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Protective effect of sesamol on cognitive impairment in *APP/PS1* mice

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ABSTRACT

To explore the effect of sesamol on the cognition of *APP/PS1* mice, 8-week-old *APP/PS1* and wild-type male mice were divided into AD model group, AD + sesamol (50 mg kg^{-1} bw) group, and Control group. Sesamol was orally administered once a day for 5 months. Morris water maze was used to evaluate the learning and memory ability of mice. The number of synapses in the hippocampal neurons was detected by Golgi staining. Nissl staining was used to observe the changes of Nissl bodies in CA1 and CA3 regions of the hippocampus. Western blotting was used to detect the expression of Aβ, SIRT1, BDNF, and p-CREB/CREB in the hippocampus and cortex. Compared with the model group, sesamol decreased the latency

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period of *APP/PS1* mice ($P < 0.05$) and increased the total number of neuronal dendritic spines in the hippocampal CA3 region, as well as increased the number of Nissl bodies ($P < 0.05$). Western blotting results showed that sesamol significantly reduced Aβ protein expression in the hippocampus and cortex, increased SIRT1 expression in the cortex, and increased BDNF expression in the hippocampus ($P < 0.05$). Sesamol improved the learning and memory abilities of *APP/PS1* mice probably through increasing the density of neuronal dendritic spines and upregulating the levels of SIRT1 and BDNF.

KEYWORDS

sesamol, Alzheimer's disease, cognition, SIRT1, BDNF

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterised by cognitive, memory, personality, and psychiatric disturbances that appear as the disease progresses (Alzeimer's Disease International, 2018). Alzheimer's Disease International (ADI) estimated a dementia prevalence of about 50 million people worldwide, projected to triple in 2050. The main pathological features of AD are the accumulation of β-amyloid protein outside the brain nerve cells, accompanied by synaptic damage, mass loss of neurons, and apoptosis. Currently, drugs to eliminate symptoms of AD may lead to high treatment costs and adverse effects (Yu et al., 2020). Thus, it is important to find alternative safe and effective interventions and treatments for AD.

Recent years have witnessed a growing academic interest in the active ingredients in natural compounds. Sesamol is a phenolic compound extracted from sesame, with strong lipophilicity and various biological activities such as anti-inflammatory, antioxidant, and neurotrophic (Kuhad and Chopra, 2008). Studies have shown that sesamol can protect the synaptic ultrastructure in the brain (Liu et al., 2021), increase the level of brain derived neurotrophic factor (BDNF) (Liu et al., 2017b), decrease Amyloid precursor protein (APP) and the overexpression of β-secretase 1 (BACE1) through blood-brain barrier, decrease Aβ formation in the brain, decrease neuronal damage and improve the spatial memory in AD mice (Liu et al., 2017a).

Sirtuin protein family is a nicotinamide adenine dinucleotide-dependent class III histone deacetylase. Among them, sirtuin1 (SIRT1) has been widely studied and found to have definite neuroprotective effects in a variety of neurodegenerative diseases such as AD, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. It is closely related to the pathogenesis of cognitive impairment (Khan et al., 2016; Wang et al., 2016, 2020; Gong et al., 2020). The Camp-response element binding protein (CREB)-SIRT1 signalling axis has a coordinated role in the pathogenesis of cognitive impairment in AD (Zhao et al., 2019). CREB is one of the key signalling molecules involved in learning and memory. It can activate the downstream BDNF signalling molecule, thereby regulates learning and memory. Targeted up-regulation of BDNF expression may prevent or treat learning and memory impairment (Belgacem and Borodinsky, 2017).

The purpose of this study was to explore the ameliorating effect of sesamol on cognitive impairment and to determine whether sesamol could improve cognitive function in *APP/PS1* mice by regulating the SIRT1/CREB/BDNF signalling pathway.

