

Schisantherin A improves learning and memory abilities partly through regulating the Nrf2/Keap1/ARE signaling pathway in chronic fatigue mice

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Abstract. Chronic fatigue is frequently accompanied by decreased learning and memory capabilities. Schisantherin A (SCA) is one of the main active monomer components in *Schisandra chinensis* lignans. In the present study, a chronic fatigue mouse model was established using the exhausted swimming approach to investigate the effects of SCA on learning and memory and its associated mechanism of action. Learning and memory abilities were tested by step through tests and water maze methods. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) in hippocampal tissue were measured by corresponding assays. The effect of SCA on the expression of kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), Bcl2, Bax and cleaved caspase-3 were determined by western blot. The present results showed that SCA can improve the learning and memory capabilities of chronic fatigue mice. SCA was found to increase the activities of SOD and CAT in addition to increasing the levels of GSH but reduced the levels

of MDA in hippocampus tissues. Furthermore, SCA treatment downregulated the protein expression levels of Keap1, Bax and cleaved caspase-3 and upregulated the protein expression levels of Nrf2, HO1 and Bcl2 in the hippocampus. These results suggested that modulations in the Nrf2-Keap1-antioxidant response element pathway, anti-oxidative and anti-apoptosis effects are the causes underlying the improvements from SCA treatment on the learning and memory abilities of chronic fatigue mice.

Introduction

Suboptimal-health is a state between health and disease (1). Long-term stress, fatigue, environmental pollution and other factors can cause suboptimal-health (2). A previous study suggested that an increasing number of people have a suboptimal level of health and that the worldwide proportion of the population with suboptimal-health is as high as 75% (3). Study of an ethnically diverse community in the US indicated that 2-11% of people experience severe fatigue with a duration of at least six months (4). As a principal manifestation and symptom of suboptimal-health, chronic fatigue appears likely to become one of the predominant factors affecting human health (5,6). Continuous excessive physiological activities, such as strenuous exercise, can cause a large number of free radicals, resulting in oxidative stress injury that can in turn induce chronic fatigue (7,8). The occurrence of chronic fatigue is frequently accompanied with reductions in organ function, especially that in the brain, which is characterized by decreases in learning and memory capabilities (4,9,10), and greatly reduces overall quality of life. Therefore, research and development into agents and health foods that can protect against fatigue will likely have a global impact.

Schisandra chinensis is the mature fruit of *Schisandra chinensis* in the *Magnoliaceae* family that was first recorded in the Shennong materia medica >2,000 years ago, which has been used as a therapeutic agent or nutritional supplement in the United States, Japan, South Korea and China (11,12). Schisantherin A (SCA) is one of the main active monomer components of the *Schisandra chinensis* lignans and has been

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Abbreviations: SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; CAT, catalase; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1

Key words: Schisantherin A, fatigue, brain, nuclear factor (erythroid-derived 2)-like 2-kelch-like ECH-associated protein 1-antioxidant response element pathway, apoptosis

reported to confer anti-inflammatory, antioxidant and memory improving effects (13). A previous study found that SCA not only significantly enhanced the exercise endurance of chronic fatigue mice (14), but also improved the learning and memory abilities of aging mice, in a D-galactose induced manner (15). However, to the best of our knowledge, no previous reports currently exist regarding the effects of SCA on the learning and memory abilities on chronic fatigue mouse models. Therefore, the present study aimed to investigate the effects of SCA on the learning and memory abilities of chronic fatigue mice, to provide a theoretical basis for the research and development into exploiting the use of *Schisandra* in functional health foods.

Materials and methods

Reagents and materials. A total of 40 male ICR mice (age, 4–6 weeks), weighing 19 ± 2 g, were provided by the Experimental Animal Research Center, Jilin University [Production license no. of experimental animals: SCXK (Ji)-2016-0003]. The research design was approved by the Animal Ethics Committee of Beihua University (Jilin City, China) and all experiments were conducted in accordance with the established guidelines for animal research [The 2010/63/EU directive (2010) on the Protection of Animals] (16,17). Mice were maintained at a temperature of 18–22°C and in a 50–60% humidity-controlled environment with a light/dark cycle of 12-h and under specific pathogen-free conditions, with free access to food and water.

SCA was provided by Sichuan Weikeyi Biotechnology Co., Ltd. Superoxide dismutase (SOD; cat. no. A001-3-2), malondialdehyde (MDA; cat. no. A003-1-2), glutathione (GSH; cat. no. A006-2-1) and catalase (CAT; cat. no. A007-1-1) activity assay kits were purchased from Nanjing Jiancheng Bioengineering Research Institute. RIPA lysis buffer (cat. no. WB-0071) was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Kelch-like ECH-associated protein 1 (Keap1; 1:1,000; diluent, TBST containing 1% Tween-20; cat. no. A17061), Nuclear factor (erythroid-derived 2)-like 2 (Nrf2; 1:1,000; diluent, TBST containing 1% Tween-20; cat. no. A0674), heme oxygenase-1 (HO-1; 1:1,000; diluent, TBST containing 1% Tween-20; cat. no. A1346), Bcl2 (1:1,000; diluent, TBST containing 1% Tween-20, cat. no. A16776), Bax (1:1,000; diluent, TBST containing 1% Tween-20; cat. no. A15646), cleaved-caspase-3 (1:1,000; diluent, TBST containing 1% Tween-20; cat. no. A0214), GAPDH (1:50,000; diluent, TBST containing 1% Tween-20; cat. no. AC033), horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG H + L (1:5,000; diluent, TBST containing 1% Tween-20; cat. no. AS014) and HRP goat anti-mouse IgG H + L (1:5,000; diluent, TBST containing 1% Tween-20; cat. no. AS003) antibodies were purchased from ABclonal Biotech Co., Ltd. ECL chromogenic solution was purchased from Vazyme Biotech Co., Ltd.

