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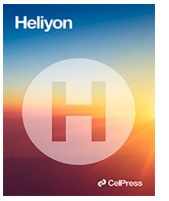

The study of therapeutic efficacy and mechanisms of *Schisandra chinensis* and *Evodia rutaecarpa* combined treatment in a rat model of Alzheimer's disease☆

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ABSTRACT

Schisandra chinensis and *Evodia rutaecarpa* are traditional Chinese herbs used to treat neurodegenerative diseases. This study investigates the combined effects of SC and ER on learning and memory in an Alzheimer's disease rat model and their underlying mechanisms.

Methods: High-performance liquid chromatography was employed to analyze the primary active constituents of Schisandra and Evodia. The effects of the combined treatment of Schisandra and Evodia on learning and memory in an Alzheimer's disease rat model were evaluated through Morris water maze and Hematoxylin-Eosin staining experiments. Immunohistochemical analysis was conducted to investigate the impact of S-E on $A\beta_{1\text{-}42}$ and P-tau proteins. Western blotting and real-time quantitative polymerase chain reaction were utilized to quantify the expression of pivotal proteins and genes within the BDNF/TRKB/CREB and GSK-3β/Tau pathways.

Results: The treatment group exhibited significant neuroprotective effects, ameliorating learning and memory impairments in the Alzheimer's disease rat model. The treatment regimen modulated the activity of the BDNF/TRKB/CREB and GSK-3β/Tau pathways by influencing the expression of relevant genes, thereby reducing the generation of $A\beta_{1\text{-}42}$ and P-Tau proteins and inhibiting the deposition of senile plaques. Furthermore, among the three treatment groups, the combined treatment demonstrated notably superior therapeutic effects on Alzheimer's disease compared to the single-drug treatment groups.

Conclusion: Compared with Schisandra or Evodia alone, the combination of the two herbs can more significantly modulate BDNF/TRKB/CREB and GSK-3β/Tau pathway activity, inhibit the production of $A\beta_{1\text{-}42}$ and P-Tau proteins, reduce the production of age spots and thus exert a stronger neuroprotective effect.

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1. introduction

Alzheimer's disease (AD) is the most common cause of dementia, associated with aging and cognitive impairment [1]. Globally, there are 46 million people affected by the disease. According to data from the United States in 2015, Alzheimer's disease impacted over 5.3 million individuals. It is projected that by 2050, the annual incidence of new Alzheimer's cases will escalate to nearly a million, with estimates ranging from 11 to 16 million cases [2]. With the progressive development of AD pathology, it gradually poses substantial threats to the structure and functionality of patients' brains, primarily manifested as cognitive deficits and memory loss [3], significantly affecting the daily lives and overall well-being of AD patients. Despite the approval by the United States Food and Drug Administration of several drugs for AD treatment, such as donepezil, galantamine, memantine, rivastigmine, and the combination of memantine with donepezil, they only provide partial relief of symptoms and may be accompanied by side effects [4].

The two primary pathological features most prominent in AD are the deposition of β-amyloid protein (Aβ) leading to senile plaques (SP) and the neurofibrillary tangles (NFTs) resulting from excessive phosphorylation of tau protein [5]. Accumulating research suggests that intracerebroventricular injection of Aβ induces significant behavioral and pathological changes resembling AD characteristics in rodent models, such as pronounced spatial memory impairments [6], excessive phosphorylation of tau protein [7], and expression of β-amyloid protein [8]. The striking resemblance of these pathological symptoms to those observed in AD patients renders intracerebroventricular Aβ injection a suitable experimental approach to explore the potential molecular and pathophysiological mechanisms of AD, as well as therapeutic interventions aimed at targeting AD pathology.

In traditional Chinese medicine, a holistic approach often involves the combination of multiple herbal ingredients to achieve a "multi-target, multi-pathway" treatment strategy for diseases. Herbal pairs or combinations are the fundamental and simplest forms of herbal formulations, holding significant importance in herbal compatibility research [9]. The Chinese herb *Schisandra chinens*is (SC) , the dried ripe fruit of *Schisandra chinensis* (Turcz.) Baill. of the Magnolia family. In current clinical practice, it is commonly used to improve cognitive function [10], and recent studies have shown that SC can reduce Aβ protein production and prevent Aβ protein-induced neuronal dysfunction by decreasing the activity of β-secretase 1 [11,12]. According to traditional Chinese medicine theory, the combination of *Schisandra chinensis* and *Evodia rutaecarpa* is believed to exhibit synergistic effects, promoting the tonification of Qi, calming the mind, enhancing cognitive function, and benefiting various aspects of neurological degenerative diseases. The Chinese medicine *Evodia rutaecarpa* (ER) is the dried, near-ripe fruit of *Evodia rutaecarpa* (Juss.) Benth. of the Rutaceae family, and modern medical studies have shown that ER can exert neuroprotective effects by inhibiting tau phosphorylation through inhibition of GSK-3β/CDK5/MAPK pathway activity. Therefore, we suggest that the pairing of SC with ER can both reduce Aβ protein production and treat AD by inhibiting tau protein phosphorylation, and more importantly, the active ingredient in SC is restricted by the blood-brain barrier when entering the brain [13], However, with the addition of ER, ER can increase the permeability of the blood-brain barrier [14], thus making the herbal pair more effective than single herbs in the treatment of AD. However, to our knowledge, there are no studies on the combination of SC and ER in the treatment of AD.

The present study aims to investigate the therapeutic effects of SC and ER on an intracerebroventricular $A\beta_{1\text{-}42}$-induced AD rat model, along with their associated molecular mechanisms, with a particular focus on the therapeutic outcomes of the combined use of these two herbal medicines. Our study provides new ideas for alternative treatment of AD and provides some references for clinical drug use. Through this study, we expect to provide more therapeutic options for AD patients and thus improve their quality of life.

2. Materials and methods

2.1. Experimental drugs

$A\beta_{1\text{-}42}$ (St. Louis, Missouri, USA) was dissolved in physiological saline and stored in equal parts at −80 °C. The Aβ solution was incubated at 37 °C for 5 days to induce aggregation before injection. *Schisandra chinensis* and *Evodia rutaecarpa* were provided by Shenyang Tongrentang Pharmaceutical Co., Ltd. (Shenyang, China) and authenticated by Professor Zhenyue Wang of the Chinese Medicinal Materials Research Center at Heilongjiang University of Chinese Medicine, following the guidance of the "Chinese Pharmacopoeia" (2020 edition). Voucher specimens (HZY-2020-7) are kept in the herbarium of the School of Pharmacy, Heilongjiang University of Chinese Medicine. The SC group, ER group, and SC-ER (4:1) group were accurately weighed, each totaling 150 g. After grinding the herbs into powder, they were extracted with 70 % ethanol through heating and condensation reflux for 2 h, twice. After combining the filtrates, they were concentrated using a rotary evaporator. The concentrated extracts were kept for further use. For the preparation of HPLC sample solutions, Schisandra and Evodia were taken in a 4:1 ratio (2 g in total). Each herb was extracted separately with 15 times the amount of 70 % ethanol through reflux extraction for 1 h, three times. After combining the filtrates and concentrating them using a rotary evaporator, they were diluted to 10 mL with methanol, filtered through a 0.22 μm filter membrane, and the filtrate was used for subsequent analysis.

2.2. Experimental animals

Eight-to nine-week-old male Sprague-Dawley (SD) rats with a body weight of 200 ± 20 g were provided by the Center for Laboratory Animals at Heilongjiang University of Chinese Medicine, China (Animal License Number: SCXK(Heilongjiang) 2019–001). All animals were housed in standard specific-pathogen-free (SPF) facilities with controlled conditions (humidity: 55 ± 5 %; temperature: 23 ± 1 °C) and were given ad libitum access to water and food. After a one-week acclimation period, 50 rats were randomly divided into five groups: the control group, the model group ($A\beta_{1\text{-}42}$), the SC group ($A\beta_{1\text{-}42}$ + 2 g/kg SC), the ER group ($A\beta_{1\text{-}42}$ + 2 g/kg ER), and

the S-E group ($A\beta_{1\text{-}42}$ + 2 g/kg S-E). Except for the control group, the remaining rats were anesthetized with pentobarbital sodium (30 mg/kg) and stereotaxically injected into the hippocampal region with reference to the "Rat Brain Stereotaxic Atlas". The coordinates for stereotactic injection were 4 mm below the dura mater, 1 mm posterior to the bregma, and 1.5 mm to the left and right of the midline. Rats in the model and treatment groups received bilateral intracerebroventricular injections of 1 μL of $A\beta_{1\text{-}42}^{8}$. An equal volume of sterile physiological saline was injected into the control group. After injection, the needle was left in place for 5 min and then slowly retracted. A medical gelatin sponge was used to cover the cannula to prevent cerebrospinal fluid and injection reflux. One week after adaptation, the rats in the treatment groups were orally gavaged daily, while the control and model group rats received an equivalent volume of sterile physiological saline for 30 consecutive days (Fig. 2A) . The experimental protocol was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine (Approval Number: 20201029001), and it was conducted in strict accordance with the "Guide for the Care and Use of Laboratory Animals" provided by the National Institutes of Health (NIH) to minimize animal usage and discomfort.

