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Original Research Article

Effect of *Evodia rutaecarpa* (Juss) Benth extract on Alzheimer disease in mice

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Abstract

Purpose: *To investigate the protective effect of Evodia rutaecarpa (Juss.) Benth. extract (ERBE) against Alzheimer's disease in 3xTg-AD mice.*
Methods: *The cognitive function of 3xTg-AD mice was assessed using Morris water maze test. The levels of amyloid beta deposits and NeuN in the mouse hippocampus were evaluated by immunohistochemistry. Brain neurotrophic derived factor (BDNF) and tyrosine kinase B (TrkB) expressions were determined by western blot analysis.*
Results: *ERBE treatment significantly ameliorated learning and memory deficits in AD mice, as shown by increased time spent in the target zone during probe tests. The escape latency in the animals treated with 400 mg/kg ERBE (20.5 ± 1.3 s) was significantly higher than untreated 3xTg-AD mice (12.4 ± 1.3 s, $p < 0.01$). In addition, ERBE significantly decreased Aβ deposits, increased NeuN-positive cells, and upregulated the expressions of BDNF (1.4 ± 0.2, $p < 0.05$) and TrkB (1.1 ± 0.2, $p < 0.05$) in 3xTg AD mice.*
Conclusion: *The results suggest that ERBE administration may be a useful strategy for treating memory impairment induced by several neurodegenerative diseases.*

Keywords: *Evodia rutaecarpa, Alzheimer, Memory impairment, NeuN-positive cells*

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly and accounts for between 50 and 75 % of all cases. By 2030 it is estimated that more than 65 million people will be living with dementia, with projections almost doubling every 20 years. Alzheimer's disease is a progressive neurodegenerative disorder characterized, at least in part, by abnormal accumulation of β-amyloid peptide (Aβ) in the brain [1]. The accumulated Aβ is believed to play an important role in the pathogenesis of AD [2]. Thus, Aβ continues to be an important target for prevention and treatment of AD [3].

Alzheimer's disease is the most common form of dementia relating to memory and cognitive decline. Alzheimer's disease is a progressive neurodegenerative disease in which dementia

symptoms gradually worsen over a number of years [4]. The classical biochemical hallmarks of AD include the accumulation of amyloid beta (Aβ) peptide oligomers and soluble hyperphosphorylated tau [5]. Brain-derived neurotrophic factor (BDNF) is a small dimeric protein, which acts through high affinity binding with its receptor, tyrosine kinase B (TrkB). Brain-derived neurotrophic factor (BDNF) modulates neuronal growth and survival, and BDNF is implicated in learning and memory processes; therefore, dysfunction in BDNF is accompanied by cognitive deficits. In particular, BDNF is involved in the AD-related decline in neurogenesis, and levels of nerve growth factor are also diminished with AD [6].

Medical treatment for AD patients is placing an increasing burden on physicians and families every year. Clinically, there are a variety of drugs available for AD, such as cholinesterase inhibitors, glutamate receptor antagonists, and free radical scavengers. However, these drugs do not closely target the pathogenesis of the disease and have numerous side effects [7]. Therefore, it is extremely important to elucidate the mechanism of AD pathophysiology to find a new drug for treatment.

Evodia rutaecarpa (Juss.) Benth., has been used in China in the treatment of various disorders, including stress-induced physiological changes, inflammation, hypertension, and cancer. *Evodia rutaecarpa (Juss.) Benth.* extract has been reported to have anti-oxidant [8], immunomodulatory [9], and anti-mutagenic activities [10]. In this study, we investigated to evaluate the neuroprotective effects of ERBE on learning and memory deficits in a triple-transgenic mouse model of Alzheimer's disease (3xTg-AD), which expresses APP_{Swe}, $PS1_{M146V}$, and tau_{P301L} [11].

