Current Medical Science
DOI https://doi.org/10.1007/s11596-022-2582-8
42(3):474-482,2022

Ginkgo Biloba Extract Ameliorates Scopolamine-induced Memory Deficits via Rescuing Synaptic Damage*

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[Abstract] Objective: Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder. Emerging evidence suggests that synaptic dysfunction is associated with the onset and progression of AD. Interestingly, Ginkgo biloba extract (EGb) is one of the most frequently investigated herbal medicines for enhancing cognition and alleviating neurodegenerative dementia. This study aimed to investigate the effect and the mechanism of EGb on AD-like synaptic disorders. **Methods:** Scopolamine (SCO)-induced rats were used to mimic AD-like memory deficits. Morris water maze test and fear conditioning test were conducted to evaluate the memory status of rats in response to different treatments. Then, the synapse alterations were assessed by Golgi staining, and Western blotting was conducted to assess the protein expression of PSD95, GluN2B, synapsin-1, and synaptophysin. Reverse transcription quantitative polymerase chain reaction was applied to detect the mRNA expression of PSD95 and the levels of miR-1-3p/miR-206-3p. **Results:** EGb supplement alleviated the learning and memory deficits induced by SCO in behavioral experiments. Moreover, EGb treatment attenuated synaptic damage elicited by SCO, manifested as increased dendritic spine density and the proportion of mushroom-type spines in hippocampal neurons. Further investigation indicated that EGb rescued the expression of synaptic-related proteins, especially PSD95, and decreased the levels of miR-1-3p/miR-206-3p in the rat hippocampus. **Conclusion:** The application of EGb effectively treats SCO-induced memory impairments probably by suppressing miR-1-3p/miR-206-3p and elevating the expression of PSD95.
Key words: Ginkgo biloba extract; Alzheimer's disease; synapse; PSD95; MiR-1; MiR-206

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive deficits. The formation of extracellular senile plaques and intracellular neurofibrillary tangles is a hallmark event for this disease. Notably, synaptic deterioration, which occurs in the early stage of AD, is commonly associated with memory impairments and subtle behavioral changes[1]. Furthermore, emerging lines of evidence have revealed that the accumulation of amyloid-β (Aβ) and hyperphosphorylated tau can directly or indirectly impair synaptic function, and even contribute to synaptic loss[2]. Accordingly, targeting the synaptic damage may be a promising research topic conducive to the early detection and treatment of AD.

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*This work was supported by the National Key Research and Development Project of China (No. 2017YFC1309601).

Ginkgo biloba (EGb) is one of the oldest living tree species in the world, and has been widely cultivated due to its health-promoting effect. The extract derived from the dried leaves of EGb contains two major constituents: flavonoids and terpene lactones. Both of them present a variety of biological activities. Substantial reports have documented that EGb has positive effects in attenuating AD and other age-related neurodegenerative disorders. For example, EGb can enhance adult hippocampal neurogenesis in an AD mouse model and alleviate the neuropathological damage of Parkinson's disease[3, 4]. Furthermore, EGb administration can suppress the excitotoxity and apoptosis in spinocerebellar ataxia type 17 cells and transgenic mice[5]. In addition, the study conducted by Zhang *et al* highlighted that supplemental treatment with EGb can mitigate the symptoms of tardive dyskinesia in schizophrenia patients[6]. Thus, EGb might be a promising medicine for the prevention and treatment of neuropsychological diseases.

Scopolamine (SCO) is a competitive nonselective

muscarinic acetylcholine receptor antagonist. A wealth of research evidence has established the paradigm that the impairment of central cholinergic systems contributes to deficits in learning and memory abilities. Furthermore, this pattern of memory dysfunction, which occur in healthy young people and rat models, is similar to that in patients who suffer from AD[7]. Hence, a number of medicines used for dementia therapy have been investigated in SCO-induced animal models. Although it has been reported that EGb, a cognitive enhancer, can reverse SCO-induced amnesia[8], its underlying molecular mechanisms has not been clearly established.

MicroRNAs (miRNAs) are highly conserved small noncoding RNA molecules, which are capable of regulating the expression of approximately 30% of all human genes at the post-transcriptional level[9]. Recently, in a 2-year National Toxicology Program, the researchers found that a number of miRNAs involved in carcinogenesis can be regulated by EGb treatment[10]. Furthermore, accumulating evidence suggests that a number of synaptic miRNAs confer important roles in modulating synaptic activities, contributing to the structural and functional organization of synapses, as well as synaptic strength and excitability[11].

The present study revealed that EGb can attenuate SCO-induced memory impairments and dendritic spine loss probably by downregulating miR-1-3p/miR-206-3p, and elevating the expression of PSD95.

1 MATERIALS AND METHODS

1.1 Reagents

The primary antibodies employed in the present study and the properties are listed in table 1. The SCO was purchased from Sigma Chemical Co. (USA). The EGb was obtained from BEST BIO-TECH Co., Ltd. (China), which contained 24% flavonol glycosides, 5%–7% terpene trilactones, and less than 5 ppm ginkgolic acids. The formulation and composition of the EGb were consistent with the standard EGb, which was registered in Germany and labeled EGb761.

1.2 Animals and Drug Administration

Male Sprague Dawley rats (grade Ⅱ, 200–250 g, 8 weeks old) were supplied by the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology (China). All rats were acclimated for one week before the experiments, and housed with free access to food and water under controlled environment and lighting conditions (12-h light-dark cycle). Afterwards, these rats were randomly divided into four groups, with 9 in each group: control (CON) group, SCO group, SCO+EGb group, and EGb group.