2.3. *High performance liquid Chromatography* (*HPLC*)

A qualitative HPLC method was established for the analysis of SC, ER, and S-E extracts. The chromatographic separation was performed on a BEH C-18 column (1.7 μm, 2.1 mm × 100 mm, Waters, USA). The detection wavelength was set at 220 nm, and the column temperature was maintained at 25 °C. The flow rate was set at 1.0 ml·min-1, and the injection volume was 20 μl. The mobile phase consisted of solvent A (water) and solvent B (methanol). The gradient elution conditions are shown in Table 1.

2.4. *Morris water maze test*

The Morris water maze test consisted of two phases: the positioning navigation test and the spatial probe test [15]. The experiment was conducted in a circular pool with a diameter of 150 cm, a height of 50 cm, and water depth of 30 cm. Ink was added to the water to make it opaque. The water temperature was maintained at (25 ± 1)°C. The pool was divided into four quadrants, with a hidden black platform located 1 cm beneath the water surface in the fourth quadrant. The positioning navigation test spanned 5 days, with each rat receiving two training sessions per day. In positioning navigation experiment test, rats were released into the second quadrant, and the time it took them to reach the hidden platform was recorded as the escape latency. If a rat failed to find the platform within 90 s, the experimenter manually placed the rat on the platform for 20 s, and the escape latency was recorded as 90 s. After 5 days, the spatial probe test was conducted. The hidden platform was removed, and the number of times the mice crossed over the original platform location within 90 s was recorded.

2.5. *Brain tissue preparation*

After completion of behavioral tests, all rats were anesthetized with pentobarbital sodium and executed. The brains were rapidly removed, and for each group, five rats were randomly selected. Their entire brains were fixed in 4 % paraformaldehyde for 24 h and then dehydrated with a sucrose gradient before being frozen-embedded in OCT compound for cryosectioning. The brains of the remaining rats were dissected to obtain the hippocampus, which was rapidly frozen at −80 °C for subsequent biochemical analysis. Prior to the biochemical analysis, the dissected hippocampi were homogenized in cold RIPA buffer containing phosphatase and protease inhibitor mix using ultrasound and then centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant was collected.

2.6. *Hematoxylin and Eosin* (*HE*) *staining*

The pre-prepared brain tissues were sectioned into 5-μm slices, fixed, and stained with hematoxylin and eosin. After dehydration with graded alcohols, the sections were embedded in neutral resin. The hippocampal neuronal damage was observed, and photographs were taken using an optical microscope [16].

2.7. *Congo red staining*

The tissue sections were blown in cold air for 30 min, rinsed in running water for 1 min, soaked in Congo red staining solution for 15 min, rinsed in running water for 2 min, differentiated in saturated lithium carbonate solution for 15 s, rinsed in running water for 1 min, hematoxylin staining solution for 2 min, rinsed in running water for 1 min, differentiated in 1 % hydrochloric acid-ethanol

Table 1
Gradient elution conditions.

Time (min)	Solvent A (%)	Solvent B (%)
0	45.0	50.0
20	30.0	70.0
30	20.0	80.0
35	10.0	90.0
40	0.0	100.0

solution for several seconds, rinsed in running water for 2 min, returned to blue in ammonia for several seconds, rinsed in running water for 1 min, and finally routinely The films were dehydrated, transparent, sealed with neutral resin, observed under a light microscope and photographed [17].

2.8. Immunohistochemistry

After tissue section washing, the sections were boiled in citrate buffer for 30 min to retrieve antigens, followed by a 10-min blockade of endogenous peroxidase. The sections were then incubated with primary antibodies against $A\beta_{1\text{-}42}$ (1:400, Abcam, Wuhan, China) and P-tau (1:200, Abcam, Wuhan, China) at 4 °C overnight. Subsequently, they were placed in an HRP-labeled broad-spectrum secondary antibody at room temperature for 30 min. Visualization was performed using diaminobenzidine (DAB) staining, followed by counterstaining with hematoxylin. Afterward, the sections were dehydrated, cleared, and coverslipped. Observation and photography were performed under an optical microscope (400 ×).

2.9. Western blot analysis

After determining the total protein levels of brain tissue or lysed cells using a BCA reagent kit (Beyotime, Suzhou, China), target proteins were separated using 12 % SDS-PAGE gels and transferred onto PVDF membranes. The PVDF membranes were blocked in TBST with 5 % skim milk at 4 °C. After overnight incubation with primary antibodies, secondary rabbit antibodies were applied at room temperature for 1 h. Protein bands were visualized using chemiluminescent reagents (Beyotime, Suzhou, China). The protein bands were scanned with a molecular imager (BIO-RAD, Hercules, CA, USA) and analyzed for band intensities using Image J software.

2.10. Real-time quantitative polymerase chain reaction

Total RNA was extracted from the hippocampus (3 per group). Complementary DNA (cDNA) was synthesized using the PrimeScript Reverse Transcriptase Kit (Takara Bio, RR037A, Japan). SYBR Premix Ex *Taq*II rt PCR Kit (Takara Bio, RR820A) was used for RT-PCR of BDNF, TRKB, CREB, GSK-3β, TAU. Transcript levels using the $2^{-\Delta\Delta CT}$ method. The sequences of these primers are listed in Table 2.

2.11. Statistical analysis

All values were expressed as mean ± standard deviation and analyzed with graph pad Prism 8.0. The significance levels of the behavioral experiment results were determined using two-way analysis of variance (ANOVA) and Bonferroni post hoc test, while the significance levels of the other experimental results were determined using one-way analysis of variance and Tukey post hoc test. The significance level was $P < 0.05$.

3. Results

3.1. HPLC analysis of SC, ER, and S-E extracts

The chromatograms of SC, ER, and S-E extracts are presented in Fig. 1. The seven compounds (Schisandrol A; Evodiamine; Schisandrol B; Rutaecarpine; Schisantherin A; Schizandrin A; Schizandrin B.) were successfully identified. SC extract exhibited abundant lignans, including Schisandrol A、Schisandrol B、Schisantherin A、Schizandrin A and Schizandrin B (Fig. 1A) . On the other hand, ER extract showed significant alkaloid content, primarily evodiamine and rutaecarpine (Fig. 1B) . The S-E extract demonstrated the presence of active components from both herbs (Fig. 1C) .

Table 2
Primer sequences.

Gene		Primer sequence	Accession number	product length
BDNF	F	CACACACAGCGCTCCTTA	NC_051338.1	774 nt
	R	AGTGGTGGTCTGAGGTTGG		
TRKB	F	TGGCGAGACATTCCAAGTTTG	NC_051352.1	2514 nt
	R	AGAGTCATCGTCGTTGCTGATG		
CREB	F	CCAAACTAGCAGTGGGCAGT	NC_051344.1	1026 nt
	R	GAATGGTAGTACCCGGCTGA		
GSK-3β	F	GGGCACCAGAGCTGATCTTT	NC_000082.7	1302 nt
	R	GCCGAAAGACCTTCGCCA		
TAU	F	CCAGGAGTTTGACACAATGGAAGAC	NC_000077.7	2304 nt
	R	CTGCTTCTTCAGCTTTTAAGCCATG		
GAPDH	F	AACGACCCCTTCATTGAC	NC_051339.1	1002 nt
	R	TCCACGACATACTCAGCA		

