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Ameliorative effect of lotus seedpod proanthocyanidins on cognitive impairment and brain aging induced by D-galactose

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ABSTRACT

This study mainly investigated the ameliorative effect of lotus seedpod proanthocyanidins (LSPC) and the mechanism underlying such effect on cognitive impairment and brain aging induced by D-galactose. Aging mice induced by D-galactose (150 mg/kg, sc injection daily for 6 weeks) were chosen for the experiment. LSPCs (30, 60, and 90 mg/kg, ig) were provided after D-galactose injection. Learning and memory functions were detected by Y-maze and step-down avoidance tests. Then, some biochemical indexes related to cognitive ability and aging were measured. Histopathological feature and P53 protein expression in the hippocampus were observed. Results showed that the three different doses of LSPC could significantly ameliorate the learning and memory abilities impaired by D-galactose. LSPC significantly reduced the levels of malondialdehyde and nitric oxide (i.e. 90 mg/kg LSPC group vs. model group, $P = 0.008$), reduced the content of β-amyloid peptide 1–42 (i.e. 90 mg/kg LSPC group vs. model group, $P = 0.009$), decreased the activities of acetylcholinesterase, monoamine oxidase B, total nitric oxide synthase (i.e. 90 mg/kg LSPC group vs. model group, $P = 0.006$), and neuronal nitric oxide synthase and synchronously increased the activities of superoxide dismutase and glutathione peroxidase in the brain. Furthermore, LSPC could prevent neuron damage and could lessen the expression of P53 protein in the hippocampus. These findings demonstrated that LSPC effectively attenuated cognitive damage and improved parameters related to brain aging in senescent mice induced by D-galactose, and may be used to treat Alzheimer's disease.

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1. Introduction

Proanthocyanidins are widely distributed in common foods including cereals, fruits, vegetables, and wines and belong to a ubiquitous group of plant polyphenols (Ronald and Liwei, 2005). Proanthocyanidins exhibit potent antioxidant capacity and possible protective effects on human health. They reduce the risk of chronic diseases, such as cardiovascular diseases and cancers (Ronald and Liwei, 2005). In addition, proanthocyanidins also exhibit vasodilatory, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immune-stimulating, anti-viral, and estrogenic activities (Debasis et al., 2000).

The lotus seedpod is the inedible part of *Nelubo nucifera* Gaertn (Ling et al., 2005). Lotus is an industrial crop that is widespread in China, and its cultivated area was >40,000 ha in 1999. Lotus seedpod proanthocyanidins (LSPC) have been first isolated and characterized in Natural Product Laboratory, Food Science and Technology Department, Huazhong Agriculture University (Wuhan, China) (Ling et al., 2005). Lotus seedpod is likely to be another important source of proanthocyanidins besides grape seed. LSPC exhibits excellent antioxidant activity (Ling and Xie, 2002a, 2002b; Duan and Xie, 2003; Ling et al., 2005; Duan et al., 2005; Gong et al., 2008; Xu et al., 2009, 2010a). Furthermore, LSPC can improve the cognitive deficits of senescence-accelerated mice (SAMP8) and aged rats because LSPC has the ability to scavenge oxygen free radicals, to stimulate antioxidant enzyme activity, to rejuvenate cholinergic system and to reverse the decreased phosphorylation of adenosine 3′,5′-monophosphate (cAMP) response element-binding protein in the hippocampus (Gong et al., 2008; Xu et al., 2009, 2010b, 2011).

Progressive neurological dysfunction is a key aspect associated with human aging. Animal models for the study of disorders that manifest late in life are difficult to develop (García et al., 2011). A large number of studies have demonstrated that long-term injection of D-galactose can mainly lead to excessive reactive oxygen species (ROS) formation, antioxidant enzyme activity decrease, neuronal damage, and cognitive impairment in rats or mice (Ho et al., 2003; Wei et al., 2005; Chen et al., 2006; Cui et al., 2006; Banji et al., 2014; Hao et al., 2014). Furthermore, this mimetic aging model has age-related advanced glycation end products (AGEs) that may enhance oxidative stress damage and abnormal phosphorylation and affect learning and memory functions

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(Reddy et al., 2002; Tsai et al., 2011). D-Galactose can impair neurogenesis in the dentate gyrus through a process similar to the natural aging in mice (Ho et al., 2003; Zhang et al., 2005). Thus, D-galactose injection has been gradually accepted as a tool for establishing an aging model in brain aging studies or in anti-aging pharmacological research (Zhang et al., 2008; Li et al., 2010, 2015; Banjia et al., 2013; Wei et al., 2014). Cognitive deficits are characteristics of aging and age-related neurodegenerative disorders that lead to a progressive loss of cognitive function, especially in spatial memory (Barnes et al., 1980). Since oxidative damage may play a role in the aging process, including the associated cognitive decline, many researchers believe that antioxidant supplements may alleviate age-related impairment in spatial learning and memory functions and delay the aging process.