Instruments. The mouse passive avoidance system (cat. no. 080057, model no. BA-200) and rat Morris water maze video analysis system (cat. no. 43513, model no. WMT-100, software version no. 1.0.0.1) were supplied by Chengdu Taimeng Software Co., Ltd. Western blot gel electrophoresis apparatus and transfer instruments were purchased from Bio-Rad Laboratories, Inc. The automatic gel imaging and

analysis system was purchased from Beijing Sage Creation Science Co., Ltd. The Infinite M200 automatic plate reader was obtained from Tecan Group, Ltd.

Animal grouping and administration. The mice were allowed to acclimatize to the laboratory environment for 7 days and then randomly divided into the following four groups (n=10 mice per group): i) Control group, (CON), which were treated with distilled water by gavage and subjected to sedentary experiments; ii) chronic fatigue model group (MOD), which were treated with distilled water by gavage and underwent exhaustive exercise training; iii) SCA control group [SCA (C)], which received 2.5 mg/kg SCA by gavage and was subjected to sedentary experiment; and iv) SCA model group [SCA (M)], which were treated with 2.5 mg/kg SCA by gavage followed by exhaustive exercise training. Mice in the SCA (C) and SCA (M) groups were given 2.5 mg/kg SCA once a day by gavage continuously for 6 weeks, whilst those in CON and MOD groups were given an equal volume of distilled water using the same approach. Subsequently, behavior of mice was observed and the related biochemical indicators were investigated. The specific experimental processes are shown in Fig. 1.

Loaded swimming training. The chronic fatigue mouse model was established using a loaded swimming training procedure as previously described (14,18,19). The mice were first placed in a plastic container, with a water depth of 20 cm at $15 \pm 2^\circ\text{C}$. In the first week, mice in each group were trained for adaptation. On weeks 2–6, the mice were then burdened with a 10% lead block attached to the tail. The training on the first day lasted 30 min, 45 min on the second day and then 60 min on each of the following days. The mice were trained for 5 days per week. Subsequently, daily swimming training sessions each lasting 60 min were performed from weeks 2–6, in which the body weight of mice was measured weekly and the loaded weight was adjusted to 10% of their body weight.

Learning and memory test

Step-through test. On day 43 of the experiment, step-through training was performed. After a further 24 h, the step-through test began. Darkness avoidance latency and the number of errors were observed and recorded over a 5 min period to investigate the effect of SCA on the learning and memory ability of chronic fatigue mice.

Morris water maze test. Morris water maze test was performed between days 45 and 50. The mice were placed in the MWT-100 Morris water maze video tracking test system. The training time was set as 120 sec maximum. If the mice were unable to reach the platform within 120 sec, this was recorded as 120 sec. The mice were trained once every 24 h successively for 5 days. On day 6, the platform for testing spatial search function was removed, following which the number of times the mice crossed the point where the platform would have been and the effective area that the mice passed through were recorded.

Detection of SOD, CAT, GSH and MDA levels in hippocampal tissue. On day 51 of the experiment, mice in all groups were euthanized with a lethal dose of pentobarbital (210 mg/kg), and their

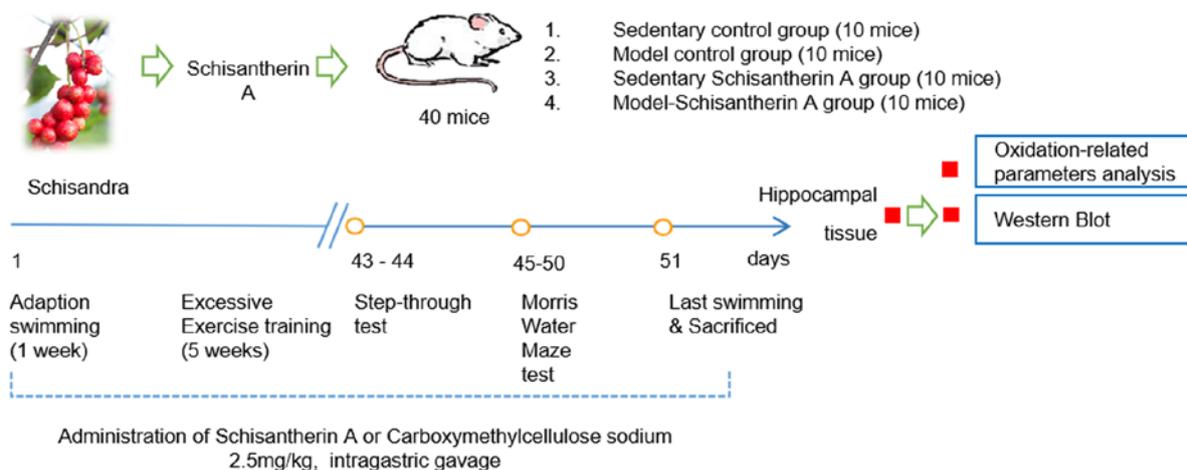


Figure 1. Flow chart of animal experiments.