2. MATERIALS AND METHODS

2.1. Animals and treatment

C57BL/6J male mice (8 weeks old) and *APP/PS1* male mice were purchased from the Hubei Center for Disease Control and Prevention (Wuhan, China). Animals were housed in rectangular cages in a controlled atmosphere with a 12 h light/dark cycle, and normal chow food was provided *ad libitum*. After two weeks adaptation, a total of 60 mice were randomly divided into 3 groups ($n = 20$ mice per group): (1) Control group: wild type mice. (2) AD group: *APP/PS1* mice. (3) AD + Sesamol: *APP/PS1* mice. AD + Sesamol group received sesamol by gavage daily (50 mg kg^{-1} body weight, Aladdin, S106853) and Control group and AD group were given an equal volume of distilled water by gavage daily (Fig. 1A). All experimental procedures followed the Guide for the Care and Use of Laboratory Animals: Eighth Edition, ISBN-10:0-309-15396-4, and the animal protocol was approved by the animal ethics committee of Wuhan University of Science and Technology. All surgeries were performed under anaesthesia and efforts were made to minimise suffering.

2.2. Behavioural tests

After treatment for five months, the Morris water maze test was used to analyse spatial learning and memory (Bromley-Brits et al., 2011). The mice were allowed to receive 3 training periods per day for 5 consecutive days. The indicators of each group were recorded. On day 6, probe test was performed. The residence time in the target quadrant and the number of times crossing the original platform were recorded. The spatial positioning ability of the tested mice was observed. All data was recorded *via* visual tracking system (SuperMaze software, Shanghai Xinruan Information Technology Co., Ltd, China).

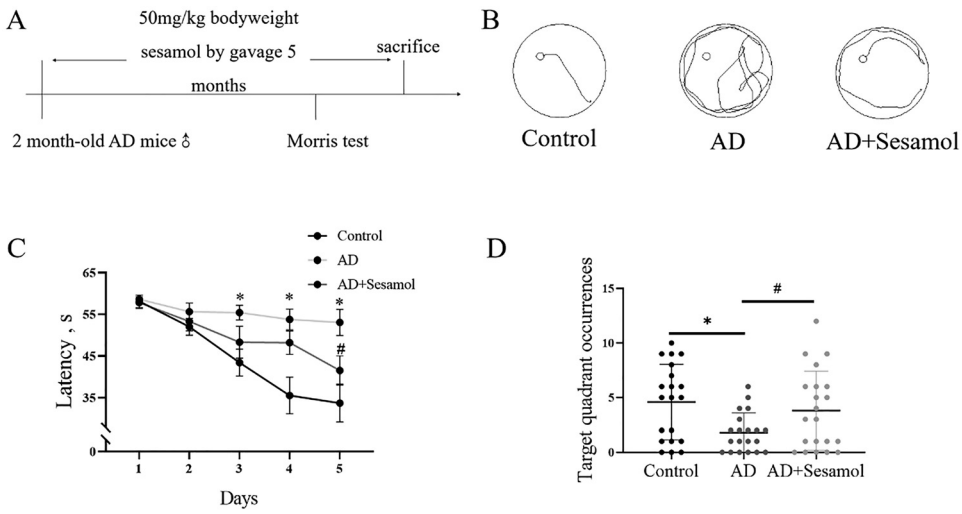

Fig. 1. (A) Schedule of animal treatment and behavioural tests. (B) Representative swim paths for each treatment group in the spatial navigation trial test. (C) Latency times in the spatial navigation test. (D) Number of target quadrant occurrences during spatial detection trials. Data are expressed as mean ± SD, $n = 20$. $^{*}P < 0.05$ compared with Control group. $^{\#}P < 0.05$ compared with AD group

2.3. Golgi staining and Nissl staining

The Golgi solution (5% potassium dichromate + 5% mercury ascending + 5% potassium chromate) was prepared 5 days in advance, and the animals were anesthetised and perfused with 0.9% saline until the blood was eliminated, and the hippocampus was taken and added to the Golgi solution and left to rest for 14 days away from light. After that, the brain tissue was transferred into 30% sucrose solution until the brain tissue sank to the bottom and then cut into 200–300 μm brain slices from the coronal using a vibrating microtome. The brain slices were transferred to gelatine-coated glass slices and stained by the following sessions: distilled water for 1 min, concentrated ammoniacal liquor for 30 min, distilled water for 1 dish, Kodak fixative for 30 min, distilled water for 1 min, 50%–100% ethanol gradient for 6 times, CXA (1,000 mL chloroform + 1,000 mL xylene + 1,000 mL ethanol) solution for 15 min, and then neutral resin for sealing the slices for observation. The images were observed by inverted microscope (Olympus IX71, Japan) and analysed by ImageJ software.

