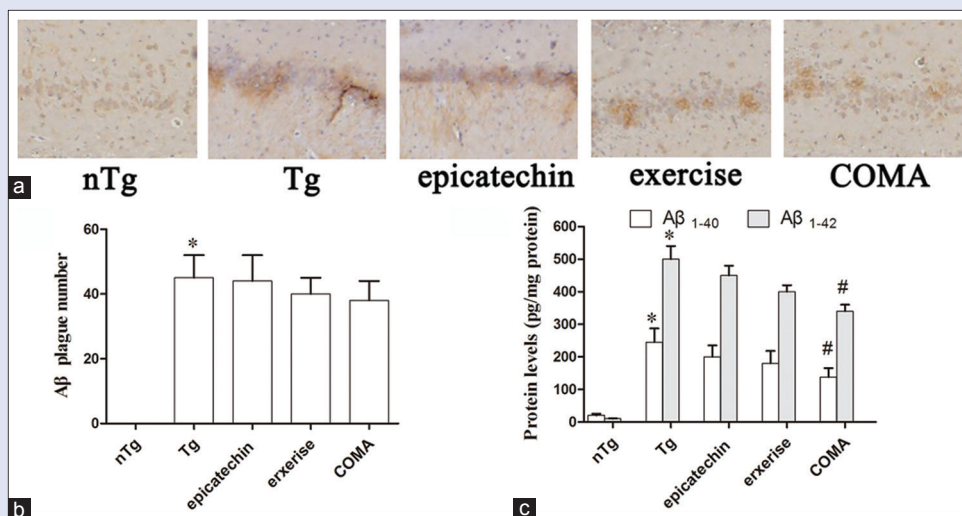


Figure 4: Effect of treadmill exercise and epicatechin on amyloid- β plaque formation and soluble amyloid- β production in the hippocampus of amyloid precursor protein/presenilin 1 mice. (a) Amyloid- β immunoreactive plaques formation in the hippocampus of amyloid precursor protein/presenilin 1 mice. Scale bar = 200 μ m. (b) Quantification of the amyloid- β -positive plaques number. (c) Soluble amyloid- β_{1-40} and amyloid- β_{1-42} production by enzyme-linked immunosorbent assay kit. Values are expressed as mean \pm standard error $n = 8$. Statistics (b and c): * $P < 0.05$ compared with wild-type littermates; # $P < 0.05$ compared with transgenic