The SCO was dissolved in 3% dimethyl sulfoxide (DMSO) before injection. The EGb was suspended in normal saline, which contained 3% Tween® 80, before intragastric administration. The doses were determined based on the descriptions in previous studies[12, 13]. All experimental procedures are presented in fig. 1. Briefly, prior to the experiment, the rats were intragastrically administered with 400 mg/kg/day of EGb or the same volume of vehicle for 14 consecutive days. On the next day, rats in the SCO group and SCO+EGb group were intraperitoneally injected with SCO at a dose of 1 mg/kg for 30 min before the behavior training and tests, while rats in the CON group and EGb group were injected with 3% DMSO. The experiment was conducted during the light period, and these rats were euthanized at 24 h after the final injection. All animal experiments were performed in accordance to the guidelines of the Animal Care and Use Committee affiliated with Huazhong University of Science and Technology (China).

1.3 Open-field Activity

The testing apparatus consisted of a square box (1.0 m×1.0 m×0.7 m), which was placed in a separate room from the experimenter, and was surrounded by blue curtains. The outer 0.25-m region of the box was considered as the periphery, while the 0.5 m×0.5 m square region at the center was considered as the center area. In each test, the rats were initially placed at the center area, and allowed to freely move round for 10 min. Meanwhile, the movement of rats was recorded by an overhead camera. The total distance and percentage of movement distance in the center square were calculated.

1.4 Morris Water Maze Test

The Morris water maze is often used to assess the learning and memory abilities of rats. Briefly, the rats were trained to find a transparent platform hidden 1 cm under water from 3 different directions for 6 consecutive days. Three tests were conducted each day with 30-s intervals using a stationary array of cues outside the pool. A digital tracking device was connected to a computer to record the swimming traces

Table 1 Summary of commercial antibodies used

Name	Type	Catalog No.	Source	Dilution
PSD95	Mono	MAB1596	Sigma-Aldrich	1:1000 for WB
Synaptophysin	Mono	ab32127	Abcam	1:1000 for WB
Synapsin-1	Poly	20258-1-AP	Proteintech	1:1000 for WB
GluN2B	Poly	21920-1-AP	Proteintech	1:1000 for WB
GAPDH	Mono	60004-1-Ig	Proteintech	1:15000 for WB

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; WB: Western blotting

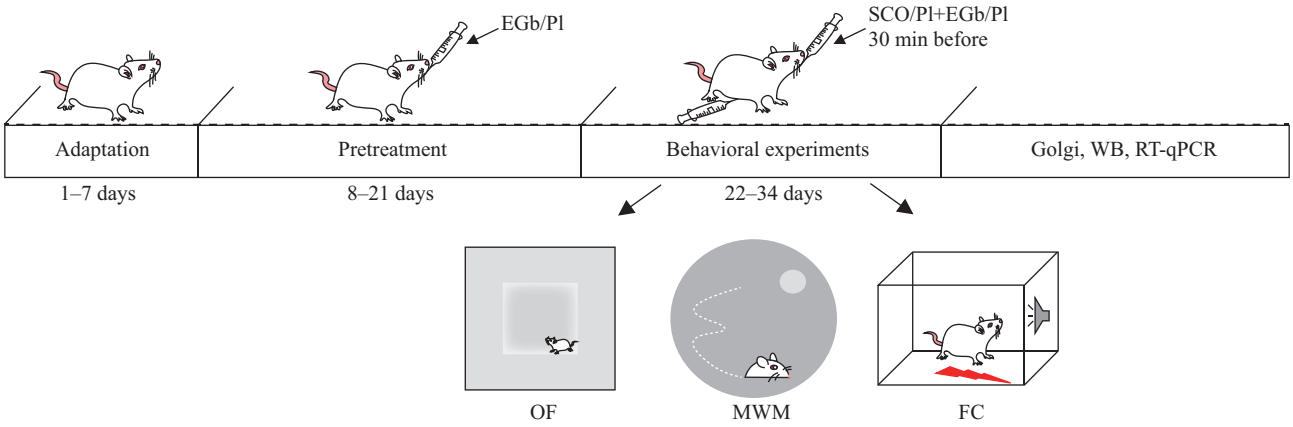

Fig. 1 Schematic illustration of the experimental design

After adaptation, a total of 18 rats were assigned to the SCO+EGb and EGb groups, and were treated with EGb for 2 weeks, while the remaining rats were assigned to the CON and SCO groups, and were administered with the same volume of vehicle. Before the behavioral tests, the SCO or vehicle was injected to rats after EGb for 30 min. Then, these rats were subjected to the behavioral tests, Western blotting, Golgi staining and reverse transcription quantitative polymerase chain reaction. EGb: Ginkgo biloba extract; SCO: scopolamine; OF: open-field activity; MWM: Morris water maze; FC: fear conditioning test. Pl: placebo

and escape latency. The test was terminated once the rats reached the platform. If the rat failed to reach the platform within 60 s, the rat was guided to the platform, and allowed to stay on the platform for 30 s. On the 7th day, the swimming traces and escape latency of rats to reach the hidden platform were recorded for comparison. On the 9th day, the number of times the rat crossed the platform and the latency of rats to reach the removed platform were recorded.

1.5 Fear Conditioning Test

Normal fear learning and memory allow animals to predict and avoid physical dangers, which is important for survival. The contextual and cued fear conditioning test is a behavioral paradigm used to assess hippocampus-, frontal cortex- and amygdala-dependent associative fear learning and memory[14]. Before the experiment, the rats were allowed to adapt to the novel environment for 60 min. Then, these rats were placed in a sound-attenuated startle chamber under light, which was equipped with a grid floor and conveyed foot shock. On the training day, each test was repeated 5 times, with a variable intertrial interval of 90–120 s, and included an acclimation for 180 s, a tone conditioned stimulus (CS) of 75 dB and 2.8 Hz for 30 s, and a 15-s trace interval following 1 mA of foot shock (US) for 2 s. After 60 s of the final shock, the rats were removed from the chamber, and the apparatus was sterilized with 75% alcohol. For the contextual fear test, rats were placed in the previous training compartments, and were allowed to freely explore the chamber for 300 s, without any CS or US presentation. For the cued fear test, rats were placed into another testing chamber with completely different properties, including color, pattern and odor. After adaptation to the new context, the activity and freezing behaviors of rats were evaluated using a video tracking system. Freezing behavior was defined as complete absence of any movement, except for respiration or heartbeat, and the duration of freezing response was recorded after immobility for one second. Freezing time (in percentage) was averaged within the same group to compare the difference among groups.