For the Nissl staining procedure, the slices were dewaxed with xylene and washed with ethanol. First, the slices were immersed in xylene I for 15 min, xylene II for 15 min, 100–70% ethanol gradient for 3 times. They were then washed in ultra-pure water three times, immersed in 1% toluidine blue for 40 min in incubator at 60 °C, and then washed with ultra-pure water three additional times. Next, they were immersed in 70–100% ethanol gradient for 3 times, 100% chloroform for 5 s, a differentiation agent for approximately 15 s, 95% ethanol for 30 s, 100% ethanol for 60 s, 100% ethanol for 60 s, and xylene for 5 min (twice). Finally, the slices were immersed in xylene for 2 h prior to being sealed with neutral gum.

2.4. Western blotting

The hippocampus and cortex proteins were lysed with the RIPA lysis buffer (Applygen, C1053). The BCA protein assay kit (Applygen, P1511-1) was used to quantify the total protein. The extracts with equal quantities were subjected to SDS-PAGE and transferred onto PVDF membranes. After blocking in 5% milk for 1 h at 37 °C, the membranes were incubated at 4 °C overnight with the following primary antibodies: CREB (Proteintech, 12208-1-AP), pCREB (Proteintech, 9198S), SIRT1 (Millipore, #07-1596), BDNF (Proteintech, 25699-1-AP), and Aβ (Santa Cruz Biotechnology, #sc-28365). Subsequently, the membranes were incubated with secondary antibodies at room temperature for 1 h. The protein levels were visualised using a Gel-Pro system (Tanon Technologies, Shanghai, China), and the band intensities were quantified with ImageJ software.

2.5. Data analysis

Data were analysed by SPSS 21. All data were reported as means ± SD. Statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

To investigate the effects of sesamol on AD mice memory impairments, the Morris water maze test was performed. Swimming paths for each group were present in the spatial navigation trial test (Fig. 1B). In the hidden platform test, there was a remarkable difference in the escape latency

time of different groups ($P < 0.05$) (Fig. 1C). Compared with Control group, mice in the AD group took longer to find the platform ($P < 0.05$). Compared with AD group, Sesamol group showed a significant decrease in escape latency time on day 5 ($P < 0.05$). During the spatial probe trial test, compared with Control group, AD group spent less time in the target quadrant and crossing the target platform, and Sesamol group crossed over the platform area more times than AD group ($P < 0.05$) (Fig. 1D), which showed sesamol improved memory and retention in AD mice. Recently, the neuroprotective effect of sesamol has been paid more and more attention. Sesamol plays a role in reducing the levels of inflammatory mediators such as TNF-α and IL-1β (Liu et al., 2017a), aging-induced oxidative stress by inhibiting malondialdehyde production, and increasing antioxidant enzymes. It was found that senile plaques appeared in the cortex and cognitive function decreased in Double transgenic *APP/PS1* mice from the age of 6 months (Aso et al., 2012). Previous studies have shown that in the Morris water maze test, sesamol restored lipopolysaccharide elicited deterioration of spatial learning abilities (Liu et al., 2017a) and reduced the oxidative stress-induced memory impairment (Ren et al., 2018).