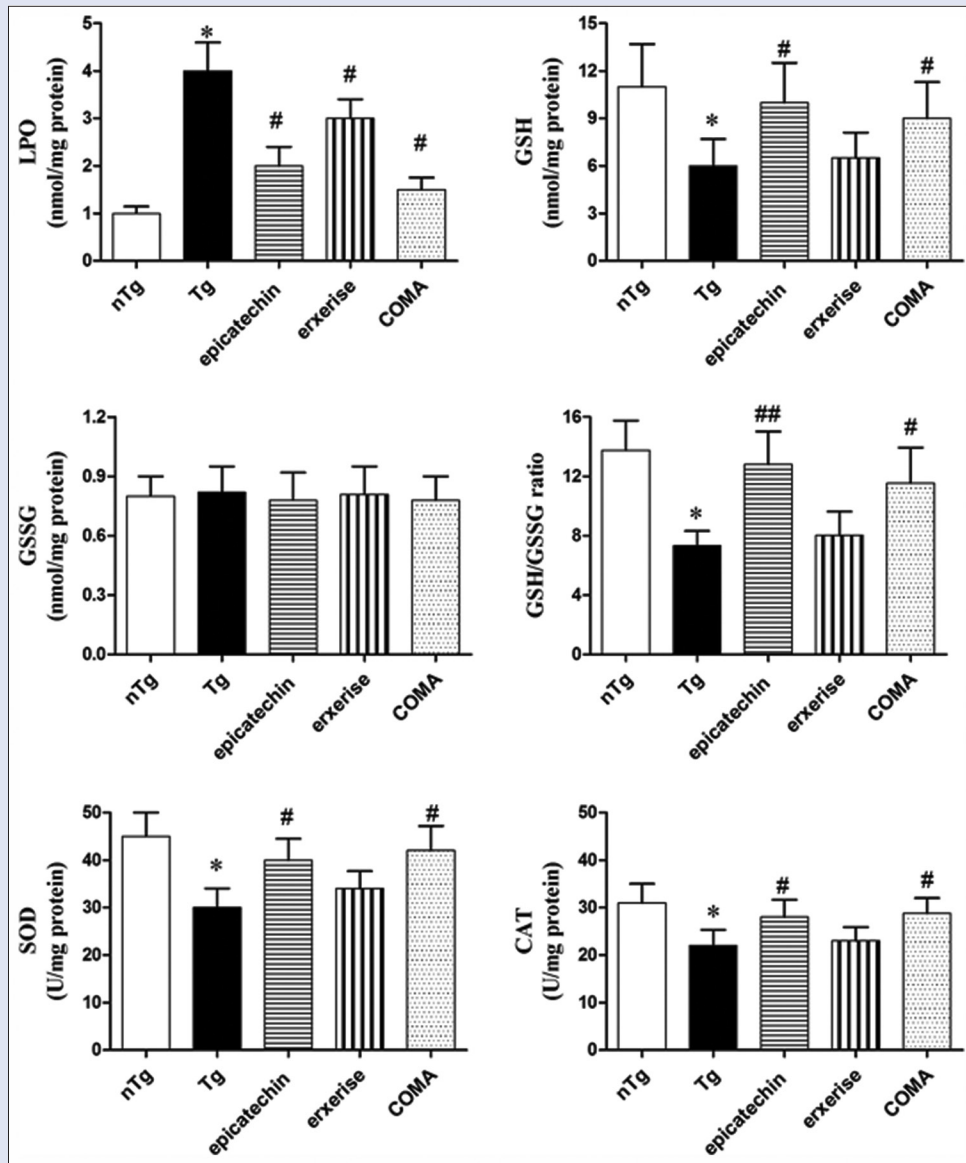


Figure 5: Effect of treadmill exercise and epicatechin on oxidative stress in the hippocampus. Glutathione, oxidized glutathione, lipoperoxidation contents, superoxide dismutase, and catalase activities were used as parameters to measure the oxidative stress. Values are expressed as mean \pm standard error $n = 8$. * $P < 0.05$ compared with wild-type littermates; # $P < 0.05$, ## $P < 0.01$ compared with transgenic

including PI3K/Akt.^[25] 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-3 β and phosphor-GSK-3 β (ser9). While no differences in the total GSK-3 β or total Akt levels was observed across all the groups ($P > 0.05$), the phosphorylation of GSK-3 β , CREB and Akt were significantly reduced in Tg mice than nTg mice. A notable reversal of the decreased phosphorylation of GSK-3 β , CREB, and Akt was observed in exercise, epicatechin, or COMA groups. There is a significant difference of phosphor-GSK-3 β 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 A β levels, while both single treatments failed to diminish A β deposition. In addition, the cognitive function was improved in the combined therapy group.

The occurrence of A β has been regarded as a mark of moderate- or mid-stage AD from about 6–12 months of age in APP/PS1 mice.^[22] At the endpoint of this study (at 12 months of age), in the measure of cognitive affections through the MWM test, there was a notable increment in escape latency for Tg mice, compared with their nTg, which means

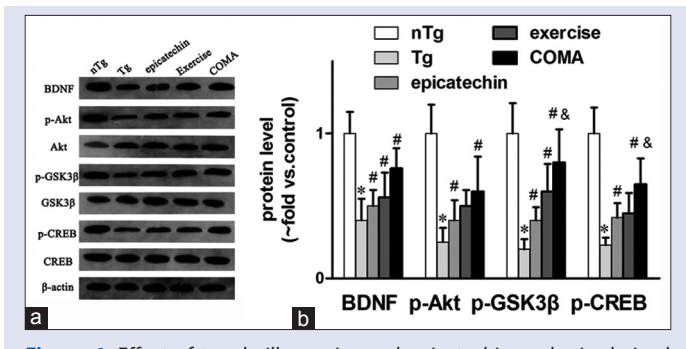


Figure 6: Effect of treadmill exercise and epicatechin on brain-derived neurotrophic factor levels and Akt/GSK-3/cAMP response element binding protein signaling pathway in the hippocampus. (a) Representative Western blot of protein expressions normalized to β -actin protein in the hippocampus. (b) Protein expressions were semi-quantified by densitometry analysis. Values are expressed as mean \pm standard error. * $P < 0.05$ compared with wild-type littermates; # $P < 0.05$ compared with transgenic; and & $P < 0.05$ compared with COMA

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.^[26] Cognitive deficits in these mice correlate with onset and progression of an AD-like pathology, associated with the aggregation of A β peptide, a primary cause of AD.^[27] As expected, immunohistochemistry results exhibited a surge of A β plaques in the hippocampus, paralleled with a significantly higher level of A β_{1-40} and A β_{1-42} 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.^[28] It is worth mentioning that not all exercise shows similar effects on learning and memory,^[29] 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.^[30] 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,^[31] NSE/APPsw mice,^[16] APP/PS1 mice, AD-like mice by intracerebroventricular (ICV) injection of A β_{25-35} ,^[32] and 3xTg-AD mice,^[33] 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-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 A β levels. There is evidence that when treadmill exercise started at 3 months of age, an obvious decline of soluble A β levels and A β deposition was observed in brains. At 17 months of age, when A β plaque has probably emerged in APP/PS1 mice, treadmill exercise beginning at this stage only brought down soluble A β levels, without prevention on A β deposition in the hippocampus.^[30] Consistently, in the current study, 4 months of combination therapy, which was initiated at 8 months of age, could reduce soluble A β levels in mid-stage APP/PS1 mice, but was not enough to reduce the A β plaque loading in the hippocampus of APP/PS1 mice. Interestingly, our findings described that long-term exercise could not lessen A β plaque, but decrease the levels of soluble