1.6 Golgi Staining and Dendritic Spine Analysis

The FD Rapid Golgi Stain kit (FD Neuro-Technologies, Inc., USA) was employed to examine the morphology of neuronal dendrites and dendritic spines. Briefly, the rats were anesthetized before euthanasia. Then, the brain tissues of these rats were removed as quickly as possible, and immersed in the impregnation solution (Solution A/B) at room temperature for 2 weeks, without light exposure. Afterwards, the above-mentioned tissues were transferred into Solution C for at least 72 h, according to manufacturer's instructions. Next, the brain tissues were sliced into 100-μm-thick sections using a Vibratome (VT1000S, Leica, Germany), and the neurons were imaged by bright field microscopy (Axio Observer, Zeiss, Germany). The images were coded, and the synaptic spines were counted using the ImageJ software.

1.7 Western Blotting

The rats were euthanized by cervical dislocation under anesthesia. Then, the hippocampus and prefrontal cortex were immediately extracted from the brain of rats, and homogenized in RIPA Lysis Buffer (Beyotime, China) with cocktail (Roche, Switzerland) on ice. Then, the mixture was boiled for 10 min. Next, the supernatant was collected after brief sonication and centrifugation at 12 000 g for 5 min. Afterwards, the protein concentration of the supernatant was measured using a bicinchoninic acid protein assay kit (Pierce, USA). After the separation by SDS-polyacrylamide gel electrophoresis (10% and 8% gels), the protein was transferred onto a nitrocellulose membrane. Then, the membrane was blocked with 5% skimmed milk at 25°C

for one hour, and incubated with the primary antibodies (table 1) at 4°C, overnight. Afterwards, anti-mouse or anti-rabbit IgG conjugated to IRDye™ (800 CW) was added to the membrane, and incubated at 25°C for one hour. Subsequently, the protein bands were visualized and quantified using the Odyssey Infrared Imaging System (LI-COR Biosciences, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference for proteins.

1.8 RNA Isolation and Reverse Transcription Quantitative Polymerase Chain Reaction

Total RNA was extracted using the TRIzol reagent (Invitrogen, Thermo Fisher Scientific, USA), according to manufacturer's instructions. Total RNA (1 μg) was employed to synthesize the complementary DNA (cDNA) of PSD95 using the Reverse Transcription Kit (Toyobo Life Science, Japan), and all samples were heated to 42°C for 60 min and 99°C for 5 min. For the miR-1-3p and miR-206-3p expression measurement, the miRcute Plus miRNA First-Strand cDNA Kit (Tiangen Biotech, China) was used for the reverse transcription from miRNA to cDNA, and the mixture was heated to 42°C for 60 min and 95°C for 3 min. All primers were designed using Primer Premier 5 and the Basic Local Alignment Search Tool, and synthesized by Sangon Biotech Co., Ltd. (China). These are listed in table 2. The RT-qPCR reaction was performed using the SYBR Green PCR Master Mix (Takara, Japan) and the CFX96 Real-Time PCR Detection System (Bio-Rad, USA). β-actin and U6 were used as internal references for the mRNAs and miRNAs. In addition, the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of genes and miRNAs of interest.

Table 2 Summary of primers used

Name	Forward	Reverse
PSD95	5′-GCTACCAAGATGAAGACACG-3′	5′-GAAGCCCAGACCTGAGTTAC-3′
β-actin	5′-GAGACCTTCAACACCCCAGC-3′	5′-GGAGAGCATAGCCCTCGTAGAT-3′
Rno-miR-1-3p	5′-TGGAATGTAAAGAAGTGTGTAT-3′	
Rno-miR-206-3p	5′-CGGAATGTAAGGAAGTGTGTGG-3′	5′-GCTGTCAACGATACGCTACG-3′
U6	5′-CGATGACACGCAAATTCGTGAA-3′	

1.9 Statistical Analysis

The data were presented as mean±standard error of the mean (SEM) of at least three independent experiments. The statistical analyses were performed in the GraphPad Prism software (version 8.0). The data of multiple groups were compared by one- or two-way analysis of variance, followed by Tukey's post-hoc test. The results were considered statistically significant when $P<0.05$.

2 RESULTS

2.1 EGb Ameliorates SCO-induced Memory Impairment

The open-field test was performed to evaluate the anxiety, depression, and spontaneous exploratory and locomotor ability of rats[15]. As shown in fig. 2A and 2B, rats in all the groups traveled the similar total distances, and had similar percentages of movement distances in the center area, indicating that these rats shared similar patterns of motor capacity that were not affected by emotions. Then, it was determined whether EGb could rescue the SCO-induced behavioral deficits. The Morris water maze test results revealed that compared with SCO-treated rats, the EGb supplement significantly attenuated the spatial learning impairment, which corresponded to the shorter escape latency to find the invisible platform (fig. 2D and 2E). On the 7th day, untreated rats in the SCO group continued to use a random search strategy, while rats in the SCO+EGb group took a nearly linear path to the platform (fig. 2C). On the 9th day, the hidden platform was removed, and the rats were allowed to swim freely during the probe test. It was found that compared to rats without SCO treatment, SCO-treated rats needed an obviously longer latency to reach the platform at the first time, and presented with fewer crossing times, while rats that were simultaneously treated with SCO and EGb exhibited similar behaviors to the control rats (fig. 2F and 2G). These above results suggest that the EGb treatment effectively improved the learning and memory impairments of SCO-induced rats.