EXPERIMENTAL

Plant material

The plant material, *Evodia rutaecarpa (Juss.) Benth* were collected from Changde City, Hunan Province in China in October 2018. Taxonomic identification of the plant was performed by Professor Lei Fu of Wuhan University, China. A voucher specimen (no. ERBE 201803007) was deposited in College of Pharmacy, Wuhan University, China for future reference.

The herbal samples *Evodia rutaecarpa (Juss.) Benth.* was dried in an oven. The abstract ERBE was obtained by steeping the dried *Evodia rutaecarpa (Juss.) Benth.* in water at 60 ℃ three times, each for one hour before first drying in an oven and then freeze-drying the ERBE thus obtained. The powder (1 g) was equivalent to about 1.7 g crude samples and the yield was 56.34 %.

Animals

Alzheimer's disease (3xTg-AD) mice carrying a mutant APP (KM670/671NL), a human mutant PS1 (M146V) knock-in, and tau (P301L) transgenes (B6; 129-*Psen1*^{tm1Mpm} Tg (APPSwe, tauP301L) 1Lfa/J) were purchased from the Animal Research Institute, Nanjing University (Nanjing, China). The non-transgenic littermates were used as wild type (WT) controls. All animals were kept in a pathogen-free environment on a 12 h light/12 h dark cycle and had access to feed and water *ad libitum*.

Animal groups

The mice were randomly divided into four groups (n = 8) as follows: (1) saline treated WT group (WT, n = 8); (2) saline-treated 3xTg group (3xTg, n = 8); (3) 200 mg/kg ERBE-treated 3xTg group (3xTgþ + ERBE 200, n = 8) and (4) 400 mg/kg ERBE-treated 3xTg group (3xTgþ + ERBE 400, n = 8). Starting at 3 months of age, mice received PBS and ERBE once a week for 3 months until they were 6 months old. Drugs were dissolved in water, and administered using a 5-ml syringe with a 2-cm long gavage needle through the mouth to the mouth once daily for 3 weeks. The animal experiment was approved by the Animal Care and Use Committee of Wuhan University (approval ref no. 20100405) and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [12].

Water maze test

A modified version of the water maze procedure described by Morris was used to test each mouse's cognitive function [13]. The water maze was a circular pool 0.9 m in diameter and constructed fiberglass. Water in the pool was maintained at 22 ± 2 ℃ and mixed with 1 kg of powdered skim milk to make the water opaque. During testing in the water maze, a platform (6 cm in diameter) was fixed 1 cm below the surface of the water at identical location within the pool. The pool was surrounded by differing extra-maze cues. All mice were subjected to four trials per day at intervals of 15 min for four consecutive days. The proportion of time spent searching for the platform in the training quadrant, i.e, the previous location of the

platform, was used as a measure of memory retention.

Western blotting

At the end of the experiment, the mice were decapitated and the brains were rapidly removed and placed on ice. The hippocampus was quickly dissected by a scalpel and stored at -80 ºC fridge until use. The hippocampal tissue was homogenized in PRO-PREPTM Protein Extraction Solution (Shanghai Shengong, Shanghai, China). The homogenate was subsequently centrifuged at 12,000 g for 10 min at 4 ℃, and the supernatant was collected for protein concentration determination using a protein assay (Bio-Rad, Hercules, CA, USA). Protein samples (30 μg) were separated on a sodium dodecyl sulfate-polyacryl-amide gel and transferred onto a nitrocellulose membrane.

The membrane was incubated with 5 % skim milk in Tris-buffered saline containing 0.1 % Tween-20 and then incubated overnight at 4 ℃ with the following primary antibodies: mouse β-actin antibody (1 : 1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit BDNF antibody (1 : 500; Santa Cruz Biotechnology), and rabbit TrkB antibody (1 : 1000; Santa Cruz Biotechnology). Subsequently, membranes were incubated for 1 h with secondary antibodies (1 : 2000; Cell Signaling), and detection was performed using the enhanced chemiluminescence (ECL) detection kit.