In order to clarify the basis of cellular morphological changes of cognitive impairment in mice, Golgi staining was used to observe the changes of neuronal processes and dendritic spines in the hippocampus. The results showed that compared with the Control group, the density of neuronal dendritic spines in AD group decreased significantly, while for Sesamol group it increased significantly compared with AD group (Fig. 2A). Nissl staining was used to further

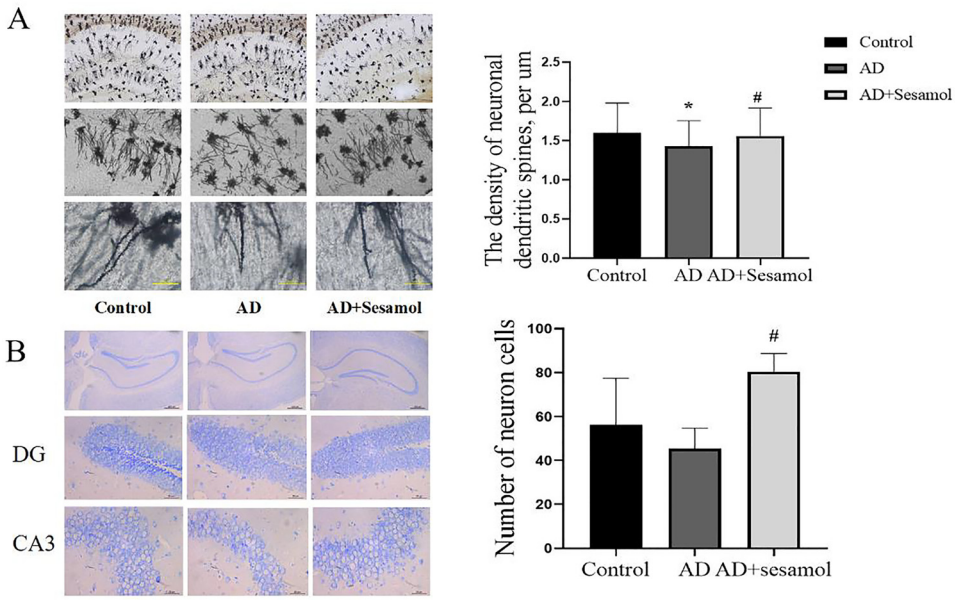

Fig. 2. (A) Representative micrographs of Golgi staining (left) and the density of neuronal dendritic spines (right). (B) Representative micrographs of Nissl staining (left) in the hippocampal and the number of neuron cells of hippocampal CA3 region (right). Data are expressed as mean ± SD, $n = 3$. $^{*}P < 0.05$ compared with Control group. $^{\#}P < 0.05$ compared with AD group

assess whether sesamol had the protective effects on neurons of the hippocampus in AD mice. As shown in Fig. 2B, the number of neurons in the hippocampal CA3 area of the Control group was higher, while the number of neurons in AD group decreased. However, sesamol increased the number of Nissl bodies in AD mice. Previous studies have shown that the cytoplasm, prominent nucleus, and nucleolus of neurons in the sesamol intervention group were well-preserved (Ren et al., 2020). Learning and memory are coordinated by dendritic spines, and the more dendritic spines, the better the learning and memory ability. The water maze results are consistent with this.

The pathogenesis of Alzheimer's disease is complex, mainly related to hyperphosphorylated Tau protein, abnormal deposition of Aβ, and genetic variants (Koutsodendris et al., 2022). Among them, the beta-amyloid cascade hypothesis is dominant. As an important component of sesame oil aroma, sesamol has neuroprotective effect *in vivo* and *in vitro* (Zhang et al., 2021). Extensive studies on sesamol have shown that it has a wide range of therapeutic properties, especially in cancer treatment with a promising therapeutic effect (Majdalawieh and Mansour, 2019). Sesamol is a potent anti-inflammatory and oxidative stress agent inhibiting NF-κB translocation into the nucleus, decreasing MAPK activation, upregulating Nrf2 signalling, and ameliorating inflammation and oxidative damage (Wu et al., 2015), thereby inhibiting tumour development. In addition, sesamol also has a certain therapeutic effect on other diseases. For example, sesamol can regulate the development of some neurodegenerative diseases through oxidative stress in a mouse model (Liu et al., 2021; Zhang et al., 2021).