Next, the fear conditioning test was performed to determine whether EGb could rescue the fear-motivated associative learning and memory impairments. In this test, the contextual fear memory and cue fear memory were evaluated at 24 and 48 h after the training session. As expected, it was observed that rats in the SCO group had a notably lower percentage of freezing time in both tests, while rats in the SCO+EGb group exhibited a similar performance to that in the CON group (fig. 2H and 2I).

These results reveal that EGb can alleviate the learning and memory deficits induced by SCO, contributing to building a precise spatial representation of the environment, and an association between aversive experiences and environmental cues.

2.2 EGb Improves SCO-induced Synaptic Abnormalities

Dendritic spines are dynamic structures, and the

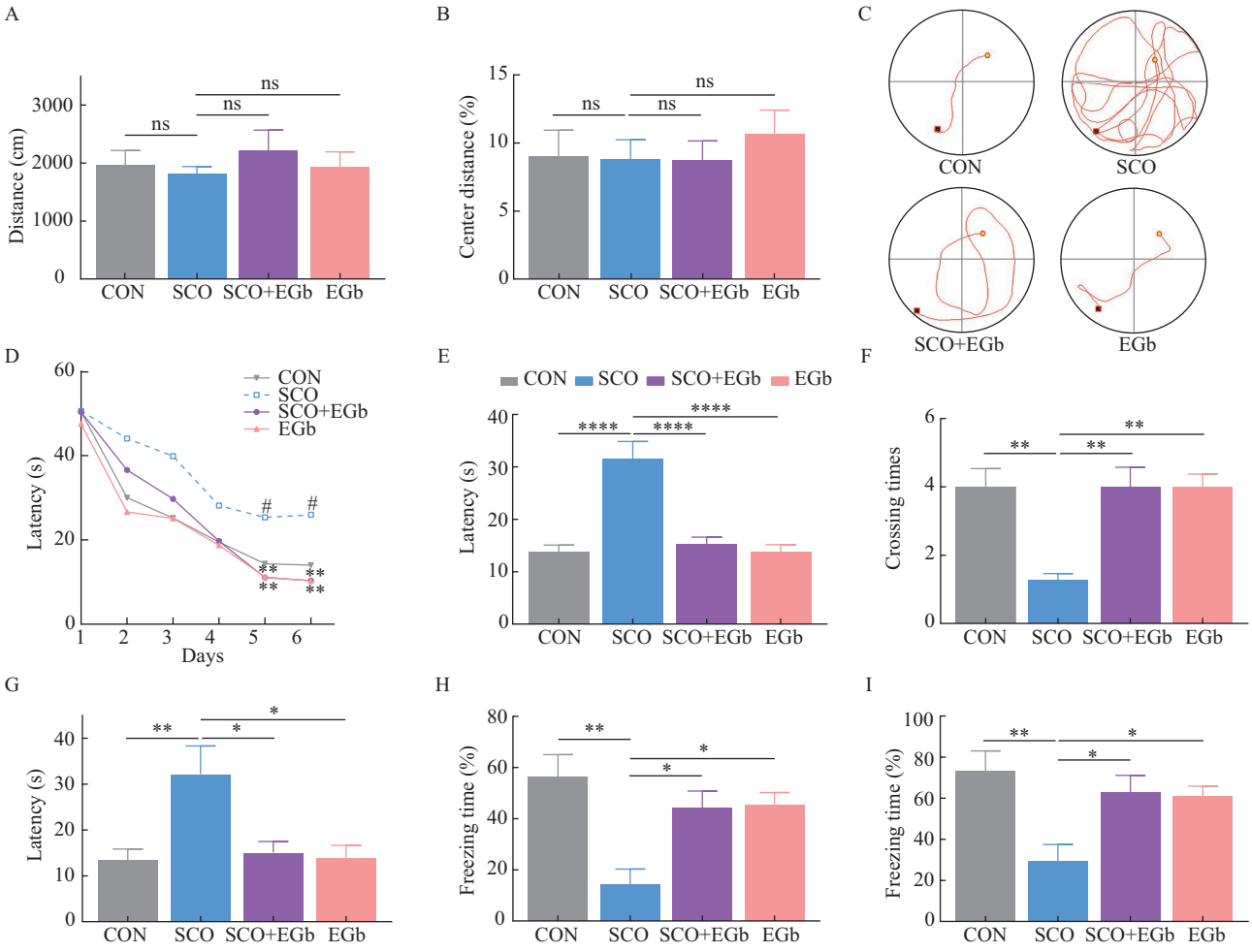

Fig. 2 EGb ameliorates scopolamine-induced memory impairments

A: the total distance that the rats moved in the open field; B: the percentage of movement distance in the center area of the open field; C: the representative searching trace on day 7 in the Morris water maze; D: the escape latency to find the invisible platform from day one to day 6; E: the escape latency to find the invisible platform on day 7; F: the number of platform crossings on day 9; G: the escape latency to initially reach the platform on day 9; H: the percentage of the freezing time in the contextual fear conditioning test; I: the percentage of the freezing time in the cue fear conditioning test. The data were presented as mean±SEM (n=9, animals per group). Ns, not significant. $^{*}P$<0.05, $^{**}P$<0.01, $^{****}P$<0.0001 *vs*. the SCO group; $^{\#}P$<0.05 *vs*. the CON group in panel 2D. CON: control group; SCO: scopolamine-treated group; SCO+EGb: scopolamine plus Ginkgo biloba extract-treated group; EGb: Ginkgo biloba extract-treated group

shape, size and density changes with the synaptic activity[16]. Furthermore, hippocampal synaptic plasticity plays a key role in learning and memory capacities. The Golgi staining method was performed to evaluate the spine morphological changes in the hippocampus. The results uncovered that after the SCO injection, the density of dendritic spines and the percentage of mushroom-type spines decreased, while these abnormalities were constrained after the EGb treatment (fig. 3).