A β_{1-40} and A β_{1-42} in aged Tg mice, indicating that the decline of soluble A β 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 A β levels in 7 months old TASTPM mice.^[9] One study of chronic treatment of epicatechin diet at 3 months of age for 9 months reported that epicatechin significantly reduced total A β 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.^[11] In the present study, 4 months of treatment of epicatechin diet at 8 months of age exerted no obvious effect on A β 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.^[34] In AD patients, SOD and CAT activities were lower in both the central nervous system and peripheral tissues.^[35] 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 A β plaques.^[36] Epicatechin is a potent scavenger of lipid peroxidation products.^[37] A single oral dose of epicatechin (30 mg/kg) can block the oxidative damage of the hippocampus induce by A β_{25-35} injection.^[38] Epicatechin can channel the reactive oxygen to itself, undergo oxidation, and subsequently protect the reactive cysteines from oxidation by interacting with them.^[12] 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.^[39] 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 A β_{25-35} .^[40] 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.^[41] As the most studied protein kinase, Akt is capable of phosphorylating and inactivating GSK-3.^[42] A β 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. A β exposure could enhance the GSK-3-mediated inhibition of CREB phosphorylation, then afterward decreasing BDNF expression.^[43] The phosphorylation of GSK-3 β , CREB, and Akt were significantly reduced in Tg mice than nTg mice. In exercise, epicatechin or COMA groups, Akt/GSK-3 β /CREB signaling pathway was activated.

In summary, treadmill exercise and epicatechin in combination may represent a practical management strategy for AD. The study was initiated

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 A β levels, improving cognitive function, and activating of Akt/GSK-3 β /CREB signaling pathway. However, in order to apply these methods in humans, further studies are needed to determine the underlying mechanisms.