To explore the molecular changes in sesamol-induced improvement of learning and memory ability, we performed western blotting experiments. The result indicated that sesamol can effectively reduce the expression of Aβ protein in the hippocampus and cortex (Fig. 3C). The

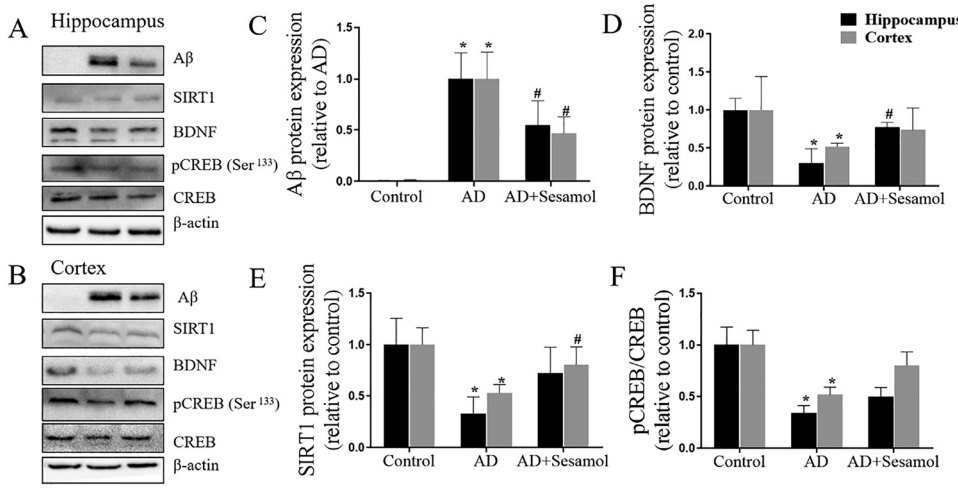

Fig. 3. (A) (B) Western blot analysis of Aβ, SIRT1, BDNF, p-CREB, CREB, and β-actin in hippocampus and cortex, respectively. (C) Densitometric analysis of Aβ. (D) Densitometric analysis of SIRT1. (E) Densitometric analysis of BDNF. (F) Densitometric analysis of p-CREB/CREB. Data are shown as mean ± SD, $n = 4$. $^{*}P < 0.05$ compared with Control group. $^{\#}P < 0.05$ compared with AD group

SIRT1 protein level in cortex was significantly up-regulated in Sesamol group compared with AD group (Fig. 3D). The BDNF protein level in the hippocampus was remarkably raised in Sesamol group compared with AD group (Fig. 3E). The phosphorylation level of p-CREB/CREB in hippocampus and cortex of AD group was significantly lower than of Control group (Fig. 3F). Abnormal anabolism of Aβ, decreased catabolism level, and transport disorder are the main causes of Aβ deposition in the brain (Ren et al., 2018). Amounting studies showed that sirtuins family is associated with cognitive dysfunction (Satoh et al., 2017) and is critically involved in the pathogenesis of cognitive impairment. SIRT1 is involved in biological processes such as neuroprotection, oxidative stress, inflammatory response, autophagy, and cellular senescence through deacetylation (Cui et al., 2022). Decreased expression of SIRT1 in brain tissue can lead to cognitive dysfunction (Papagno and Trojano, 2018). BDNF can regulate the plasticity of neurons and synapses, has great significance for the proliferation and repair of central neurons, and promote the formation of learning and memory (El Hayek et al., 2019). The synthesis and transcription of BDNF depend on the expression of CREB, and the phosphorylation of CREB can promote the expression of BDNF. BDNF and CREB, two important molecules, form a self-regulated positive feedback loop (Esvald et al., 2020). Sesamol reduced the expression of Aβ protein and ameliorated cognitive impairment in AD mice *via* the SIRT1/CREB/BDNF signalling pathway. However, the relationship between Aβ protein expression and SIRT1/CREB/BDNF signalling pathway need to be further investigated.

4. CONCLUSIONS

Our data show that sesamol could significantly improve the cognitive decline of spatial learning during the progression of AD and improve neuronal function through the SIRT1/CREB/BDNF signalling pathway. This provides evidence that sesamol may improve cognitive levels in AD mice and may provide important strategies for the future development of natural compound drugs for AD.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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