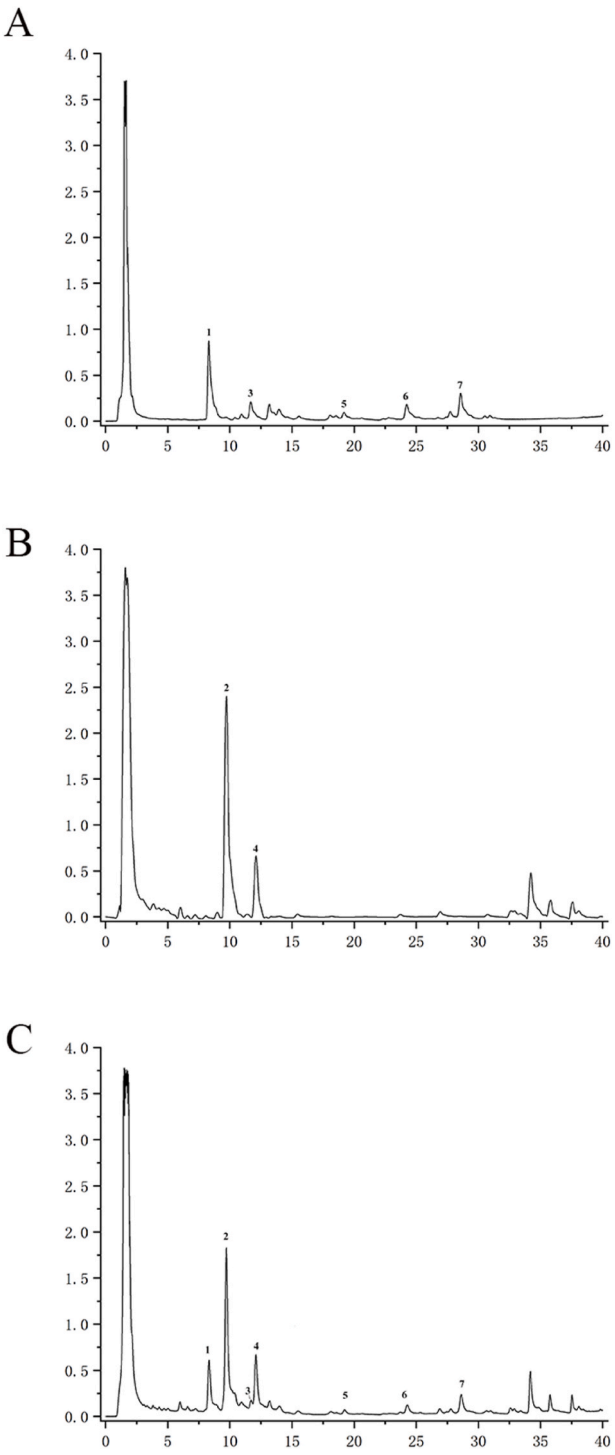

Fig. 1. HPLC chromatograms: A: SC extract; B: ER extract; C: S-E extract; Peaks: 1. Schisandrol A; 2. Evodiamine; 3. Gomisin A; 4. Rutaecarpine; 5. Schisantherin A; 6. Schizandrin A; 7. Schizandrin B.

3.2. The combined use of SC and ER provided stronger protection against neurological injury in AD model rats compared to SC or ER alone

To investigate the effect of combined SC and ER on neurological damage in a rat model of AD, we performed the Morris water maze experiment. We first measured the mean swimming speed of the rats to exclude the effect of rat locomotor ability on the experimental results. The results showed that there was no significant difference in the locomotor ability of the rats in each group ($F(4, 25) =$

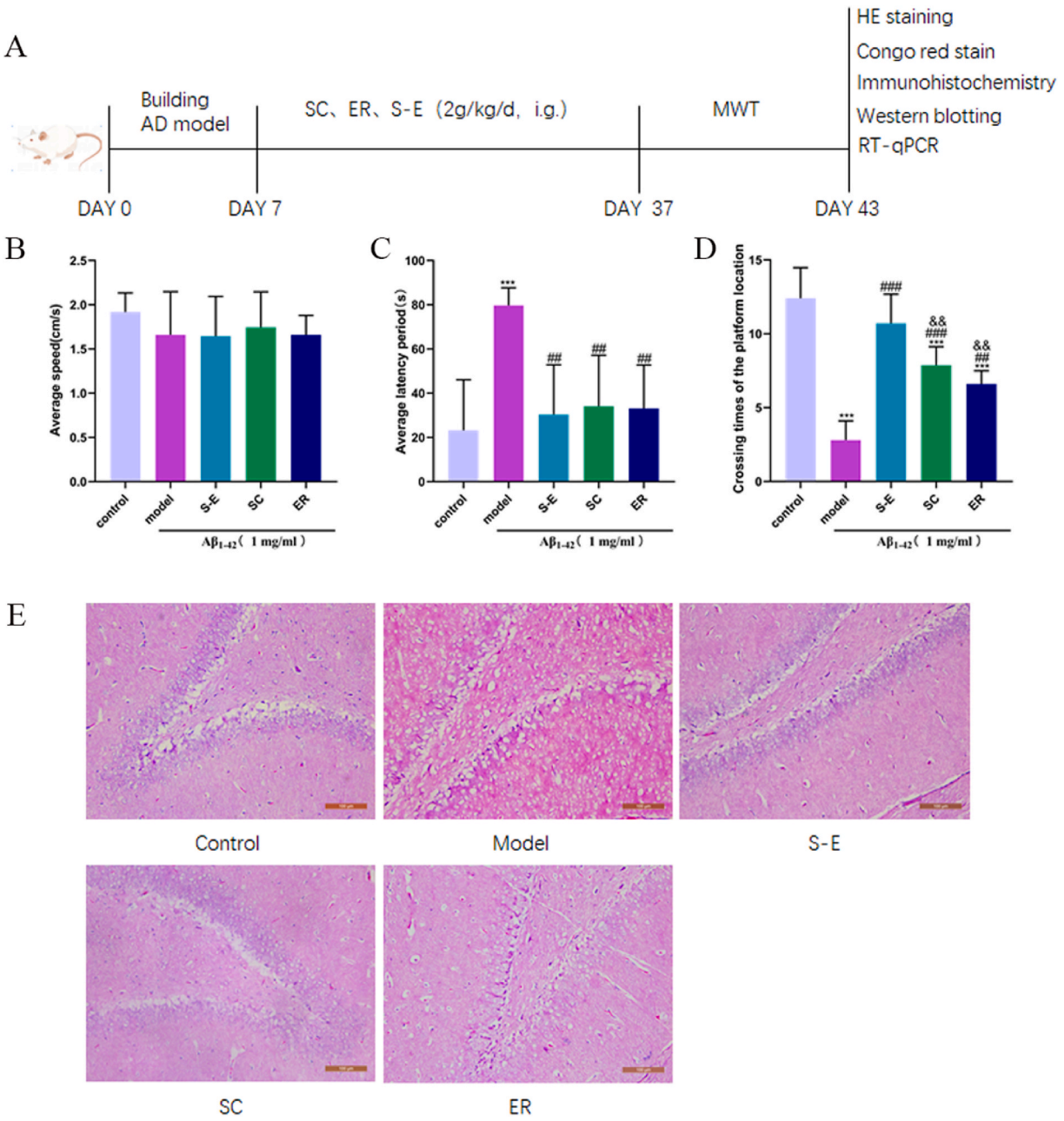

Fig. 2. Experimental procedure and neuroprotective effects of SC and ER on $A\beta_{1\text{-}42}$ induced AD rat model. A: Experimental flow diagram; B: Mean swimming speed of rats in the water maze experiment; C: Latency of rats in the water maze experiment; D: Number of times rats crossed the location of the platform in the positioning navigation experiment; E: Brain tissue sections of rats undergoing HE staining; Model: $A\beta_{1\text{-}42}$, i.c.v.; S–E: Schisandra: Evodia treatment group. i. g.; SC: SC treatment group, i. g.; ER: ER treatment group, i. g.; Data expressed as mean ± standard deviation (n = 6–8 per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with model group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, compared with the S-E group.).