Financial support and sponsorship

Talents research start-up funding of Hainan Medical University (No. 2010012).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Kalra J, Khan A. Reducing A β load and tau phosphorylation: Emerging perspective for treating Alzheimer's disease. *Eur J Pharmacol* 2015;764:571-81.
- Qiu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer's disease: Occurrence, determinants, and strategies toward intervention. *Dialogues Clin Neurosci* 2009;11:111-28.
- Alva G, Cummings JL. Relative tolerability of Alzheimer's disease treatments. *Psychiatry (Edgmont)* 2008;5:27-36.
- 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.
- Nichol KE, Poon WW, Parachikova AI, Cribbs DH, Glabe CG, Cotman CW. Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. *J Neuroinflammation* 2008;5:13.
- Seo TB, Cho HS, Shin MS, Kim CJ, Ji ES, 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.
- 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.
- García-Mesa Y, Colie S, Corpas R, Cristófol R, Comellas F, Nebreda AR, *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.
- Cox CJ, Choudhry F, Peacey E, Perkinson MS, Richardson JC, Howlett DR, *et al.* Dietary (-)-epicatechin as a potent inhibitor of β gamma-secretase amyloid precursor protein processing. *Neurobiol Aging* 2015;36:178-87.
- Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, Pollard A, *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.
- Zeng YQ, Wang YJ, Zhou XF. Effects of (-) epicatechin on the pathology of APP/PS1 transgenic mice. *Front Neurol* 2014;5:69.
- George RC, Lew J, Graves DJ. Interaction of cinnamaldehyde and epicatechin with tau: Implications of beneficial effects in modulating Alzheimer's disease pathogenesis. *J Alzheimers Dis* 2013;36:21-40.
- Gomez-Pinilla F. Collaborative effects of diet and exercise on cognitive enhancement. *Nutr Health* 2011;20:165-9.
- Arcoverde C, Deslandes A, Moraes H, Almeida C, Araujo NB, Vasques PE, *et al.* Treadmill training as an augmentation treatment for Alzheimer's disease: A pilot randomized controlled study. *Arq Neuropsiquiatr* 2014;72:190-6.
- McGowan E, Sanders S, Iwatsubo T, Takeuchi A, Saido T, Zehr C, *et al.* Amyloid phenotype characterization of transgenic mice overexpressing both mutant amyloid precursor protein and mutant presenilin 1 transgenes. *Neurobiol Dis* 1999;6:231-44.
- Cho JY, Um HS, Kang EB, Cho IH, Kim CH, Cho JS, *et al.* The combination of exercise training and alpha-lipoic acid treatment has therapeutic effects on the pathogenic phenotypes of Alzheimer's disease in NSE/APPsw-transgenic mice. *Int J Mol Med* 2010;25:337-46.
- Kim HB, Jang MH, Shin MC, Lim BV, Kim YP, Kim KJ, *et al.* Treadmill exercise increases cell proliferation in dentate gyrus of rats with streptozotocin-induced diabetes. *J Diabetes Complications* 2003;17:29-33.
- Li XH, Lv BL, Xie JZ, Liu J, Zhou XW, Wang JZ. AGEs induce Alzheimer-like tau pathology and memory deficit via RAGE-mediated GSK-3 activation. *Neurobiol Aging* 2012;33:1400-10.
- Guo C, Wang T, Zheng W, Shan ZY, Teng WP, Wang ZY. Intranasal deferoxamine reverses iron-induced memory deficits and inhibits amyloidogenic APP processing in a transgenic mouse model of Alzheimer's disease. *Neurobiol Aging* 2013;34:562-75.
- Chakrabarty P, Jansen-West K, Beccard A, Ceballos-Diaz C, Levites Y, Verbeeck C, *et al.* Massive gliosis induced by interleukin-6 suppresses Abeta deposition *in vivo*: Evidence against inflammation as a driving force for amyloid deposition. *FASEB J* 2010;24:548-59.
- Song J, Zhong R, Huang H, Zhang Z, Ding D, Yan H, *et al.* Combined treatment with *Epimedium koreanum* Nakai extract and gefitinib overcomes drug resistance caused by T790M mutation in non-small cell lung cancer cells. *Nutr Cancer* 2014;66:682-9.
- Xu ZQ, Zhang LQ, Wang Q, Marshall C, Xiao N, Gao JY, *et al.* Aerobic exercise combined with antioxidative treatment does not counteract moderate- or mid-stage Alzheimer-like pathophysiology of APP/PS1 mice. *CNS Neurosci Ther* 2013;19:795-803.
- Bero AW, Yan P, Roh JH, Cirrito JR, Stewart FR, Raichle ME, *et al.* Neuronal activity regulates the regional vulnerability to amyloid- β deposition. *Nat Neurosci* 2011;14:750-6.
- Hoppe JB, Coradini K, Frozza RL, Oliveira CM, Meneghetti AB, Bernardi A, *et al.* Free and nanoencapsulated curcumin suppress β -amyloid-induced cognitive impairments in rats: Involvement of BDNF and Akt/GSK-3 β signaling pathway. *Neurobiol Learn Mem* 2013;106:134-44.
- Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, *et al.* Age-dependent accumulation of soluble amyloid beta (A β) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP (alpha)) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3 β pathway in Alzheimer mouse model. *J Biol Chem* 2011;286:18414-25.
- 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.
- 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.
- Akbaraly TN, Portet F, Fustini 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;73:854-61.
- Lin TW, Chen SJ, Huang TY, Chang CY, Chuang JI, Wu FS, *et al.* Different types of exercise induce differential effects on neuronal adaptations and memory performance. *Neurobiol Learn Mem* 2012;97:140-7.
- 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;298:357-66.
- 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.
- 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.
- García-Mesa Y, López-Ramos JC, Giménez-Llort L, Revilla S, Guerra R, Gruart A, *et al.* Physical exercise protects against Alzheimer's disease in 3xTg-AD mice. *J Alzheimers Dis* 2011;24:421-54.
- Sultana R, Mecocci P, Mangialasche F, Cecchetti 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 dementing disorder. *J Alzheimers Dis* 2011;24:77-84.
- Chen G, Tang J, Ni Z, Chen Q, Li Z, Yang W, *et al.* Antiasthmatic effects of resveratrol in ovalbumin-induced asthma model mice involved in the upregulation of PTEN. *Biol Pharm Bull* 2015;38:507-13.
- Tung BT, Rodríguez-Bies E, Talero E, Gamero-Estévez E, Motilva V, Navas P, *et al.* Anti-inflammatory effect of resveratrol in old mice liver. *Exp Gerontol* 2015;64:1-7.
- Zhu Q, Zheng ZP, Cheng KW, Wu JJ, Zhang S, Tang YS, *et al.* Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chem Res Toxicol* 2009;22:1721-7.
- 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-beta 25-35 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 neurotrophic 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.



Houcai Huang

ABOUT AUTHOR

Houcai Huang is a Professor in Jiangsu Provincial Academy of Chinese Medicine, Nanjing, China. His research focuses on the area of screening bioactive components from the Chinese medicine against Alzheimer's disease, as well as exploring anti-aging mechanism of *Uncaria rhynchophylla*. In addition, he has done a lot of profound work in establishing animal models. He is an expert panel member in Jiangsu province experimental animal association and a quality inspector in Jiangsu province with respect to experimental animals.