Densitometry analysis

Coronal sections of the hippocampus were examined from the rostral anteroposterior (- 2.1 mm) to the anteroposterior (- 4.5 mm) direction, as defined by the bregma of the brain atlas. Images were obtained at ×10 magnification using the IMAGE PRO PLUS System (version 4.0; Media Cybernetics, Silver Spring, MD, USA) on a computer attached to a light microscope (Zeiss Axioskop, Oberkochen, Germany), which interfaced with a charge-coupled device video camera (Kodak Mega Plus model 1.4 I). To determine the density of the Aβ-immunoreactive staining in the hippocampus, a square frame of 500 × 500 μm^2 was placed in the dorsal part of the hippocampus. A second square frame of 200 × 200 μm^2 was placed in the corpus callosum to measure the background. As previously described, variations in background illumination were controlled by subtracting the average background density of the corpus callosum from the average density of the hippocampus in each section analyzed [14].

Statistical analysis

Data are presented as mean ± standard deviation (SD). The results were analyzed statistically with one-way ANOVA followed by Tukey's multiple comparison using SPSS 16.0 software for Windows. Differences were considered statistically significant at $p < 0.05$.

RESULTS

ERBE reverse spatial learning deficits in 3xTgAD mice

To examine spatial memory, the performance of animals in the probe trial was compared to the time animals spent swimming to the platform. All animals were examined by a retention test that involved removing the platform from the pool on the fourth trial day. From Table 1, the escape latency in animals treated with 400 mg/kg ERBE was significantly increased when compared to the 3xTg-AD mice.

3xTg-AD mice displayed severely impaired spatial cognition in the water maze test, when compared with the WT controls, and the administration of ERBE ameliorated these learning and memory deficits.

Table 1: Effect of ERBE on behavior in a Morris water maze (n = 6)

Group	Escape latency (s)
Control	23.6 ± 1.6
3xTg	12.4 ± 1.3**
ERBE-L	13.9 ± 0.9
ERBE-H	20.5 ± 1.3##

Escape latency was determined using the hidden platform test. Significance was determined by Student's t test (**$p < 0.01$, compared with the control group; ##$p < 0.01$, compared with the 3xTg group)

Effect of ERBE on Aβ pathology in 3xTg-AD mice

In 3xTg-AD mice, Aβ is present in the hippocampus at 6 months of age. Therefore, to investigate the link between neurogenesis and the development of AD pathology, we assessed the Aβ burden in brains from 3xTg-AD mice that began receiving ERBE treatments 3 month prior to 6 months of age. Aβ deposits in the CA1 region of the hippocampus were significantly increased in 6-month-old 3xTg animals compared to the age-matched WT group ($p < 0.01$). In the CA3 region of the hippocampus, Aβ deposits were significantly increased in the 3xTg group ($p < 0.001$) compared to the WT group ($p < 0.01$). Compared with the 3xTg group, Aβ

deposits in the CA1 region were significantly decreased by ERBE of 400 mg treatment ($p < 0.05$). In addition, compared with the 3xTg group, Aβ deposition in the CA3 was significantly decreased by ERBE 200 mg treatment ($p < 0.05$). However, there were no significant differences between the 3xTg group and the 3xTgþ ERBE (400 mg/kg) groups (Table 2).

Table 2: Effect of ERBE on the deposition of Aβ in the hippocampus (n = 6)

Group	Amyloid beta deposits in CA1 (% of 3xTg)	Amyloid beta deposits in CA3 (% of 3xTg)
control	51.5 ± 2.2	71.7 ± 1.5
3xTg	103.7 ± 3.4**	102.8 ± 2.3**
ERBE-L	92.3 ± 2.5	89.2 ± 2.1
ERBE-H	75.8 ± 2.1#	77.4 ± 1.7#

Data are shown as mean ± SEM; **$p < 0.01$, when compared with control mice; #$p < 0.05$ compared with 3xTg group (one-way ANOVA and Tukey's post-hoc tests)