In order to further investigate the protective effect of EGb against the synaptic damage elicited by SCO, Western blotting was performed to measure the expression of synaptic-related proteins, including PSD95, GluN2B in post-synapse and synapsin-1, and synaptophysin in pre-synapse. As shown in fig. 4A–4D, SCO dramatically suppressed the PSD95 expression in the rat hippocampus, while EGb significantly reversed this effect. In addition, in both the hippocampus and prefrontal cortex, the expression levels of GluN2B, synapsin-1 and synaptophysin exhibited a similar trend, but there were no significant differences between the SCO group and SCO+EGb group. These data suggest that EGb can improve the SCO-induced synaptic disorder, which is possibly involved in PSD95 dysregulation.

2.3 Effect of EGb on PSD95 Expression Probably Involves the Regulation of MiR-1-3p/miR-206-3p

Next, the study shifted to elucidate how the EGb treatment restored the PSD95 expression. RT-qPCR was performed to analyze the mRNA level of PSD95 in the hippocampus. Interestingly, no significant differences were found in all groups (fig. 5C). This suggests that EGb does not participate in the gene transcriptional regulation in this process. As it is known, miRNAs play an important role in post-

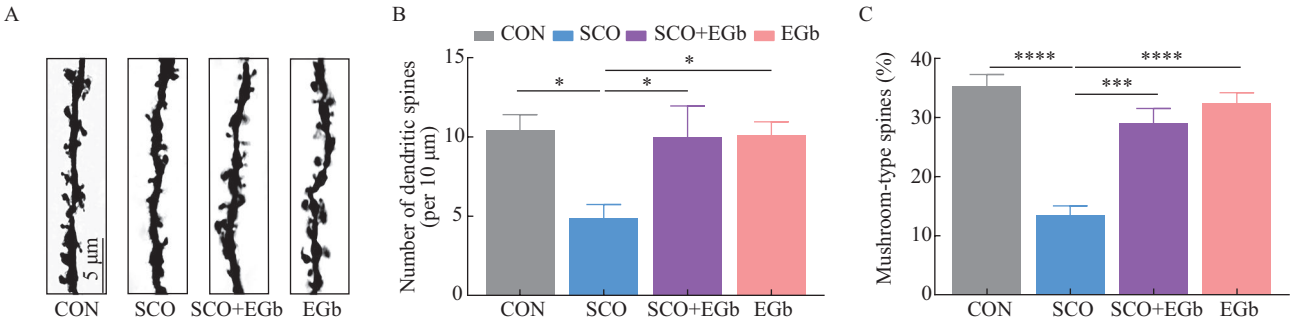

Fig. 3 Golgi staining was used to evaluate the effect of EGb on scopolamine-induced changes in dendritic spines

A: the representative images for dendritic spines; B: the quantification of dendritic spine density; C: the ratio of mushroom-type spines. The data were presented as mean±SEM (n=20, dendrites obtained from 5 animals per group). $^{*}P$<0.05, $^{***}P$<0.001, $^{****}P$<0.0001 *vs*. the SCO group. CON: control group; SCO: scopolamine-treated group; SCO+EGb: scopolamine plus Ginkgo biloba extract-treated group; EGb: Ginkgo biloba extract-treated group

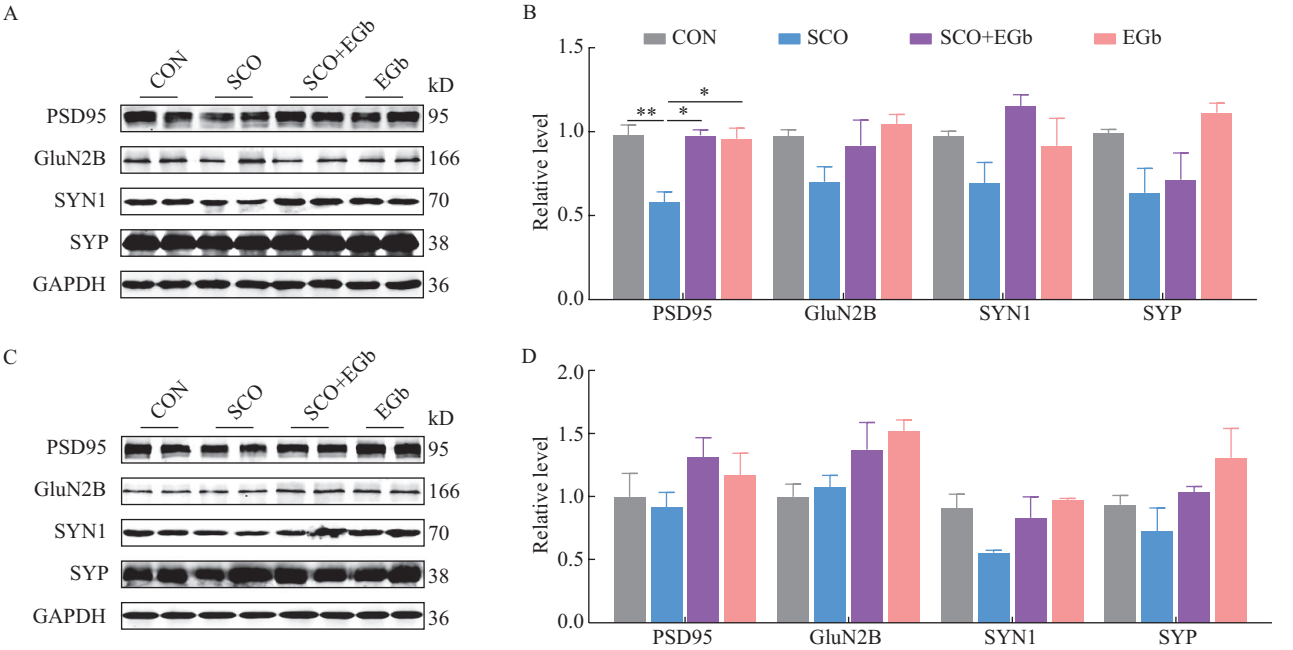

Fig. 4 Western blotting for the expression level of the synapse-related protein

A: the representative protein levels of PSD95, GluN2B, synapsin-1 and synaptophysin in the hippocampus; B: the quantification for the protein levels of PSD95, GluN2B, synapsin-1 and synaptophysin in the hippocampus; C: the representative protein levels of PSD95, GluN2B, synapsin-1 and synaptophysin in the prefrontal cortex; D: the quantification for the protein levels of PSD95, GluN2B, synapsin-1 and synaptophysin in the prefrontal cortex. The data were presented as mean±SEM (n=5, animals per group). $^{*}P$<0.05, $^{**}P$<0.01 *vs*. the SCO group. CON: control group; SCO: scopolamine-treated group; SCO+EGb: scopolamine plus Ginkgo biloba extract-treated group; EGb: Ginkgo biloba extract-treated group. SYN1: synapsin-1; SYP: synaptophysin