0.471 , $p > 0.05$, Fig. 2B). The results of the positioning navigation experiment showed that the Model group had a longer latency period compared with the Control group, and the difference was significant ($F(4, 25) = 7.594$, $p < 0.01$, Fig. 2C). In contrast, the latency period was shorter in both the herb administration group compared with the Model group, and the difference was significant ($p < 0.05$). In the spatial exploration experiment, the number of times rats crossed the original platform location was significantly reduced in the Model group compared with the Control group ($F(4, 25) = 13.571$, $p < 0.05$, Fig. 2D), while the number of times rats crossed the original platform was significantly increased in all herb administration groups ($p < 0.05$). Among them, the number of rats crossing the original platform was significantly increased in the S-E group compared with the SC and ER groups ($p < 0.05$). In the

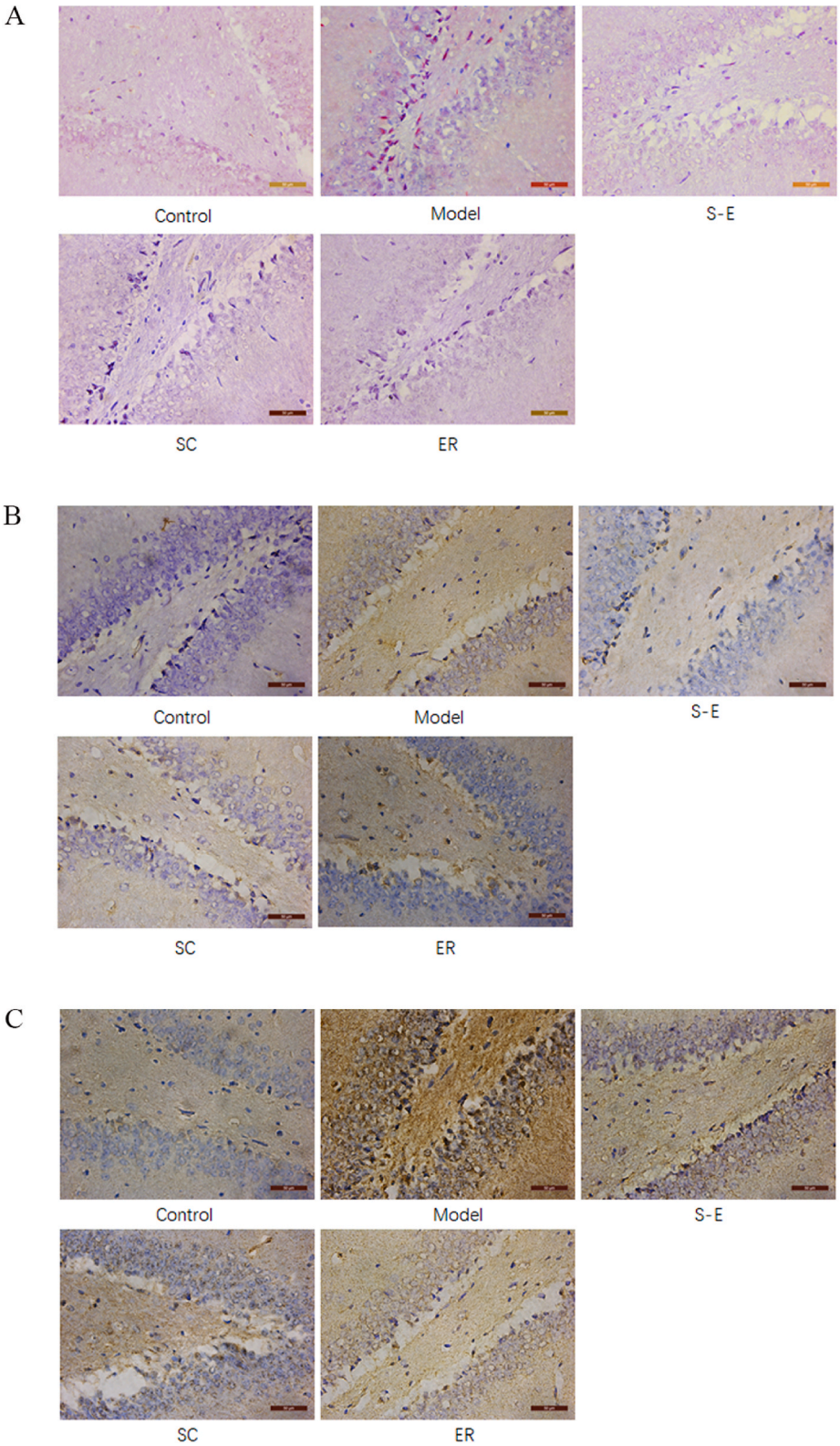

Fig. 3. S-E inhibits the production of $A\beta_{1\text{-}42}$ and P-Tau proteins in the hippocampus of a rat model of AD. A: Congo red staining for age spots; B: Immunohistochemical assay for $A\beta_{1\text{-}42}$ protein; C: Immunohistochemical assay for P-tau protein; Model: $A\beta_{1\text{-}42}$, i. c.v.; S–E: Schisandra: Evodia = 4:1 group, i. g.; SC: SC treatment group, i. g.; ER: ER treatment group, i. g.

HE staining experiment(Fig. 2E), the Control group exhibited well-organized and tightly packed hippocampal neurons with regular morphology, showing no signs of inflammation. In comparison, the Model group displayed irregularly shaped hippocampal neurons, loosely arranged with deformities, increased intercellular spaces, noticeable tissue cavities, and significant signs of inflammation. Compared to the Model group, the S-E group demonstrated relatively regular cellular morphology and neater arrangement with minimal inflammation. The SC and ER groups displayed somewhat irregular cell morphology and arrangement, accompanied by mild inflammation and a few tissue cavities. In summary, S-E exhibited a protective effect against neuronal damage in AD rats, with a superior therapeutic effect compared to individual use.

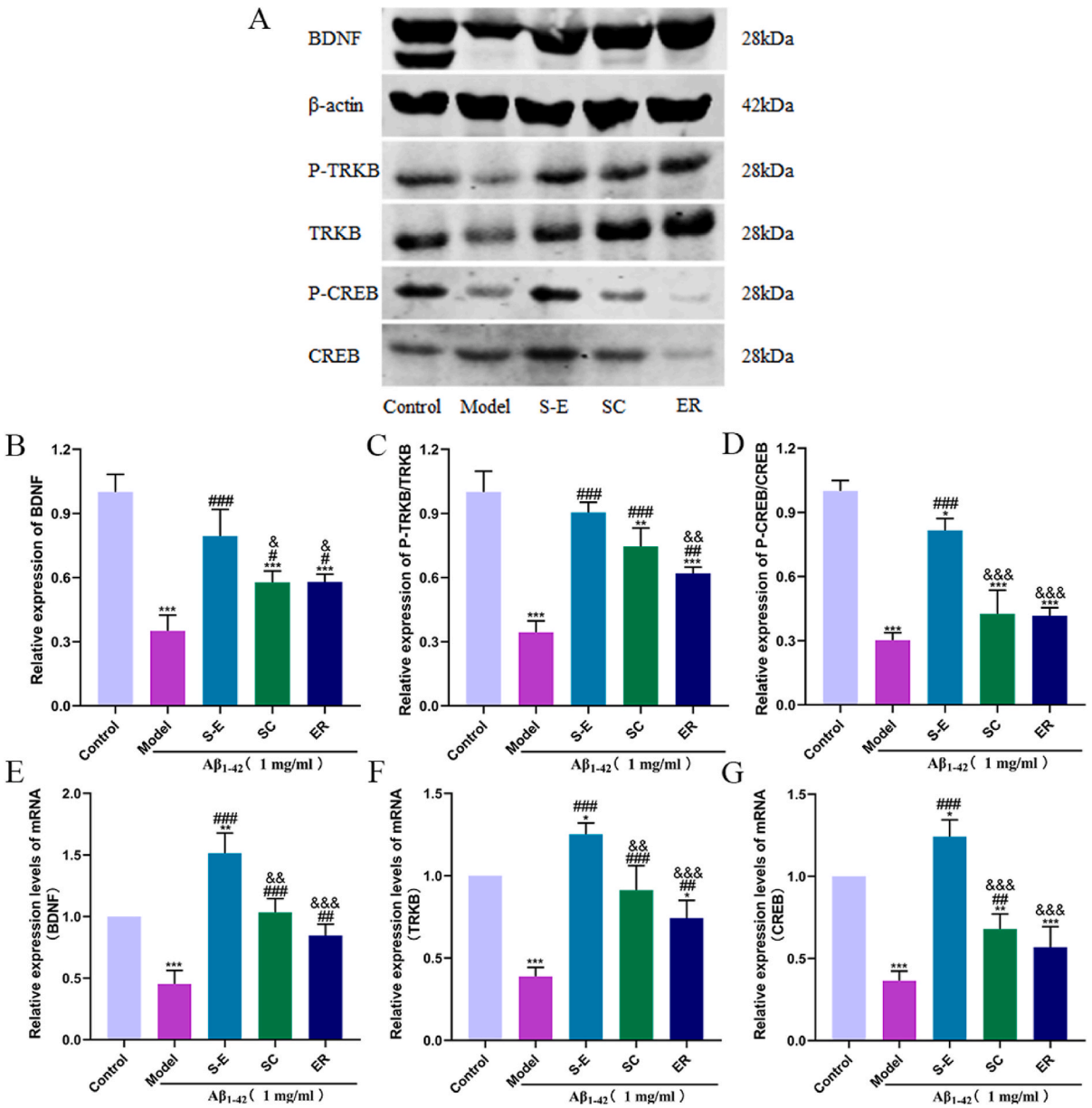

Fig. 4. SC paired with ER increases the expression of BDNF/TRKB/CREB pathway proteins and related genes. A: Western blot for representative expression of BDNF, P-TRKB, TRKB, P-CREB, CREB proteins (Supplementary Material 1) ; B, C, D: Quantitative analysis of BDNF (B), P-TRKB/TRKB (C) and P-CREB/CREB (D) protein expression; E, F, G: BDNF (E), TRKB (F), CREB (G) mRNA Quantitative analysis of expression; Data are expressed as mean ± standard deviation (n = 3 per group,*P < 0.05,**P < 0.01, ***P < 0.001, compared with Control group;$^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001, compared with Model group;$^{\&}$ P < 0.05, $^{\&\&}$P < 0.01, $^{\&\&\&}$P < 0.001, compared with S-E group).