hippocampi were taken to prepare hippocampal homogenate. The hippocampus was homogenized in a glass homogenizer with x10 saline on ice, centrifuged at 160 x g, for 10 min, and then the supernatant was collected. Hippocampal tissue homogenate was then diluted with x10 saline for the determination of the activities of SOD and CAT, as well as the concentration of GSH and MDA, in the hippocampus homogenate were then determined according to the protocols of the kits.

Detection of Keap1, Nrf2, HO-1, Bcl2, Bax and cleaved-caspase-3 protein expression levels in the hippocampus using western blotting. Protein lysis buffer (RIPA Lysis Buffer) was added to the hippocampus homogenates. Subsequently, the protein concentration was detected using bicinchoninic acid protein assay and 10% SDS-PAGE gel electrophoresis was performed on the samples (60 μ g of protein per lane). Proteins were then transferred onto PVDF membranes and blocked with blocking buffer (TBST buffer containing 5% skim milk powder) for 1 h at room temperature before the primary antibodies (1:1,000) were added and incubated overnight at 4°C. Secondary antibodies (1:5,000) were then added onto the membranes after washing with TBST and incubated at room temperature for 1 h. ECL chromogenic solution was used to develop the bands after the membranes were washed. ImageJ (version 1.51j8; National Institutes of Health) was used to perform the western blotting densitometric analysis.

Statistical analysis. SPSS 20.0 software (IBM Corp.) was used to analyze the data. Mice behavioral data, SOD and CAT activities, in addition to GSH and MDA expression levels in the hippocampus tissue of mice and protein expression levels, were all expressed as the mean \pm SD. The data between the two groups were compared using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of SCA on the learning and memory abilities of mice. Step-through tests and water maze tests are classic methods that can be used to assess the learning and memory ability

of animals (20). The results of the step-through test (Fig. 2) showed that compared with that in the CON group, in the MOD group, the darkness avoidance latency of mice was significantly shortened ($P < 0.01$), whereas the number of errors was significantly increased ($P < 0.01$). No significant difference was observed between the number of errors committed and the latency time in the SCA (C) group or the SCA (M) group compared with that in the CON group. Compared with that in the MOD group, the latency time was found to be significantly prolonged ($P < 0.01$ and $P < 0.01$) and the number of errors was significantly decreased ($P < 0.01$ and $P < 0.05$) in the SCA (C) group and SCA (M) groups respectively. No significant difference was observed between the SCA (M) group and the SCA (C) group in the number of errors committed or the latency time.

Morris water maze test results (Fig. 3) showed that in the orientation navigation test, compared with that in the CON group, the time that mice took to find the platform on day 5 ($P < 0.01$) and the total swimming distance ($P < 0.05$) were significantly prolonged in the MOD group. Compared with the CON group, there were no significant differences between the time that the mice took to find the platform on day 5 or the latency time in either the SCA (C) group or the SCA (M) group. However, compared with those in the MOD group, the time that mice took to find the platform on the 5th day ($P < 0.01$) was significantly shorter in the SCA (C) group and both the time that mice took to find the platform on the 5th day ($P < 0.01$) and the total swimming distance ($P < 0.05$) were significantly shorter in the SCA (M). There was no significant difference between the SCA (C) group and the SCA (M) group in the time that the mice took to find the platform on the 5th day or the total swimming distance. In the space exploration test, compared with that in the CON group, the number of mice crossing the platform was significantly reduced ($P < 0.01$), whilst the residence time in the target quadrant was significantly shortened ($P < 0.01$) and the latency of space exploration was significantly prolonged ($P < 0.01$) in the MOD group. In comparison with the CON group, the number of mice crossing the platform, the residence time in the target quadrant and the latency of space exploration of both the SCA (C) group and the SCA (M) were not significantly different. The number of

