

Neuroprotective effects of polysaccharide of Schisandrae Chinensis Fructus in aging mice

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Abstract. This study aimed to investigate the effects of Schisandra Chinensis Fructus polysaccharide (SCP) on learning and memory, hippocampal antioxidant activity and Keap1/Nrf2 signal transduction pathway in an aging mouse model. The step-down test was used to observe learning and memory. Biochemical analysis was used to detect Superoxide dismutase (SOD) and malondialdehyde (MDA) levels. *Nrf2*, *HO-1*, and *Keap1* gene expression in the hippocampus were measured by quantitative real-time polymerase chain reaction (qRT-PCR). SCP can improve the learning and memory ability ($P < 0.05$), increase hippocampal SOD activity, decrease MDA levels, up-regulate *Nrf2* and *HO-1* gene expressions, down-regulate *Keap1* gene expression ($P < 0.05$). Our findings suggest that SCP can improve brain damage in D-galactose-induced aging mice, improve antioxidant enzyme activity, reduce lipid peroxidation, and has obvious anti-aging effects. Its anti-aging activity may be related to activating the Keap1/Nrf2 signal transduction pathway in the hippocampus.

1 Introduction

Alzheimer's disease (AD) is a chronic degenerative disease of the central nervous system^[1]. The pathogenesis of AD includes senile plaques (SP) formed by β -amyloid protein (A β) precipitation, neurofibrillary tangles (NFT) formed by excessive phosphorylation of tau protein, neuroinflammation, mitochondrial dysfunction, autophagy dysfunction, and oxidative stress^[2].

Studies have shown that excessive deposition of A β can produce a large number of free radicals^[3], leading to oxidative stress^[4] and mediating nuclear factor E2-related factor 2 (Nrf2)/(Kelch-like epichlorohydrin-related protein-1) Keap1 signaling pathways^[5].

Recent studies have revealed that polysaccharides are the main bioactive part of Schisandra Chinensis Fructus^[6]. SCP has certain anti-fatigue, anti-oxidative aging, anti-inflammation, immune enhancement, anti-tumor, and liver-protecting effects^[7-10]. However, the mechanism of the neuroprotective effects of SCP is still unclear, and further research is required.

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2 Materials and methods

2.1 Construction of an aging mouse model

Kunming mice weighing 23 ± 2 g were purchased from the Laboratory Animal Center of Qingdao University (Certification No. SCXK (Ji)2016-0003). Animals were housed in standard temperature ($25\pm 2^\circ\text{C}$), humidity ($55\pm 5\%$), and 12h light/dark cycle. All animals were housed under these conditions for 1w before experiments. Rats were randomly divided into the normal group, the model group, and the SCP group. Mice in the normal group were intraperitoneally injected with normal saline every day for seven weeks. Mice in the model group were intraperitoneally injected with 120 mg/kg/day D-galactose for seven weeks. After intraperitoneal injection of D-galactose, mice in the SCP group were intragastric with SCP (400 mg/kg/day) for seven weeks, and all samples were dissolved in normal saline. SCP was provided by the College of Pharmacy, Jilin Medical University. All animal experiments were carried out with the approval of the Animal Ethics Committee of Beihua University.

2.2 Step-down test

Mice were placed in the jumping platform instrument. After adapting to the environment for 3 minutes, the grid plate at the bottom was energized. The time of mice jumping on the platform (reaction period) and the number of electric shocks suffered by mice within 5 minutes (number of errors) were recorded as learning results. After 24h, the mice were placed on the platform, and the grid plate at the bottom was energized.

2.3 Superoxide dismutase and malondialdehyde were detected in the hippocampus of mice

Hippocampus homogenate (10%) was prepared in a homogenizer, centrifuged at 3000 r/min for 10 min, and then the supernatant was removed^[11]. The SOD and MDA activity was measured according to the kit's instructions.

2.4 qRT-PCR

Total RNA was isolated from the tumor tissues using TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). First-strand cDNA was synthesized by reverse transcription of 2 μg total RNA using an RNA PCR kit (Takara Bio., Inc., Shiga, Japan). Reactions were run in an ABI PRISM 7900 HT thermocycler (Applied Biosystems, Foster City, CA, USA) for 35 cycles.

2.5 Statistical analysis

All data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). The data were expressed as means \pm SEM. One-way ANOVA was used to compare the three groups of RT-qPCR. Plots were drawn by GraphPad Prism 6 (GraphPad Software, San Diego, California, USA). $P < 0.05$ was considered statistically significant.

3 Results

3.1 Results of the step-down test

The model group had a significantly prolonged response period of learning training ($P<0.05$) (Fig. 1-A) and an increased number of errors than the normal group ($P<0.05$) (Fig. 1-B). The SCP group had a significantly shortened response period ($P<0.05$) (Fig. 1-A) and a reduced number of errors ($P<0.05$) (Fig. 1-B) than the model group. The memory test after 24h showed that the latency period of the model group was significantly shortened ($P<0.05$) (Fig. 2-C), and the number of errors was significantly increased than the normal group ($P<0.05$) (Fig. 2-D). The SCP group had a significantly prolonged latency period ($P<0.05$) (Fig. 2-C) and a reduced number of errors than the model group ($P<0.05$) (Fig. 2-D).