3.3. Stronger inhibition of $A\beta_{1\text{-}42}$ and P-tau protein by SC in combination with ER compared to SC or ER alone

To investigate the effects of SC and ER paired use on $A\beta_{1\text{-}42}$ and P-tau proteins in the brain of AD rat model, we performed Congo red and immunohistochemical experiments. The results of Congo red experiments showed (Fig. 3A) that there was almost no SP staining in the Control group, while the positive expression was significantly increased in the Model group. Both the SC and ER groups showed a decrease in positive expression compared to the Model group, while the S-E group showed the most significant improvement compared to the Model group, with only a small amount of positive SP expression. In immunohistochemical experiments, the expression of $A\beta_{1\text{-}42}$ and P-Tau protein was significantly increased in the Model group compared with the Control group (Fig. 3B、C) . The positive expression levels of $A\beta_{1\text{-}42}$ and P-Tau proteins were significantly lower in all dosing groups compared to the Model group. Among the three dosing groups, the positive expression levels of $A\beta_{1\text{-}42}$ and P-Tau protein were most reduced in the S-E group, which was most similar to the Control group. The above experimental results indicate that the use of SC with ER not only inhibits the production and aggregation of $A\beta_{1\text{-}42}$, thus reducing the production of SP, but also prevents the hyperphosphorylation of tau protein.

3.4. Stronger agonistic effect of SC with ER on the expression of BDNF/TRKB/CREB pathway proteins and their related genes compared to SC or ER alone

We performed WB and RT-qPCR experiments to reveal the molecular mechanism of the neuroprotective effects of SC and ER in AD. The results of WB experiments showed (Fig. 4A–D) that the expression of BDNF ($F(4, 10) = 28.541$, $P < 0.05$) , P-TRKB/TRKB ($F(4, 10) = 43.486$, $P < 0.05$) , and P-CREB/CREB ($F(4, 10) = 66.427$, $P < 0.05$) proteins decreased significantly in the Model group rats, while the expression of related proteins increased significantly after herb administration. Compared with the S-E group, the treatment effect of both the SC and ER groups decreased to different degrees, while the related protein expression of the S-E group was not significantly different from that of the Control group. RT-qPCR experiments showed that the expression of BDNF ($F(4, 10) = 36.699$, $P < 0.05$) , TRKB ($F(4, 10) = 49.561$, $P < 0.05$) , CREB ($F(4, 10) = 37.961$, $P < 0.05$) genes decreased after Aβ1-42 treatment, while the related gene expression was significantly increased in each herb administration group (Figure E–G) . Notably, the activation effect of the S-E group on the expression of related genes was significantly stronger than that of

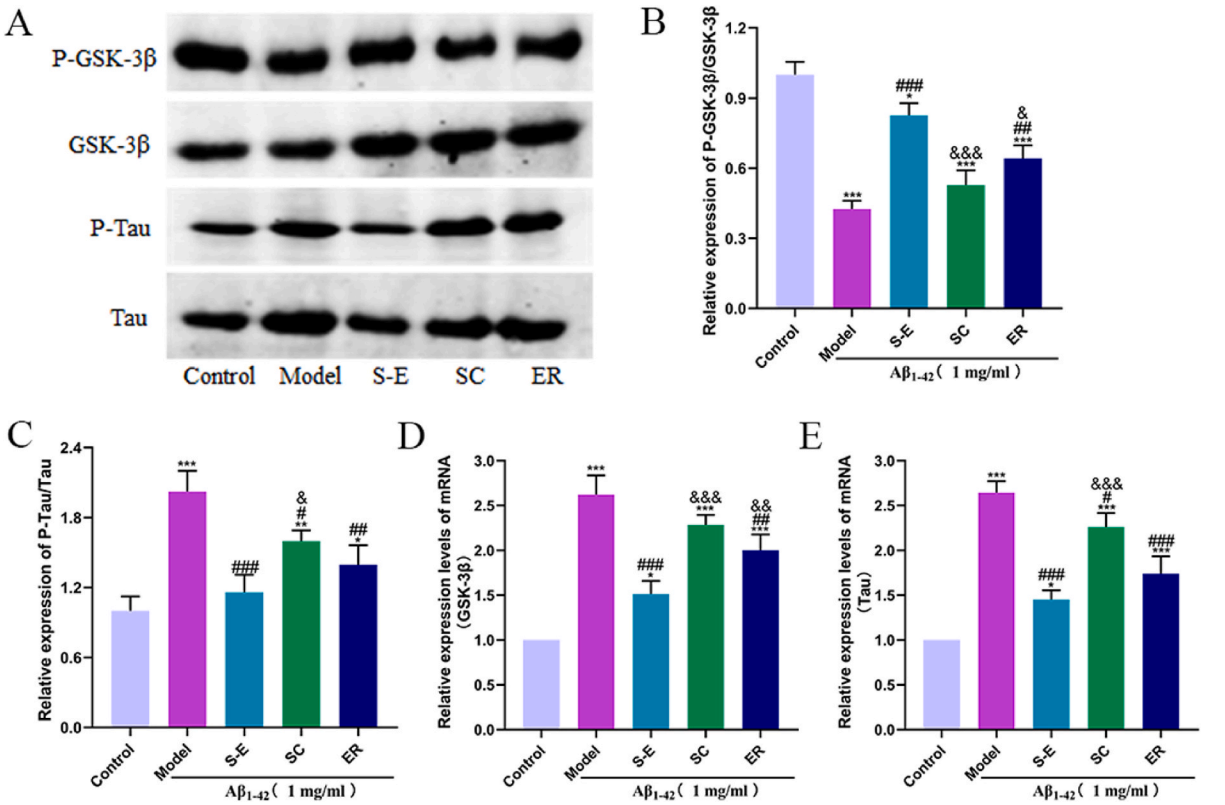

Fig. 5. SC and ER mating inhibit GSK-3β/Tau pathway protein and related gene expression. A: Western blot for representative expression of P-GSK-3β, GSK-3β, P-Tau, and Tau proteins (Supplementary Material 2) ; B, C: Quantitative analysis of P-GSK-3β/GSK-3β(B) and P-Tau/Tau (C) protein expression; D, E: Quantitative analysis of GSK-3β/Tau mRNA expression; data are expressed as mean ± standard deviation (n = 3 per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with Control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with Model group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, compared with S-E group).

the SC and ER groups.

3.5. Compared to SC or ER alone, SC paired with ER exhibited stronger inhibition of GSK-3β/tau pathway protein and its related gene expression

Finally, we explored the effects of SC and ER on GSK-3β/Tau pathway. the results of WB experiments showed (Fig. 5A–C) that P-GSK-3β/GSK-3β ($F(4, 10) = 57.944$, $P < 0.05$) protein expression was significantly decreased and P-Tau/Tau ($F(4, 10) = 22.694$, $P < 0.05$) protein expression was significantly increased in the Model group rats, and the administration of the herb reversed this trend. In addition, compared with the S-E group, the treatment effects of both the SC and ER groups were RT-qPCR experiments showed that GSK-3β ($F(4, 10) = 56.066$, $P < 0.05$) and Tau ($F(4, 10) = 71.755$, $P < 0.05$) gene expression were significantly increased in the Model group compared with the Control group, and each administration group significantly inhibited GSK-3β and Tau gene expression (Fig. 5D、E) , but the inhibition of related gene expression in the S-E group was significantly stronger than that in the SC and ER groups.