Effect of ERBE on expressions of BDNF and TrkB and in mouse hippocampus

The results are shown in Table 3. The expression of hippocampal BDNF and rkB of 3xTg mice treatment with saline was significantly lower compared with that of WT mice ($p < 0.05$). It was also demonstrated that ERBE treatment significantly increased the expression of hippocampal BDNF and TrkB. These results show that the induction of AD reduced BDNF and TrkB expressions in the hippocampus, whereas, ERBE treatment enhanced BDNF and TrkB expressions in the hippocampus of the 3xTg mice.

Table 3: Effect of ERBE on BDNF and TrkB expressions in the hippocampus

Group	TrkB/beta action ratio	BDNF/beta action ratio
Control	1.3 ± 0.3	1.2 ± 0.3
3xTg	0.7 ± 0.1*	0.8 ± 0.2*
ERBE-L	0.9 ± 0.3	0.9 ± 0.2
ERBE-H	1.1 ± 0.2#	1.4 ± 0.2#

Asterisk indicate significant difference at *$p < 0.05$, compared to control mice; #$p < 0.05$, compared to 3xTg mice (one-way ANOVA and Tukey's post-hoc tests)

DISCUSSION

The present study demonstrated that ERBE increased spatial learning, memory abilities, and the expression of hippocampal BDNF and TrkB in 3xTg-AD mice. In this study, we chose to use the 3xTg-AD mice, a model derived from $APPS_{we}$, $PS1_{M146V}$, and tau_{P301L} transgenes. The 3xTg-AD mice develop a progressive, age-related neuropathological phenotype that includes plaque and tangle pathologies. These hallmark lesions are limited mainly to the hippocampus, amygdala, and cerebral cortex – the brain structures most impacted by AD pathology [15].

Cognitive impairment has been detected in 2-month-old 3xTg-AD mice [16], and Aβ deposits in the hippocampus and cortex have been found in 6-month-old 3xTg-AD mice [17-19]. These findings indicate that the pathological features that imitate AD in 3xTgAD mice remain stable. Classic symptoms of AD include problems with spatial learning and memory deficits. This study demonstrated that treatment with ERBE resulted in a significant restoration of spatial learning and memory function in AD mice. These results suggest that ERBE treatment may be effective in ameliorating cognitive impairment caused by AD.

Neuron-specific nuclear antigen (NeuN) is a neuronal-specific nuclear protein [20,21]. The expression of NeuN is observed in most neuronal cell types throughout the nervous system, with the exception of some neuronal populations that are NeuN-negative, but does not stain non-neuronal cells [22]. NeuN is a soluble nuclear protein that is localized to the cell nucleus and in the neuronal cytoplasm of postmitotic neurons. Within the hippocampus, NeuN can be used as a marker of postmitotic cells and labels both “normal” post-mitotic neurons and newly generated post-mitotic neurons. BDNF plays pivotal roles in learning, memory, and neuronal plasticity.

The levels of BDNF, and its main receptor TrkB, have been reported to lessen in AD. It was hypothesized that BDNF and its receptor may be involved in the protective role of ERBE against memory impairment.

The results of the present study have demonstrated that ERBE intake significantly increases the expression of BDNF and its main receptor TrkB, in the brain, which is in agreement with the hypothesis. There is evidence to support the results. It has been demonstrated that BDNF and TrkB are capable of protecting against memory impairment and regulate neurogenesis in the hippocampus of AD. A recent study also supports the role of BDNF signaling through TrkB in the pathophysiology and cognitive and its receptor involving ERBE in AD [23].

CONCLUSION

The findings of this study reveal that ERBE attenuates learning and memory deficits in 3xTg-AD mice. Thus, the herbal extract can potentially be developed as an alternative therapeutic agent for the management of AD.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ang Cai and Liu Xiao equally contributed to this work, and they are co-first authors.

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