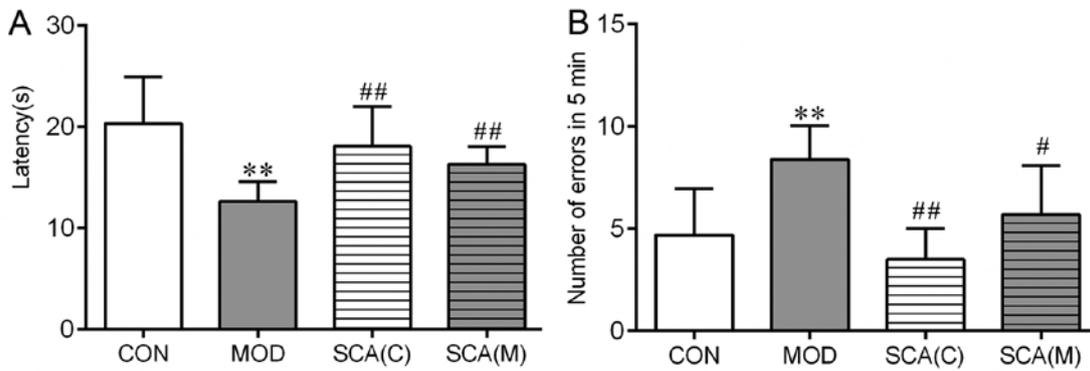


Figure 2. Effect of SCA on learning and memory capabilities of chronic fatigue model mice in the step-through test. (A) Latency and (B) number of errors in 5 min. Mean ± SD, n=10. **P<0.01 vs. CON; #P<0.05, ##P<0.01 vs. MOD. SCA, Schizanthrin A; CON, control; MOD, model; SCA (C), Schizanthrin A (control); SCA (M), Schizanthrin A (model).

platform crossings was found to be significantly increased (P<0.01, P<0.05) and the residence time in the target quadrant was significantly increased in the SCA (C) group and the SCA (M) group (P<0.01, P<0.05) compared with those in the MOD group. In addition, compared with that in MOD group, the latency of space exploration was also significantly shortened (P<0.01, P<0.05) in the SCA (C) group and the SCA (M) group. No significant differences were observed between the SCA (M) group and the SCA (C) group in these results.

Effects of SCA on the activity of SOD and CAT, in addition to the content of GSH and MDA in the hippocampus tissue of mice. SOD, CAT and GSH are important antioxidants in the body whereas MDA is the product of lipid peroxidation (21). The activity and content of these biomarkers reflects the degree of oxidative damage (22). At the end of the behavioral experiments, the hippocampal tissue of mice in each treatment group was taken for the detection of SOD, CAT, GSH and MDA. The results showed that compared with those in the CON group, the activities of SOD (P<0.05) and CAT (P<0.01), as well as the concentration of GSH (P<0.01), were significantly decreased in the MOD group. However, MDA levels were significantly increased (P<0.01) in the MOD group compared with those in the CON group (Fig. 4). The activities of SOD and CAT, as well as the GSH and MDA levels in the SCA (C) group and the SCA (M) group were not significantly different from those in the CON group. SOD (P<0.01, P<0.05) and CAT (P<0.01, P<0.05) activities and GSH concentration (P<0.01, P<0.05) were significantly increased, whilst the MDA concentration was significantly decreased (P<0.01, P<0.05) in the SCA (C) group and the SCA (M) group respectively. No significant difference was observed between the SCA (M) group and the SCA (C) group in the SOD, CAT, GSH or MDA levels.

Effects of SCA on the protein expression levels of Keap1, Nrf2 and HO-1 in the hippocampus tissue. Western blotting was used to detect the protein expression levels of Keap1, Nrf2 and HO-1 in the hippocampus tissue of the mice used in the present study. The results showed that compared with those in the CON group, the protein expression levels of Keap1 were significantly higher (P<0.05), whilst Nrf2 (P<0.05) and HO-1 (P<0.01) protein expression levels were significantly lower, in the hippocampal tissue of mice in the MOD group (Fig. 5).

Additionally, the expression levels of Keap1, HO-1 and Nrf2 were not significantly different in the hippocampal tissue of mice in the SCA (C) group and SCA (M) group compared with those in the CON group. The expression levels of Keap1 were significantly lower (P<0.05), whilst those of Nrf2 (P<0.01) were significantly higher in the SCA (C) group compared with those in the MOD group. The expression levels of Keap1 were significantly lower (P<0.05), whilst those of Nrf2 (P<0.05) and HO-1 (P<0.05) were significantly higher, in the hippocampus tissue of mice in the SCA (M) group compared with those in the MOD group. There was no significant difference between the SCA (M) group and the SCA (C) group in the Keap1, Nrf2 and HO-1 levels.

Effects of SCA on the expression levels of Bcl2, Bax and cleaved caspase-3 in the hippocampus tissue. Western blotting was used to detect the expression levels of Bcl2, Bax and cleaved caspase-3 in the hippocampus tissue of the chronic fatigue mice. As shown in Fig. 6, compared with that in the CON group, the ratio of Bcl2/Bax was significantly decreased (P<0.05) whereas the protein expression levels of cleaved caspase-3 were significantly increased (P<0.05) in the hippocampus tissue of mice in the MOD group. The ratio of Bcl2/Bax and the protein expression levels of cleaved caspase-3 in the SCA (C) group and the SCA (M) group were not significantly different from the CON group. Compared with the MOD group, the expression level of cleaved caspase-3 was significantly decreased (P<0.05) in the hippocampal tissue of mice in the SCA (C) group. The ratio of Bcl2/Bax was significantly increased (P<0.05) and the expression levels of cleaved caspase-3 were significantly decreased (P<0.05) in the hippocampal tissue of mice in the SCA (M) group compared with those in the MOD group. There was no significant difference between the SCA (M) group and the SCA (C) group in the ratio of Bcl2/Bax or the protein expression levels of cleaved caspase-3.