transcription regulation in eukaryotic cells, and the dysfunction of miRNAs is associated with synapse disorders[17]. In this study, potential miRNAs that might regulate the PSD95 expression were predicted using the Targetscan[18], microT-CDS[19] and miRDB[20] databases. Then, a Venn diagram was prepared to enhance the analysis reliability. The results revealed that miR-1-3p/miR-206-3p might bind to the 3′untranslated region (3′UTR) of PSD95, and that the binding site was highly conserved. Furthermore, the RT-qPCR results revealed that the miR-1-3p and miR-206-3p levels in the rat hippocampus were obviously enhanced after SCO treatment, while the supplemental EGb treatment completely negated the impact of the SCO treatment (fig. 5A, 5B and 5D). Thus, these present results indicate that the effect of EGb on PSD95 may be attributed to the decrease in miR-1-3p/miR-206-3p.

3 DISCUSSION

Ginkgo biloba has a long history of medical use. Previous studies reported that EGb exerts a positive effect against various cognition deficits in elderly db/db (–/–) diabetic mouse and rats with chronic cerebral hypoperfusion[21, 22]. The present study revealed that EGb can ameliorate SCO-induced memory impairments. Existing evidence has revealed that EGb can modulate the pre-synaptic choline uptake and

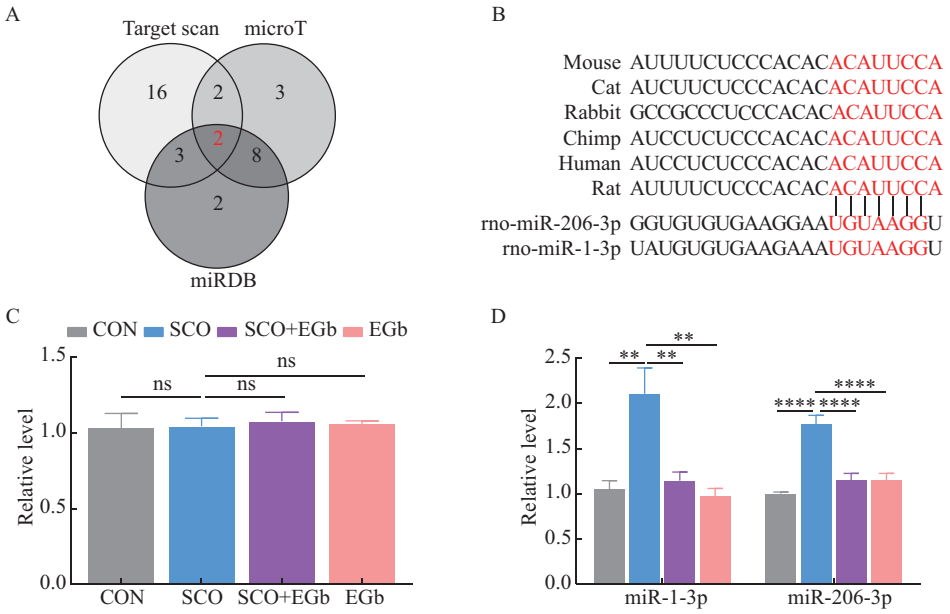

Fig. 5 The effect of EGb on the PSD95 expression probably involved the regulation of miR-1-3p/miR-206-3p

A: the intersection of possible miRNAs that target the rat PSD95 3′UTR based on the conservation prediction; B: the binding sites of miR-1-3p/miR-206-3p on the PSD95 3′UTR; C: the relative mRNA expression levels of PSD95; D: the relative expression levels of miR-1-3p/miR-206-3p, as measured by reverse transcription quantitative polymerase chain reaction. The data were presented as mean±SEM (n=5, animals per group). $^{**}P$<0.01, $^{****}P$<0.0001 *vs*. the SCO group. ns: not significant; CON: control group; SCO: scopolamine-treated group; SCO+EGb: scopolamine plus Ginkgo biloba extract-treated group; EGb: Ginkgo biloba extract-treated group

acetylcholine release, and upregulate post-synaptic muscarinic receptors to improve cognitive function[23]. In addition, it has been reported that EGb can improve the energy metabolism, stabilize the mitochondrial membrane, decrease cell apoptosis, and inhibit Aβ-induced neurotoxicity[24]. Furthermore, EGb can increase the level of brain-derived neurotrophic factors, and replicate the environment required for the neural differentiation of stem cells[25]. Moreover, recent studies have revealed that EGb may activate the PI3K/Akt/mTOR pathway to promote neurite growth and improve the level of drebrin protein, and partially inactivate cofilin to prevent dendritic spine degeneration[22, 26]. The structural plasticity of dendritic spines serves as a vital fundamental of learning. This observation suggests that EGb can improve SCO-induced synaptic abnormalities, which may involve PSD95 regulation. This finding may further confirm and clarify the neuroprotective effects of EGb.