4. Discussion

In recent years, SC and ER have received much attention for their therapeutic potential for AD [18,19]. However, there is a gap in research on the therapeutic use of these two herbs in combination for AD. In our study, we found that the combination of SC and ER was more effective in improving spatial memory and neuronal damage in AD model rats compared with the herbs alone. In addition, the combination of SC and ER significantly inhibited the production and aggregation of $A\beta_{1\text{-}42}$ and P-Tau proteins in the hippocampus of rats. Finally, we found that S-E could simultaneously regulate BDNF/TRKB/CREB and GSK-3β/Tau pathway activities and their related gene expression, thus exerting neuroprotective effects on AD (Fig. 6).

Both SC and ER are traditional Chinese medicines with multiple active ingredients and the ability to exert a wide range of pharmacological effects. To identify the major active components, we conducted HPLC analysis. The results showed that SC primarily contains lignans as its active components, while ER is rich in alkaloids. The combined extract of S-E contains a combination of the active components from both herbs. Previous studies have indicated that extracts of SC and its lignans can serve as novel herbal treatments for various neurodegenerative diseases [20]. Oral administration of lignans from SC has been shown to restore neurotransmitter levels in AD rats, thereby ameliorating their learning and memory impairments[26]. Similarly, ER contains active components such as evodiamine and rutaecarpine, which also hold potential therapeutic value for AD [21–23]. In this experiment, an Alzheimer's disease model was established through intracerebroventricular injection of $A\beta_{1\text{-}42}$. Firstly, the effects of treatment on spatial learning and memory in the AD rat model were assessed using the classic behavioral test, Morris water maze. Additionally, the therapeutic effects of SC and ER on neuronal damage in AD model rats were observed through HE staining. Our experimental results are consistent with previous research, demonstrating that both the SC and ER groups significantly ameliorated neuronal damage and

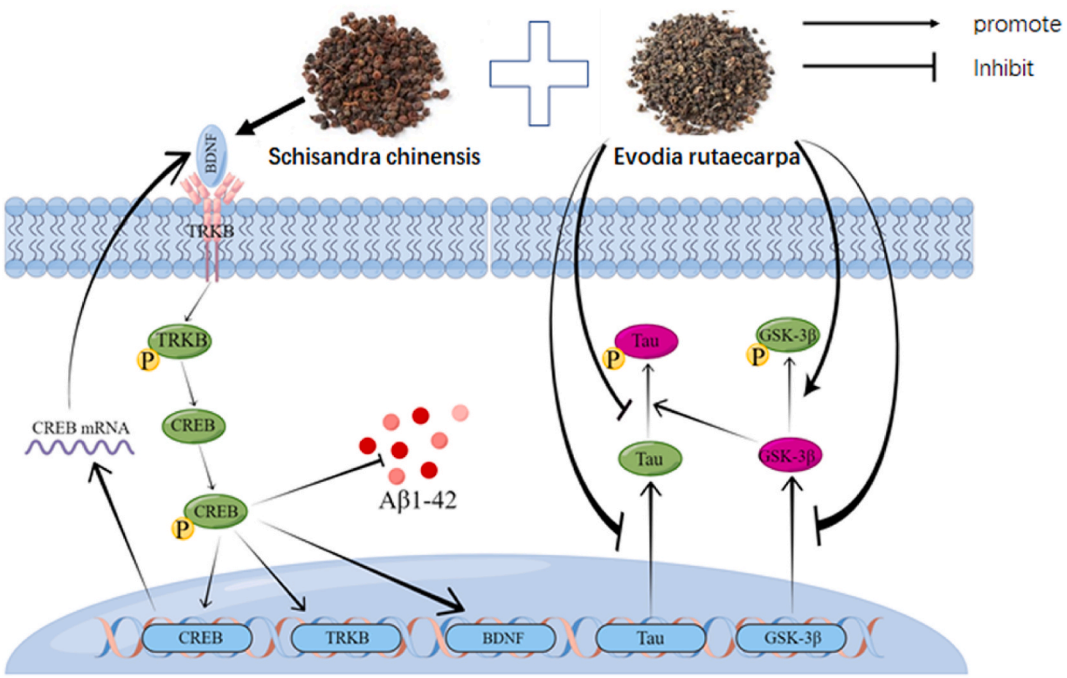

Fig. 6. Mechanism of action of S-E to inhibit $A\beta_{1\text{-}42}$ and P-Tau protein.

improved spatial learning and memory impairments in the AD rat model. Remarkably, the combined use of SC and ER exhibited even more pronounced therapeutic effects on AD, approaching levels comparable to the control group. This outcome strongly suggests that the combined use of SC and ER enhances therapeutic efficacy, exerting significant neuroprotective effects against AD, surpassing the effects of individual treatments.

Although many hypotheses have been proposed regarding the pathological mechanisms and biomarkers of AD, such as the Aβ cascade hypothesis, the neuroinflammatory hypothesis, the oxidative stress hypothesis [24], the clinical diagnosis of AD is still based on the assessment of cognitive function and intracerebral imaging of Aβ and Tau. $A\beta_{1\text{-}42}$ is the most misfolded and aggregated form of Aβ protein monomer and is the major form of seed starch aggregates and oligomer formation [25], excess P-Tau is more likely to form neuronal fiber tangles while mediating neurotoxicity leading to apoptosis; therefore, we chose $A\beta_{1\text{-}42}$ and P-Tau protein as pathological markers to detect the efficacy of SC paired with ER. Several previous studies have shown that SC is able to reduce Aβ protein production by inhibiting BACE1 expression [26], but not for Tau proteins, whereas ER contains evodiamine, rutaecarpine, all of which have the ability to inhibit Tau protein hyperphosphorylation [27,28]. Through Congo red staining and immunohistochemistry results, we found that the SC group primarily inhibited the production of $A\beta_{1\text{-}42}$ protein, while the ER group focused on reducing the positive expression of P-Tau protein. However, it has been reported that targeting Aβ or P-tau alone may not halt the pathological process, as the AD pathology likely involves interactions between Aβ and tau, where these proteins mutually amplify toxic effects rather than strictly following a hierarchical interaction pattern [29]. Hence, simultaneously targeting both these pathological factors might be a more effective approach for AD treatment. In our study, the S-E group exhibited a significantly stronger effect in reducing the generation and aggregation of $A\beta_{1\text{-}42}$ and P-Tau proteins compared to the monotherapy groups. Therefore, we believe that the combination of SC and ER comprehensively exploits the effects of both herbs, concurrently inhibiting the production of Aβ and P-Tau proteins, thereby reducing the neurotoxicity mediated by these proteins and safeguarding damaged neurons, resulting in a more potent neuroprotective effect. However, the molecular mechanisms underlying the neuroprotective action of SC and ER in combination remain unclear in our study.

Brain-derived neurotrophic factor (BDNF) is a dominant neurotrophic factor in the brain that is involved in neuronal differentiation, regeneration and plasticity mechanisms. It was found that BDNF is neuroprotective against Aβ protein-induced toxicity in AD [30], prevents neuronal death, reverses neuronal atrophy and improves cognitive deficits [31] One of the prerequisites for the action of BDNF is the activation of high-affinity pro-myosin-related kinase B (TRKB) receptors, which in turn promotes phosphorylation of cAMP response element binding protein (CREB) [32]. Yan et al. showed that SC exerts antidepressant-like effects not only through the BDNF/TRKB/CREB signaling pathway in mice induced by repetitive corticosterone [33]. SC is also involved in the inhibition of oxidative stress, neuroinflammation, and apoptosis through the BDNF/Nrf2/NF-κB signaling pathway, thereby preventing DA neurodegeneration in 6-OHDA-induced PD mice [34]. In our experiments, the S-E group significantly upregulated the BDNF/TRKB/CREB pathway-related proteins and their gene expression, indicating that S-E can increase the expression of BDNF, TRKB, and CREB proteins and promote the phosphorylation of related proteins, thus activating the BDNF/TRKB/CREB pathway to inhibit the production of Aβ proteins, alleviate neurotoxicity, and exert neuroprotective effects. Glycogen synthase kinase-3β (GSK-3β) is a highly conserved protein serine/threonine kinase, GSK-3β is closely related to the pathological process of AD, and it is a key molecular link between SP and NFTs [35], a key kinase that drives tau protein hyperphosphorylation [36]. However, GSK-3β activity is regulated by phosphorylation and dephosphorylation, and its activity is inhibited by the production of P-GSK-3β [37]. In a previous study, Evodiamine in ER were able to enhance the phosphorylation of GSK-3β protein expression inhibiting Tau protein phosphorylation [38], therefore, we used WB experiments to detect the protein expression of P-GSK-3β/GSK-3β and P-Tau/Tau, and the results showed that S-E significantly upregulated P-GSK-3β/GSK-3β expression and downregulated P-Tau/Tau expression, indicating the ability of S-E to prevent Tau protein hyperphosphorylation by inhibiting GSK-3β activity and thus Tau protein hyperphosphorylation in RT-qPCR experiments, the S-E group inhibited the expression of GSK-3β and Tau genes, indicating that S-E inhibited the hyperphosphorylation of Tau protein while suppressing the expression of GSK-3β and total Tau protein. The above experimental results showed that S-E not only upregulated BDNF/TRKB/CREB pathway activity to inhibit $A\beta_{1\text{-}42}$ protein production, but also inhibited GSK-3β/Tau pathway activity to prevent Tau protein hyperphosphorylation. In addition, almost all experimental results showed that the S-E group possessed more powerful therapeutic effects compared with the SC or ER groups, and therefore, we believe that the use of SC in combination with ER can better treat AD.