Discussion

Lignans is the main active component within *Schisandra chinensis* (23). Lignans has been reported to confer multiple properties, including protection against liver injury, reduction in lipid levels, anti-inflammation, anti-oxidation

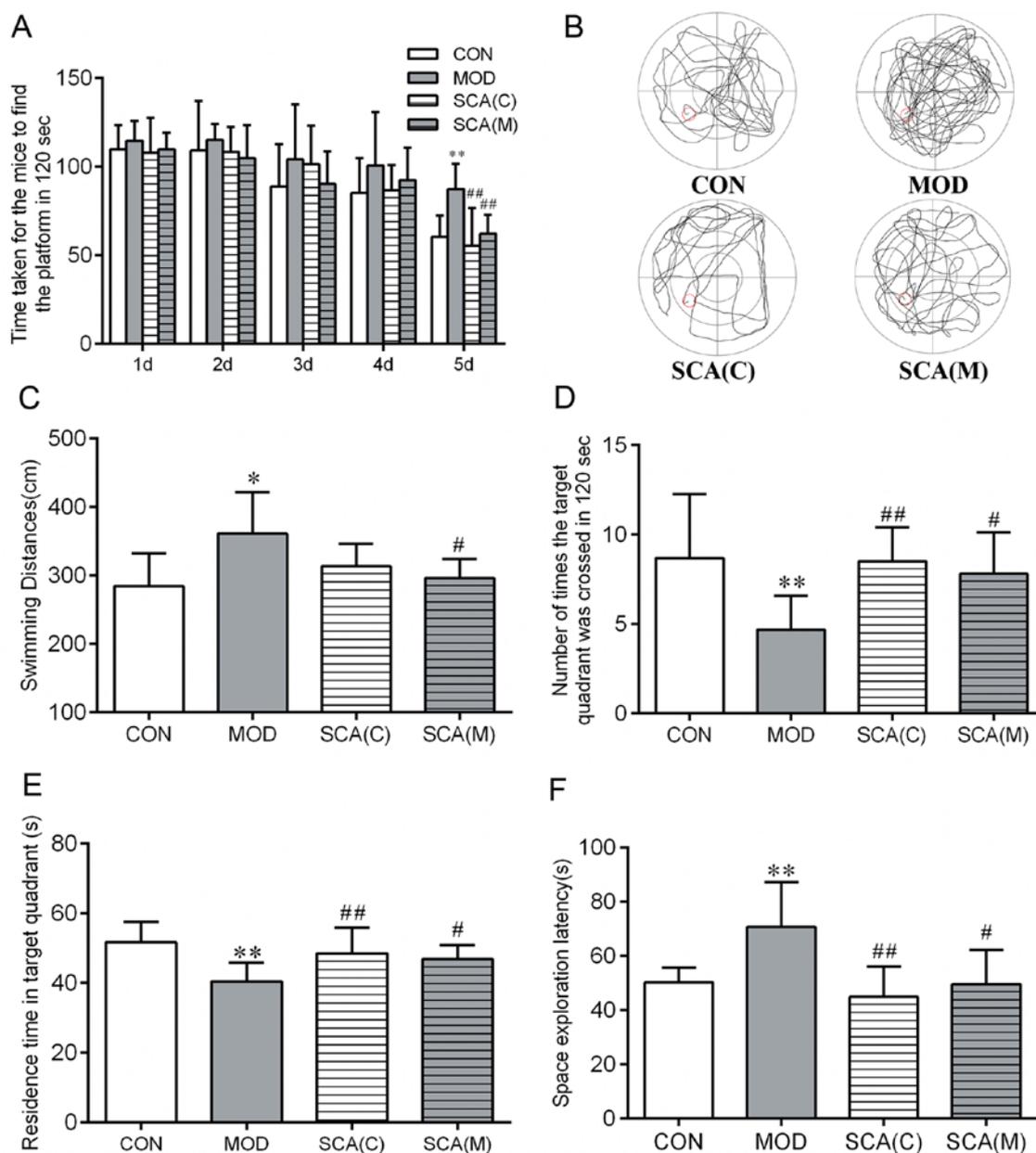


Figure 3. Effects of SCA on the learning and memory abilities of chronic fatigue mice from the Morris water maze test. (A) The time taken for the mice to find the platform on the 5th day in the orientation navigation test when given 120 sec. (B) The movement tract of mice on the 5th day in the orientation navigation test. (C) The total swimming distance in the orientation navigation test. (D) The number of times the mice crossed the target quadrant in the space exploration test. (E) Residence time in the target quadrant in the space exploration test. (F) The space exploration time in the space exploration test. Mean \pm SD, n=10. *P<0.05, **P<0.01 vs. CON; #P<0.05, ##P<0.01 vs. MOD. SCA, Schizanthrin A; CON, control; MOD, model; SCA (C), Schizanthrin A (control); SCA (M), Schizanthrin A (model).

and improvements in learning and memory (13). Among different types of lignans, SCA is one of the main active components that can be found in *Schisandra chinensis* (14). A previous study found that SCA can significantly enhance exercise endurance in chronic fatigue mice, enhance glycogen content in the liver and muscles, reduce the content of urea nitrogen and reduce MDA content in the blood, resulting in a significant anti-fatigue effect (14). In the present study, a mouse classical chronic fatigue model was established using a previously established procedure of 6-week loaded swimming training (14,18,19). The effects of SCA on learning and memory abilities of chronic fatigue mice were observed and the underlying mechanism of action

was then explored from two perspectives of anti-oxidation and anti-apoptosis.