At present, miRNAs have emerged as essential regulators of synaptic homeostasis and plasticity processes. A growing body of evidence has revealed the presence of specific miRNAs in axons and dendrites, such as miR-9, miR-15b, miR-16 and miR-204, which may locally regulate protein levels, and synaptic structure and function[27]. Interestingly, miR-135a-5p, a synaptic-associated miRNA, is abnormally downregulated in AD, accompanied by dendritic abnormalities and memory impairments[28]. MiR-206 is a member of the miR-1 family. There are only four nucleotides that are different from miR-206 and miR-1 outside the seed region, and the similarity between these two miRNAs indicate that these may have the same or similar target genes. MiR-206 is usually recognized as a muscle-enriched miRNA that promotes muscle development[29]. However, a recent study revealed that miR-206 is aberrantly increased in mild cognitive impairment subjects[30]. Furthermore, a previous report clarified that miR-206 overexpression in Tg2576 mice negatively regulates the brain-derived neurotrophic factor[31]. Moreover, the relative increase in expression of miR-1 in the hippocampus is in line with the memory deficits induced by SCO administration or stress[32]. It is noteworthy that a genetic variation of miR-206 was found in schizophrenia (SCZ) patients[33]. In addition, a previously conducted exome sequencing study of SCZ patients also revealed mutations of PSD, including PSD95[34]. The present study revealed that EGb increases the expression of PSD95 probably by downregulating miR-1-3p/miR-206-3p, in order to rescue the SCO-induced memory impairments. This further points out the potential regulatory role of miR-206/miR-1 in AD.

PSD95, a postsynaptic density protein, stabilizes filopodia and aids spine formation through recruitment or modification by a second messenger[35]. The present study revealed that SCO downregulated the level of PSD95 in the hippocampus, while GluN2B, synapsin-1 and synaptophysin were not significantly altered in the hippocampus and prefrontal cortex. One

reason can be due to the experimental conditions, in which the SCO induced mild synaptic dysfunction and memory impairments. Some authors have reported that compared with the pre-synaptic element, the post-synaptic portion appears to have greater vulnerability and earlier degradation, since the expression levels of the presynaptic protein may increase or have no change in the early and late stages of AD[36]. In a study, the memory deficit of Swiss strain albino mice induced by a dose of 2 mg/kg of SCO was correlated with the decline in the expression of GluN2B in the hippocampus and prefrontal cortex[37], while the expression level of GluNR1, an obligatory component of NMDA receptors, was higher in the SCO group at a dose of 1 mg/kg, when compared to the control group[38]. Hence, the effect of SCO on the expression of NMDA receptors may be worthy of further investigation.

SCO dementia models have many strengths. For example, the relatively good quality and low costs make the model amenable to high-throughput screening approaches[39]. Nevertheless, some investigators have proposed that antimuscarinic agents provide low construct validity in AD research, since SCO impairments are acute and primarily postsynaptic, while the pathological features of dementia are chronic and primarily presynaptic. This may also partly explain the unremarkable difference of synapsin-1 and synaptophysin in this study. In addition, the interruption of memory deficits by SCO may be confused by the effect of attention or other general impairments[40]. Despite controversies, this model has retained its popularity in drug discovery programs.

Overall, these present findings reveal that EGb can rescue the SCO-induced memory deficits and synaptic disorders, which probably involves the repression of the expression of miR-1-3p/miR-206-3p and the enhancement of PSD95 expression. Thus, the present study provides interesting targets for the prevention and treatment of synaptic disorders. Future studies are warranted to understand the mechanistic link and validation of these changes in other study cohorts.

Conflict of Interest Statement

The authors declare that there were no conflicts of interest. All authors agreed to submit the manuscript to Current Medical Science.

Author Xiao-ping LUO is a member of the Editorial Board for Current Medical Science, and Wei WU is a member of the Young Editorial Board for Current Medical Science. The paper was handled by other editors and has undergone rigorous peer review process. Authors Xiao-ping LUO and Wei Wu were not involved in the journal's review of, or decision related to, this manuscript.

REFERENCES

1 Arendt T. Synaptic degeneration in Alzheimer's disease. Acta Neuropathol, 2009,118(1):167-179
2 Rajmohan R, Reddy PH. Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. J Alzheimers Dis, 2017,57(4):975-999
3 Tchantchou F, Xu Y, Wu Y, *et al*. EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. Faseb J, 2007,21(10):2400-2408
4 Tanaka K, Galduróz RF, Gobbi LT, *et al*. Ginkgo biloba extract in an animal model of Parkinson's disease: a systematic review. Curr Neuropharmacol, 2013,11(4):430-435
5 Huang DS, Lin HY, Lee-Chen GJ, *et al*. Treatment with a Ginkgo biloba extract, EGb 761, inhibits excitotoxicity in an animal model of spinocerebellar ataxia type 17. Drug Des Devel Ther, 2016,10:723-731
6 Zhang WF, Tan YL, Zhang XY, *et al*. Extract of Ginkgo biloba treatment for tardive dyskinesia in schizophrenia: a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry, 2011,72(5):615-621
7 Chopin P, Briley M. Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. Psychopharmacology (Berl), 1992,106(1):26-30
8 Zhang J, Wang J, Zhou GS, *et al*. Studies of the Anti-amnesic Effects and Mechanisms of Single and Combined Use of Donepezil and Ginkgo Ketoester Tablet on Scopolamine-Induced Memory Impairment in Mice. Oxid Med Cell Longev, 2019,2019:8636835
9 Felekkis K, Touvana E, Stefanou C, *et al*. microRNAs: a newly described class of encoded molecules that play a role in health and disease. Hippokratia, 2010,14(4):236-240
10 Yamashita H, Surapureddi S, Kovi RC, *et al*. Unique microRNA alterations in hepatocellular carcinomas arising either spontaneously or due to chronic exposure to Ginkgo biloba extract (GBE) in B6C3F1/N mice. Arch Toxicol, 2020,94(7):2523-2541
11 Ye Y, Xu H, Su X, *et al*. Role of MicroRNA in Governing Synaptic Plasticity. Neural Plast, 2016,2016:4959523
12 Zeng K, Li M, Hu J, *et al*. Ginkgo biloba Extract EGb761 Attenuates Hyperhomocysteinemia-induced AD Like Tau Hyperphosphorylation and Cognitive Impairment in Rats. Curr Alzheimer Res, 2018,15(1):89-99
13 Wang X, Wang ZH, Wu YY, *et al*. Melatonin attenuates