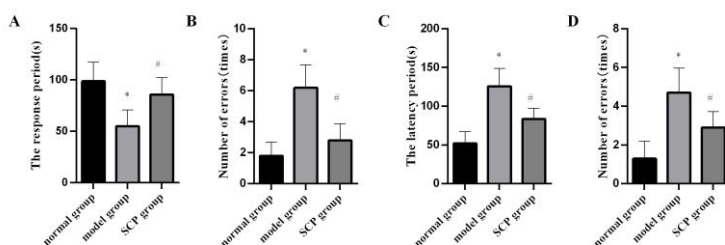


Fig. 1: Learning and memory ability of SCP in the step-down test. A response period of learning training; B Number of errors of learning training; C latency period of memory test; D Number of errors of memory test. Compared with the normal group: * $P<0.05$; Compared with model group # $P<0.05$.

3.2 Results of SOD activity and MDA content

The model group had significantly decreased SOD activity in the hippocampus of mice ($P<0.05$) (Fig. 2-A) and significantly increased MDA content than the normal group ($P<0.05$) (Fig. 2-B). The SCP intervention significantly increased SOD activity in the hippocampus of mice ($P<0.05$) (Fig. 2-A) and decreased MDA content ($P<0.05$) (Fig. 2-B).

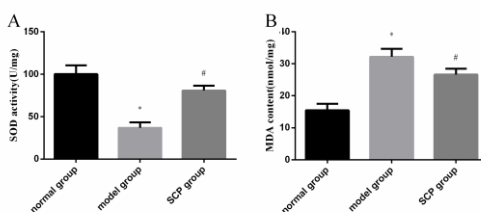


Fig. 2: Effects of SCP on hippocampal antioxidant capacity. Results of SOD activity; B Results of MDA content. Compared with the normal group: * $P<0.05$; Compared with the model group # $P<0.05$.

3.3 Effects of SCP on Nrf2/Keap1 signaling pathway in the hippocampus

The model group had significantly down-regulated *Nrf2* gene and *HO-1* gene expressions in the hippocampus of mice ($P<0.05$) and significantly up-regulated *Keap1* gene expression ($P<0.05$) than the normal group. The SCP group had significantly up-regulated *Nrf2* gene and *HO-1* gene expressions in the hippocampus of mice ($P<0.05$) and significantly up-

regulated Keap1 gene expression than the model group ($P < 0.05$). The results are shown in Fig. 3.

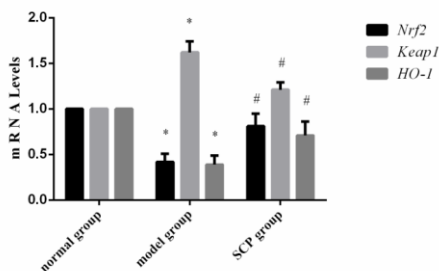


Fig. 3: Effects of SCP on *Nrf2*, *Keap1*, and *HO-1* gene expressions in the hippocampus. Compared with the normal group: * $P < 0.05$; Compared with model group # $P < 0.05$.

4 Discussion

AD is a chronic disease with progressive mental impairment and memory loss in old age. Subcutaneous or intraperitoneal injection of D-gal is currently the most commonly used method of inducing the subacute aging model of AD^[12, 13]. In this study, continuous injection of a specific dose of D-gal was used to stimulate mice to construct aging AD animal models.

The step-down test is most widely used in measuring the learning and memory function of animals. As a one-time training method of passive avoidance conditioned reflex, it can objectively reflect animal memory acquisition and consolidation^[14]. The results showed that the learning and memory ability of mice in the model group was significantly decreased compared with the normal group, while the learning and memory ability of mice in the SCP group was enhanced, indicating that SCP had neuroprotective effects on aging mice.

Oxidative stress is caused by the imbalance between the oxidation and the antioxidant systems. SOD is a highly active antioxidant enzyme, which can inhibit oxidative stress reactions, while MDA is the product of lipid peroxidation, which can aggravate the process of oxidative reaction^[15]. This study found that the intervention of SCP increased SOD activity in the hippocampus of mice, decreased MDA levels, and significantly improved the general state, learning ability, and memory of mice, indicating that SCP can slow down the degree of D-Gal-induced oxidative damage in the body, and thus has a good repairing effect on aging-induced learning and memory decline.

Activating the Keap1/Nrf2 signaling pathway can also produce a series of endogenous enzymes, such as SOD, CAT, glutathione peroxidase, and peroxidase reductase. These free radical scavenging enzymes participate in the body's antioxidant defense mechanism. In addition, Keap1/Nrf2 is one of the essential signaling pathways in oxidative stress and the first line of defense against exogenous substances and cellular oxidative damage, reducing oxidative stress and mediating antioxidants^[16-18]. Keap1 protein contains cysteine residues, which play a crucial role in maintaining the REDOX balance of cells. Transcription factor Nrf2 is the core of cell resistance to environmental stress and plays a central role in oxidative stress^[19]. Our findings revealed that SCP intervention in the brain tissue of model mice down-regulated Keap1 expression levels and up-regulated HO-1 expression levels. This suggests that the anti-aging effects of SCP are related to activating the Keap1/Nrf2 signaling pathway.

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