Step-through test and water maze test are classic methods that can be used to evaluate the learning and memory ability of animals (21). In the present study, the learning and memory abilities of mice were evaluated using the two aforementioned methods. The results showed that the learning and memory ability of mice in the MOD group was significantly lower compared with that in the CON group in both the step-through and in the Morris water maze tests, consistent with previous reports on the effects of chronic fatigue on learning and memory disorders (24,25). Results from the present study also showed that the learning and memory ability of chronic fatigue

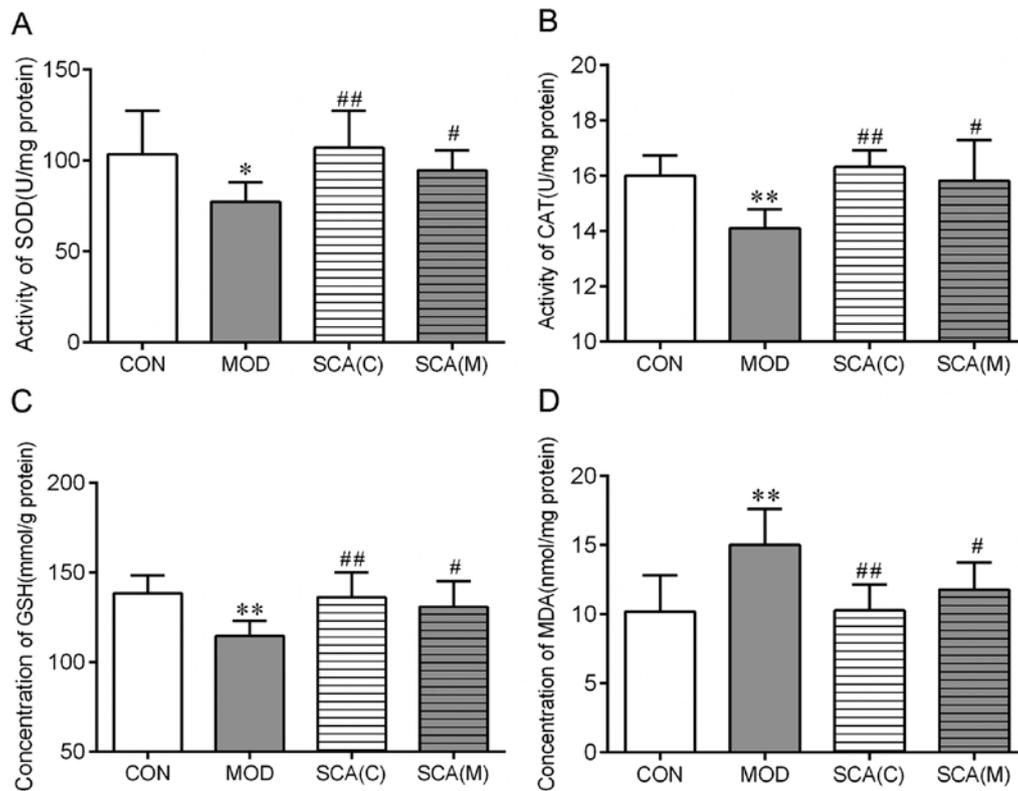


Figure 4. Effects of SCA on the parameters of antioxidants in mouse hippocampal tissues. Activities of (A) SOD and (B) CAT, as well as the concentration (C) of GSH and (D) MDA were measured in the hippocampus tissue of chronic fatigue mice. Mean \pm SD, $n=10$. * $P<0.05$, ** $P<0.01$ vs. CON; # $P<0.05$ and ## $P<0.01$ vs. MOD. CAT, catalase; GSH, glutathione; MDA, malondialdehyde; SCA, Schizanthrin A; SOD, superoxide dismutase; CON, control; MOD, model; SCA (C), Schizanthrin A (control); SCA (M), Schizanthrin A (model).

mice was significantly improved after the administration of SCA, suggesting that SCA can improve learning and memory abilities. A previous study showed that learning and memory disorders of mice induced by D-galactose were improved after the administration of SCA (15). These findings, along with results of the present study, suggest that SCA can be used as a supplement for the prevention and treatment of chronic fatigue- and D-galactose-induced learning and memory disorders.