scopolamine-induced memory/synaptic disorder by rescuing EPACs/miR-124/Egr1 pathway. Mol Neurobiol, 2013,47(1):373-381
14 Shoji H, Takao K, Hattori S, *et al*. Contextual and cued fear conditioning test using a video analyzing system in mice. J Vis Exp, 2014,85:50871
15 Tatem KS, Quinn JL, Phadke A, *et al*. Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. J Vis Exp, 2014(91):51785
16 Khanal P, Hotulainen P. Dendritic Spine Initiation in Brain Development, Learning and Diseases and Impact of BAR-Domain Proteins. Cells, 2021,10(9):2392
17 Kumar S, Reddy PH. The role of synaptic microRNAs in Alzheimer's disease. Biochim Biophys Acta Mol Basis Dis, 2020,1866(12):165937
18 McGeary SE, Lin KS, Shi CY, *et al*. The biochemical basis of microRNA targeting efficacy. Science, 2019, 366(6472):eaav1741
19 Kiriakidou M, Nelson PT, Kouranov A, *et al*. A combined computational-experimental approach predicts human microRNA targets. Genes Dev, 2004,18(10):1165-1178
20 Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res, 2015,43(Database issue):D146-152
21 Guan ZF, Zhang XM, Tao YH, *et al*. EGb761 improves the cognitive function of elderly db/db(–/–) diabetic mice by regulating the beclin-1 and NF-κB signaling pathways. Metab Brain Dis, 2018,33(6):1887-1897
22 Yao ZH, Wang J, Yuan JP, *et al*. EGB761 ameliorates chronic cerebral hypoperfusion-induced cognitive dysfunction and synaptic plasticity impairment. Aging (Albany NY), 2021,13(7):9522-9541
23 Nathan P. Can the cognitive enhancing effects of ginkgo biloba be explained by its pharmacology? Med Hypotheses, 2000,55(6):491-493
24 Singh SK, Srivastav S, Castellani RJ, *et al*. Neuroprotective and Antioxidant Effect of Ginkgo biloba Extract Against AD and Other Neurological Disorders. Neurotherapeutics, 2019,16(3):666-674
25 Ren C, Ji YQ, Liu H, *et al*. Effects of Ginkgo biloba extract EGb761 on neural differentiation of stem cells offer new hope for neurological disease treatment. Neural Regen Res, 2019,14(7):1152-1157
26 Lejri I, Grimm A, Eckert A. Ginkgo biloba extract increases neurite outgrowth and activates the Akt/mTOR pathway. PLoS One, 2019,14(12):e0225761
27 Siedlecki-Wullich D, Miñano-Molina AJ, Rodríguez-Álvarez J. microRNAs as Early Biomarkers of Alzheimer's Disease: A Synaptic Perspective. Cells, 2021,10(1):113
28 Zheng K, Hu F, Zhou Y, *et al*. miR-135a-5p mediates memory and synaptic impairments via the Rock2/Adducin1 signaling pathway in a mouse model of Alzheimer's disease. Nat Commun, 2021,12(1):1903
29 Ma G, Wang Y, Li Y, *et al*. MiR-206, a key modulator of skeletal muscle development and disease. Int J Biol Sci, 2015,11(3):345-352
30 Piscopo P, Lacorte E, Feligioni M, *et al*. MicroRNAs and mild cognitive impairment: A systematic review. Ageing Res Rev, 2019,50:131-141
31 Lee ST, Chu K, Jung KH, *et al*. miR-206 regulates brain-derived neurotrophic factor in Alzheimer disease model. Ann Neurol, 2012,72(2):269-277
32 Eivani M, Alijanpour S, Arefian E, *et al*. Corticolimbic analysis of microRNAs and protein expressions in scopolamine-induced memory loss under stress. Neurobiol Learn Mem, 2019,164:107065
33 Coley AA, Gao WJ. PSD95: A synaptic protein implicated in schizophrenia or autism? Prog Neuro-psychopharmacol Biol Psychiatry, 2018,82:187-194
34 Hansen T, Olsen L, Lindow M, *et al*. Brain expressed microRNAs implicated in schizophrenia etiology. PLoS One, 2007,2(9):e873
35 Prange O, Murphy TH. Modular transport of postsynaptic density-95 clusters and association with stable spine precursors during early development of cortical neurons. J Neurosci, 2001,21(23):9325-9333
36 Liu J, Chang L, Roselli F, *et al*. Amyloid-β induces caspase-dependent loss of PSD-95 and synaptophysin through NMDA receptors. J Alzheimers Dis, 2010,22(2): 541-556
37 Rai R, Singh HK, Prasad S. A Special Extract of Bacopa monnieri (CDRI-08) Restores Learning and Memory by Upregulating Expression of the NMDA Receptor Subunit GluN2B in the Brain of Scopolamine-Induced Amnesic Mice. Evid Based Complement Alternat Med, 2015,2015:254303
38 Falsafi SK, Deli A, Höger H, *et al*. Scopolamine administration modulates muscarinic, nicotinic and NMDA receptor systems. PLoS One, 2012,7(2):e32082
39 Terry AV, Jr. In: Levin ED, Buccafusco JJ, editors. Animal Models of Cognitive Impairment. Boca Raton (FL): CRC Press/Taylor & Francis; 2006.
40 Svoboda J, Popelikova A, Stuchlik A. Drugs Interfering with Muscarinic Acetylcholine Receptors and Their Effects on Place Navigation. Front Psychiatry, 2017,8: 215

(Received May 7, 2021; accepted Nov. 1, 2021)