5. Conclusion

This study addresses the gaps in previous research by introducing for the first time the combined treatment of SC and ER in Alzheimer's disease. Our findings reveal that the combined application of these herbs significantly modulates the activity of the BDNF/TRKB/CREB and GSK-3β/Tau pathways, leading to the suppression of $A\beta_{1\text{-}42}$ and P-Tau protein generation and a reduction in senile plaques, ultimately resulting in enhanced neuroprotection. Nevertheless, our study does have certain limitations. For instance, the Morris water maze experiment may not entirely assess the impairment of animal models' recognition memory function. Additionally, we didn't conduct more in-depth in vitro experiments to validate the involved pathways, potentially affecting our understanding of key mechanisms. Hence, further extensive research is warranted to better comprehend the therapeutic effects and mechanisms of S-E in AD, providing more reliable insights for future clinical treatments.

Ethical approval and consent to participate

Animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the

experimental protocol was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine (approval number: 20201029001).

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Data availability statements

The data associated with this study has not been stored in a publicly accessible repository; therefore, the relevant data will be provided upon request.

CRediT authorship contribution statement

Qingyu Cao: Data curation, Methodology, Software, Visualization, Writing – original draft. **Jiaqi Liu:** Conceptualization, Data curation, Methodology, Validation, Visualization. **Chengguo Pang:** Methodology, Validation. **Kemeng Liu:** Investigation, Methodology, Writing – review & editing. **Ruijiao Wang:** Methodology, Supervision, Writing – review & editing. **Yuanjin Chen:** Methodology, Validation. **Xu Yuan:** Methodology, Validation. **Meng Zhang:** Methodology, Validation. **Jiating Ni:** Methodology, Validation, Visualization. **Peiliang Dong:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Hua Han:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21942.

References

[1] N.A. Bishop, T. Lu, B.A. Yankner, Neural mechanisms of ageing and cognitive decline, Nature 464 (7288) (2010) 529–535, https://doi.org/10.1038/nature08983 [published Online First: 2010/03/26].

[2] S.S. Ahmad, H. Khan, S.M. Danish Rizvi, S.A. Ansari, R. Ullah, L. Rastrelli, H.M. Mahmood, M.H. Siddiqui, Computational study of natural compounds for the clearance of amyloid-betaeta: a potential therapeutic management strategy for Alzheimer's disease, Molecules 24 (18) (2019), https://doi.org/10.3390/molecules24183233 [published Online First: 2019/09/08].

[3] B. De Strooper, E. Karran, The cellular phase of Alzheimer's disease, Cell 164 (4) (2016) 603–615, https://doi.org/10.1016/j.cell.2015.12.056 [published Online First: 2016/02/13].

[4] L.B. Gao, X.F. Yu, Q. Chen, D. Zhou, Alzheimer's Disease therapeutics: current and future therapies, Minerva Med. 107 (2) (2016) 108–113 [published Online First: 2016/03/05].

[5] G.K. Wilcock, M.M. Esiri, Plaques, tangles and dementia. A quantitative study, J. Neurol. Sci. 56 (2–3) (1982) 343–356, https://doi.org/10.1016/0022-510x(82)90155-1 [published Online First: 1982/11/01].

[6] L.J. Min, Y. Kobayashi, M. Mogi, K. Tsukuda, A. Yamada, K. Yamauchi, F. Abe, J. Iwanami, J.Z. Xiao, M. Horiuchi, Administration of bovine casein-derived peptide prevents cognitive decline in Alzheimer disease model mice, PLoS One 12 (2) (2017), e0171515, https://doi.org/10.1371/journal.pone.0171515 [published Online First: 2017/02/06].

[7] S.J. Dai, J.Y. Zhang, Y.T. Bao, X.J. Zhou, L.N. Lin, Y.B. Fu, Y.J. Zhang, C.Y. Li, Y.X. Yang, Intracerebroventricular injection of Abeta(1-42) combined with two-vessel occlusion accelerate Alzheimer's disease development in rats, Pathol. Res. Pract. 214 (10) (2018) 1583–1595, https://doi.org/10.1016/j.prp.2018.07.020 [published Online First: 2018/08/09].

[8] A. Kasza, B. Penke, Z. Frank, Z. Bozso, V. Szegedi, A. Hunya, K. Nemeth, G. Kozma, L. Fulop, Studies for improving a rat model of Alzheimer's disease: icv administration of well-characterized beta-amyloid 1-42 oligomers induce dysfunction in spatial memory, Molecules 22 (11) (2017), https://doi.org/10.3390/molecules22112007 [published Online First: 2017/11/22].

[9] S. Wang, Y. Hu, W. Tan, X. Wu, R. Chen, J. Cao, M. Chen, Y. Wang, Compatibility art of traditional Chinese medicine: from the perspective of herb pairs, J. Ethnopharmacol. 143 (2) (2012) 412–423, https://doi.org/10.1016/j.jep.2012.07.033 [published Online First: 2012/08/09].

[10] E.J. Jeong, H.K. Lee, K.Y. Lee, B.J. Jeon, D.H. Kim, J.H. Park, J.H. Song, J. Huh, J.H. Lee, S.H. Sung, The effects of lignan-riched extract of Shisandra chinensis on amyloid-beta-induced cognitive impairment and neurotoxicity in the cortex and hippocampus of mouse, J. Ethnopharmacol. 146 (1) (2013) 347–354, https://doi.org/10.1016/j.jep.2013.01.003 [published Online First: 2013/01/22].