Vigorous exercise for an extended period will enhance the metabolism and oxygen consumption of the body and increase the production of excessive free radicals in various tissues (26). This can exceed the clearance ability of the body's own antioxidant defense system, causing oxidative stress damage. Oxidative stress in brain tissues will lead to impairments in learning and memory (27). Antioxidants serve an important role in the prevention of oxidative stress-induced memory defects (28,29). SOD, CAT and GSH are important anti-oxidants for scavenging free radicals in the body (21). MDA is the product of lipid peroxidation, the content of which can reflect the degree of lipid peroxidation and in turn the degree of cell damage (20). Since the hippocampal region of the brain is closely associated with learning and memory (30), hippocampal tissue of chronic fatigue mice was selected for the detection of the aforementioned indicators in the present study. The results showed that SCA could increase the activities of SOD and CAT, as well as the GSH content but reduce the levels of MDA, suggesting increased antioxidant capacity. It has been previously found that SCA can significantly improve antioxidant capacity in both livers in fatigue mice and brain

of aging mice induced by D-galactose (14,15). These results suggest that SCA improves the learning and memory abilities of chronic fatigue mice by enhancing antioxidant capacity to protect the brain from oxidative damage.

The Nrf2/ARE signaling pathway is an important regulatory pathway involved in the antioxidant response (31). Nrf2 is a key regulator of cell oxidation (32). It can effectively resist oxidative stress injuries when activated (32,33). By contrast, Keap1 is a negative regulator of Nrf2, and its ubiquitination and phosphorylation can activate Nrf2 (34,35). HO-1 is a strong antioxidant in the body that is regulated by Nrf2 (36). When Nrf2 is activated, HO-1 expression has been indicated to be significantly increased (37). In the present study, the activity of these three components of the Nrf2/ARE signaling pathway in the hippocampus tissue of mice was investigated. The results showed that SCA could downregulate the protein expression levels of Keap1 whilst upregulating the protein expression levels of Nrf2 and HO-1 in the hippocampus of chronic fatigue mice. This suggests that SCA may alleviate learning and memory disorders caused by chronic fatigue through the Nrf2/ARE signaling pathway. However, the exact binding site and the precise mode of action on this pathway induced by SCA remain unknown, which is a limitation of the present study.

The accumulation of a large number of free radicals can lead to apoptosis through lipid peroxidation, protein denaturation and DNA damage (38). A direct consequence of neuronal apoptosis in the brain is impairments in learning and memory (39). Therefore, protection against this process is important for the

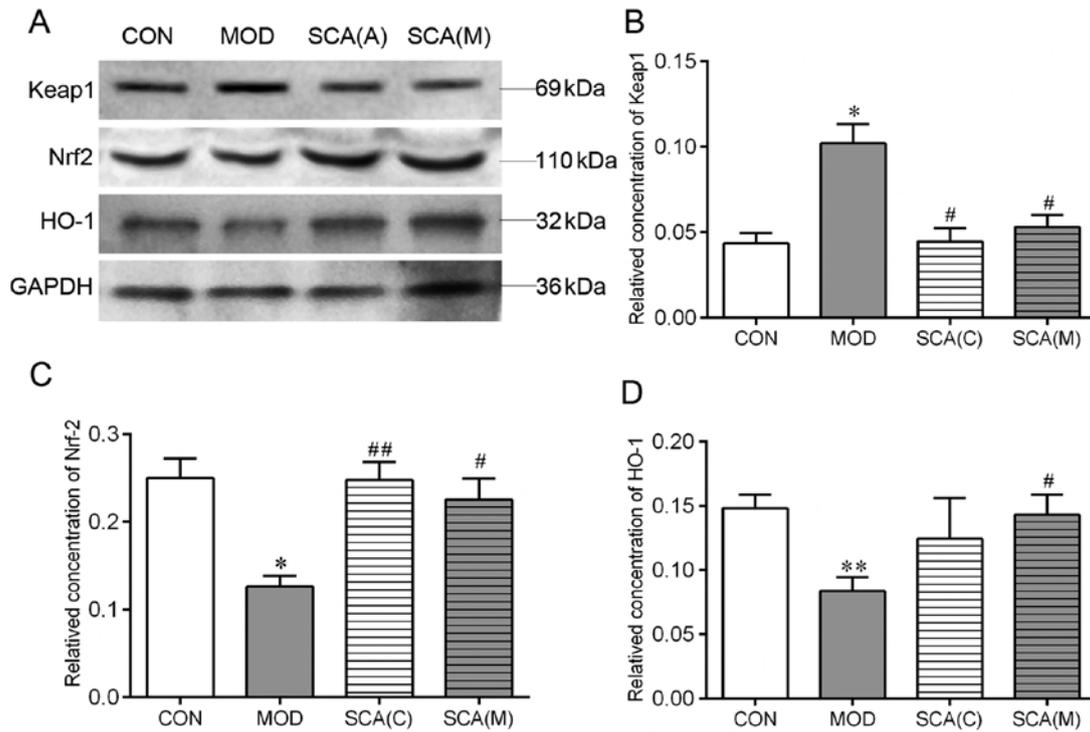


Figure 5. Effects of SCA on the parameters of redox signaling in mouse hippocampal tissues. (A) Representative western blotting images showing Keap1, Nrf2 and HO-1 expression in the hippocampal tissue of chronic fatigue mice. Protein expression levels of (B) Keap1, (C) Nrf2 and (D) HO-1 in the hippocampal tissue of chronic fatigue mice were then quantified. Mean \pm SD, n=3. *P<0.05, **P<0.01 vs. CON; #P<0.05, ##P<0.01 vs. MOD. HO-1, heme oxygenase 1; Keap1, kelch like ECH associated protein 1; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; SCA, Schizanthrin A; CON, control; MOD, model; SCA (C), Schizanthrin A (control); SCA (M), Schizanthrin A (model).

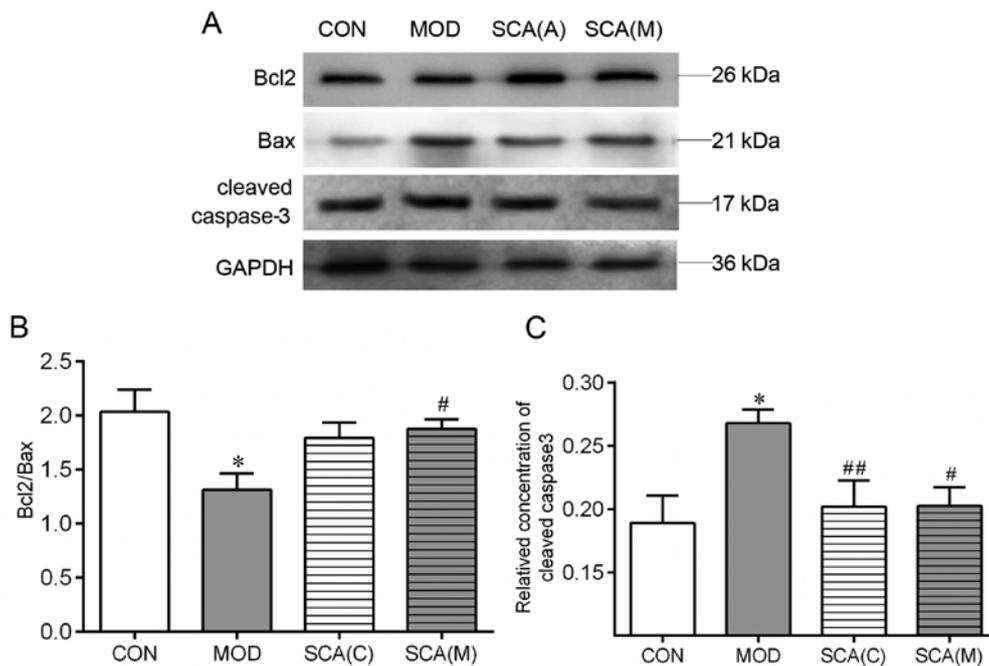


Figure 6. Effects of SCA on the parameters of apoptosis in mouse hippocampal tissues. (A) Representative western blotting images showing Bcl2, Bax and cleaved caspase-3 expression in the hippocampal tissue of chronic fatigue mice. (B) Bcl2/Bax ratio and (C) the protein expression levels of cleaved caspase3 in the hippocampal tissue of chronic fatigue mice were then quantified. Mean \pm SD, n=3. *P<0.05 vs. CON; #P<0.05 and ##P<0.01 vs. MOD. SCA, Schizanthrin A; CON, control; MOD, model; SCA (C), Schizanthrin A (control); SCA (M), Schizanthrin A (model).

protection of brain functions (39). Bcl2 and Bax are important apoptotic regulators, with Bcl2 being anti-apoptotic and Bax being pro-apoptotic, rendering the ratio of Bcl2/Bax useful

for determining the extent of apoptosis (40). Previous studies have shown that Nrf2 can regulate the expression levels of Bcl2 and the process of apoptosis by binding to the anti-oxidant

response element of the Bcl2 gene (41,42). Administration of Nrf2 inhibitors has also been found to reduce the expression levels of Bcl2 and the Bcl2/Bax ratio (42), suggesting that changes in Nrf2 expression levels may directly affect apoptosis. Consistent with these previous observations, the results of the present study showed that SCA could upregulate the protein expression levels of Nrf2 and Bcl2 whilst downregulating the protein expression levels of Bax and increasing the Bcl2/Bax ratio in the hippocampus of chronic fatigue mice, suggesting that SCA exerts an anti-apoptotic effect. Cleaved caspase-3 is another key factor for inducing apoptosis, with levels of cleaved caspase-3 directly associated with the degree of apoptosis (43,44). The present results showed that SCA could reduce the expression levels of cleaved caspase-3 in the hippocampus of chronic fatigue mice, further supporting the notion that SCA may enhance learning and memory abilities by inhibiting the apoptosis of hippocampal neurons.

In conclusion, the present study suggests that SCA treatment may improve the learning and memory abilities of chronic fatigue mice. This may be associated with its observed modulation of the Nrf2/ARE signaling pathway and antioxidant role, in addition to the inhibition of apoptosis of hippocampal neurons in chronic fatigue mice. The present study may provide an experimental basis for the application of *Schisandra* or SCA as drugs and health foods to alleviate fatigue and to improve learning and memory.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SJ and HL conceived and designed the study. HJL, XZ, JLL, LY and JL performed the animal experiments. HL, CW, JS and JC performed the data analysis. The final version of the manuscript was read and approved by all authors.

Ethics approval and consent to participate

The animal experiments were approved by the Institutional Animal Care and Use Committee of Beihua University (Jilin, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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