[11] V.V. Giridharan, R.A. Thandavarayan, S. Arumugam, M. Mizuno, H. Nawa, K. Suzuki, K.M. Ko, P. Krishnamurthy, K. Watanabe, T. Konishi, Schisandrin B ameliorates ICV-infused amyloid beta induced oxidative stress and neuronal dysfunction through inhibiting RAGE/NF-kappaB/MAPK and up-regulating HSP/beclin expression, PLoS One 10 (11) (2015), e0142483, https://doi.org/10.1371/journal.pone.0142483 [published Online First: 2015/11/12].
[12] X. Zhao, C. Liu, M. Xu, X. Li, K. Bi, Y. Jia, Total lignans of Schisandra chinensis ameliorates abeta1-42-induced neurodegeneration with cognitive impairment in mice and primary mouse neuronal cells, PLoS One 11 (4) (2016), e0152772, https://doi.org/10.1371/journal.pone.0152772 [published Online First: 2016/04/02].
[13] Y. Zhang, X. Lv, J. Qu, X. Zhang, M. Zhang, H. Gao, Q. Zhang, R. Liu, H. Xu, Q. Li, K. Bi, A systematic strategy for screening therapeutic constituents of Schisandra chinensis (Turcz.) Baill infiltrated blood-brain barrier oriented in lesions using ethanol and water extracts: a novel perspective for exploring chemical material basis of herb medicines, Acta Pharm. Sin. B 10 (3) (2020) 557–568, https://doi.org/10.1016/j.apsb.2019.10.008 [published Online First: 2020/03/07].
[14] T. Zhao, X. Zhang, Y. Zhao, L. Zhang, X. Bai, J. Zhang, X. Zhao, L. Chen, L. Wang, L. Cui, Pretreatment by evodiamine is neuroprotective in cerebral ischemia: up-regulated pAkt, pGSK3beta, down-regulated NF-kappaB expression, and ameliorated BBB permeability, Neurochem. Res. 39 (8) (2014) 1612–1620, https://doi.org/10.1007/s11064-014-1356-5 [published Online First: 2014/06/13].
[15] M.Z. Othman, Z. Hassan, A.T. Che Has, Morris water maze: a versatile and pertinent tool for assessing spatial learning and memory, Exp. Anim. 71 (3) (2022) 264–280, https://doi.org/10.1538/expanim.21-0120 [published Online First: 2022/03/23].
[16] Q. Du, X. Zhu, J. Si, Angelica polysaccharide ameliorates memory impairment in Alzheimer's disease rat through activating BDNF/TrkB/CREB pathway, Exp Biol Med (Maywood) 245 (1) (2020) 1–10, https://doi.org/10.1177/1535370219894558 [published Online First: 2019/12/19].
[17] X. Ma, M. Song, Y. Yan, G. Ren, J. Hou, G. Qin, W. Wang, Z. Li, Albiflorin alleviates cognitive dysfunction in STZ-induced rats, Aging (Albany NY) 13 (14) (2021) 18287–18297, https://doi.org/10.18632/aging.203274 [published Online First: 2021/07/29].
[18] C.H. Chou, C.R. Yang, Neuroprotective studies of evodiamine in an okadaic acid-induced neurotoxicity, Int. J. Mol. Sci. 22 (10) (2021), https://doi.org/10.3390/ijms22105347 [published Online First: 2021/06/03].
[19] J. Fu, H. Zhang, S. Liu, J. Wu, Y. Zhang, Y. Gao, F. Song, Y. Qin, X. Hu, Z. Liu, An integrated strategy using LC-MS/MS combined with in vivo microdialysis for the simultaneous determination of lignans of Schisandra chinensis (Turcz.) Baill. Fructus and endogenous neurotransmitters: application in pharmacokinetic and pharmacodynamic studies, Food Funct. 12 (19) (2021) 8932–8945, https://doi.org/10.1039/d1fo01682b [published Online First: 2021/10/05].
[20] K. Sowndhararajan, P. Deepa, M. Kim, S.J. Park, S. Kim, An overview of neuroprotective and cognitive enhancement properties of lignans from Schisandra chinensis, Biomed. Pharmacother. 97 (2018) 958–968, https://doi.org/10.1016/j.biopha.2017.10.145 [published Online First: 2017/11/16].
[21] J.F. Liao, W.F. Chiou, Y.C. Shen, G.J. Wang, C.F. Chen, Anti-inflammatory and anti-infectious effects of Evodia rutaecarpa (Wuzhuyu) and its major bioactive components, Chin. Med. 6 (1) (2011) 6, https://doi.org/10.1186/1749-8546-6-6 [published Online First: 2011/02/16].
[22] H. Yu, H. Jin, W. Gong, Z. Wang, H. Liang, Pharmacological actions of multi-target-directed evodiamine, Molecules 18 (2) (2013) 1826–1843, https://doi.org/10.3390/molecules18021826 [published Online First: 2013/02/26].
[23] Z. Fang, Y. Tang, J. Ying, C. Tang, Q. Wang, Traditional Chinese medicine for anti-Alzheimer's disease: berberine and evodiamine from Evodia rutaecarpa, Chin. Med. 15 (2020) 82, https://doi.org/10.1186/s13020-020-00359-1 [published Online First: 2020/08/11].
[24] X. Du, X. Wang, M. Geng, Alzheimer's disease hypothesis and related therapies, Transl. Neurodegener. 7 (2018) 2, https://doi.org/10.1186/s40035-018-0107-y [published Online First: 2018/02/10].
[25] R.J. O'Brien, P.C. Wong, Amyloid precursor protein processing and Alzheimer's disease, Annu. Rev. Neurosci. 34 (2011) 185–204, https://doi.org/10.1146/annurev-neuro-061010-113613 [published Online First: 2011/04/05].
[26] M. Wang, F. Lin, X. Zhang, M. Zhang, T. Yan, B. Wu, Y. Du, B. He, Y. Jia, Combination of alpinia oxyphylla fructus and Schisandra chinensis fructus ameliorates aluminum-induced Alzheimer's disease via reducing BACE1 expression, J. Chem. Neuroanat. 126 (2022), 102180, https://doi.org/10.1016/j.jchemneu.2022.102180 [published Online First: 2022/10/29].
[27] B. Zhao, Y. Wang, R. Liu, X.L. Jia, N. Hu, X.W. An, C.G. Zheng, C. Chen, H.T. Sun, F. Chen, J.J. Wang, X.H. Li, Rutaecarpine ameliorated high sucrose-induced Alzheimer's disease like pathological and cognitive impairments in mice, Rejuvenation Res. 24 (3) (2021) 181–190, https://doi.org/10.1089/rej.2020.2349 [published Online First: 2020/09/08].
[28] J.H. Peng, C.E. Zhang, W. Wei, X.P. Hong, X.P. Pan, J.Z. Wang, Dehydroevodiamine attenuates tau hyperphosphorylation and spatial memory deficit induced by activation of glycogen synthase kinase-3 in rats, Neuropharmacology 52 (7) (2007) 1521–1527, https://doi.org/10.1016/j.neuropharm.2007.02.008 [published Online First: 2007/04/17].
[29] I. Grundke-Iqbal, K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, L.I. Binder, Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology, Proc Natl Acad Sci U S A 83 (13) (1986) 4913–4917, https://doi.org/10.1073/pnas.83.13.4913 [published Online First: 1986/07/01].
[30] A.H. Nagahara, D.A. Merrill, G. Coppola, S. Tsukada, B.E. Schroeder, G.M. Shaked, L. Wang, A. Blesch, A. Kim, J.M. Conner, E. Rockenstein, M.V. Chao, E. H. Koo, D. Geschwind, E. Masliah, A.A. Chiba, M.H. Tuszynski, Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease, Nat Med 15 (3) (2009) 331–337, https://doi.org/10.1038/nm.1912 [published Online First: 2009/02/10].
[31] Y. Shang, X. Wang, F. Li, T. Yin, J. Zhang, T. Zhang, rTMS ameliorates prenatal stress-induced cognitive deficits in male-offspring rats associated with BDNF/TrkB signaling pathway, Neurorehabil Neural Repair 33 (4) (2019) 271–283, https://doi.org/10.1177/1545968319834898 [published Online First: 2019/04/14].
[32] M. Kaushik, P. Kaushik, S. Parvez, Memory related molecular signatures: the pivots for memory consolidation and Alzheimer's related memory decline, Ageing Res. Rev. 76 (2022), 101577, https://doi.org/10.1016/j.arr.2022.101577 [published Online First: 2022/02/02].
[33] T. Yan, M. Xu, S. Wan, M. Wang, B. Wu, F. Xiao, K. Bi, Y. Jia, Schisandra chinensis produces the antidepressant-like effects in repeated corticosterone-induced mice via the BDNF/TrkB/CREB signaling pathway, Psychiatry Res 243 (2016) 135–142, https://doi.org/10.1016/j.psychres.2016.06.037 [published Online First: 2016/07/09].
[34] T. Yan, Q. Mao, X. Zhang, B. Wu, K. Bi, B. He, Y. Jia, Schisandra chinensis protects against dopaminergic neuronal oxidative stress, neuroinflammation and apoptosis via the BDNF/Nrf2/NF-kappaB pathway in 6-OHDA-induced Parkinson's disease mice, Food Funct. 12 (9) (2021) 4079–4091, https://doi.org/10.1039/d0fo02836c [published Online First: 2021/05/13].
[35] M. Llorens-Martin, J. Jurado, F. Hernandez, J. Avila, GSK-3beta, a pivotal kinase in Alzheimer disease, Front. Mol. Neurosci. 7 (2014) 46, https://doi.org/10.3389/fnmol.2014.00046 [published Online First: 2014/06/07].
[36] J.C. Gandy, M. Melendez-Ferro, G.N. Bijur, F. Van Leuven, J.K. Roche, B. Lechat, H. Devijver, D. Demedts, E. Perez-Costas, R.C. Roberts, Glycogen synthase kinase-3beta (GSK3beta) expression in a mouse model of Alzheimer's disease: a light and electron microscopy study, Synapse 67 (6) (2013) 313–327, https://doi.org/10.1002/syn.21642 [published Online First: 2013/02/08].
[37] P.T. Ly, Y. Wu, H. Zou, R. Wang, W. Zhou, A. Kinoshita, M. Zhang, Y. Yang, F. Cai, J. Woodgett, W. Song, Inhibition of GSK3beta-mediated BACE1 expression reduces Alzheimer-associated phenotypes, J. Clin. Invest. 123 (1) (2013) 224–235, https://doi.org/10.1172/JCI64516 [published Online First: 2012/12/04].
[38] D. Wang, C. Wang, L. Liu, S. Li, Protective effects of evodiamine in experimental paradigm of Alzheimer's disease, Cogn Neurodyn 12 (3) (2018) 303–313, https://doi.org/10.1007/s11571-017-9471-z [published Online First: 2018/05/17].