Therefore, we investigated the effect of LSPC on learning and memory impairment and brain aging and the mechanism underlying such effect in animal models induced by 6-week subcutaneous injection of D-galactose. A certain amount of data was provided to support that LSPC could attenuate Alzheimer's disease (AD) development.

2. Materials and methods

2.1. Reagents and drugs

D-Galactose was purchased from Shanghai Bo'ao Biological Technology Co., Ltd. (Shanghai, China) and dissolved in 0.9% saline at concentration of 1.5%. Lotus seedpod proanthocyanidins were separated by our lab and dissolved in physiological saline. Commercial kits used for determining malondialdehyde (MDA), nitric oxide (NO), and protein levels, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), nitric oxide synthase (NOS), acetylcholinesterase (AChE), and monoamine oxidase B (MAO-B) activities were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). β-Amyloid peptide 1–42 ($A\beta_{1-42}$) ELISA assay kit was purchased from the Genetics Co. (Schlieren, Switzerland). P53 antibody and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Strept Avidin-Biotin Complex (SABC) and 3,3V-diaminobenzidine tetrachloride (DAB) immunohistochemistry kits were purchased from Wuhan Boster Bio-engineering Limited Company (Wuhan, China).

2.2. Preparation of lotus seedpod proanthocyanidins (LSPC)

Lotus seedpods of *N. nucifera* Gaertn were collected in Honghu Lake (Hubei, China). This variety of *N. nucifera* Gaertn is named Number 2 Wuhan plant and was authenticated by the Department of Botany, Wuhan Plant Institute of the Chinese Academy of Science.

LSPCs were isolated by using the method of Ling et al. (2005). LSPCs were extracted with Me_2CO/H_2O and purified by Sephadex LH-20 column chromatography with a purity of 98%, as measured by the method reported by Porter et al. (1986). ESI-MS analysis indicates that the extract contains monomers, dimers, and tetramers of proanthocyanidins, in which the amounts of dimmers are greatest, and catechin and epicatechin are the base units.

2.3. Animals and drug administration

Kunming mice were used. The numbers of males and females were equal. The mice were aged 3 months old, weighed 27 ± 2 g, and were obtained from Center for Laboratory Animal Sciences, Southern Medical University (Guangdong, China). In this experiment, a total of 50 mice were used. Animals were housed five per cage under conditions free of specific pathogens at a temperature of 25 ± 1 °C and relative humidity of 55%–60% and exposed to a daily cycle of 12 h light/dark (07:00 on and 19:00 off). A normal solid diet and water were provided ad libitum, and animals were allowed free access to food and water.

The mice were randomly divided into five groups consisting of 10 animals each: control, model, and three LSPC groups. Except for the control group, the mice were subcutaneously injected with D-galactose at a dose of 150 mg/kg body weight once daily for 6 weeks, whereas mice in the control group were treated with the same volume of physiological saline. At the same time, LSPC groups' mice were provided with LSPC dissolved in physiological saline at three doses of 30, 60, and 90 mg/kg body weight respectively by oral gavage after injection of D-galactose. Mice in the control and model groups were administered with same volume of physiological saline.

2.4. Behavioral tests

2.4.1. Y-maze test

To evaluate the learning and memory performances of the mice, a Y-maze (MG-2, manufactured by Zhangjiagang biomedical instrument plant of Jiangsu province, China) test was performed at the end of the treatment. The procedure used was a modified version of Heyser's method (Heyser et al., 1999). The mice were put into the Y-maze separately. The test was performed in a sound-isolated and dark room. The Y-maze apparatus consisted of three identical arms (three compartments of 30 cm × 15 cm × 10 cm with connector 10 cm × 6 cm × 10 cm), connected into a Y shape. There was a lamp at the end of each arm and the bottom of each arm was covered with electric net. However, the lamp of only one of the three arms was turned on. At this moment, there was no electric current at the bottom of this arm, which was considered as a safe area. The lamps in the other two arms were not turned on, and there was electric current (50–70 V) on the base (considered as unsafe area). Safe and unsafe areas were alternated randomly.

At the beginning of the experiment, no lamp was turned on. Each mouse was given 2–3 min to be acclimatized to the circumstances and subsequently subjected to electric shock with the same intensity and duration. Then, one of the three lamps was switched and the mouse was placed in this safe area, where the mouse adapted to the condition for 10 s. Afterward, the other lamp was switched to force the mouse to run to the safe area. Such a trial process was continued. During the experiment, if the mouse ran to the safe arm directly, it was a correct response. Otherwise, it was an incorrect response. Nine correct responses out of ten consecutive trials were considered as learning criterion. Trial continued until each mouse reached the learning criterion. The total number of trials needed to reach the criterion was recorded to show the learning ability of the mouse. The fewer the total number, the stronger the learning ability of the mouse. After 24 h, the electric shock test was repeated 10 times for each mouse, and the incorrect responses were recorded to reflect the memory ability of the mouse. The fewer the incorrect responses, the stronger the memory ability of the mouse.

2.4.2. Step-down avoidance test

The apparatus consisted of five paralleled 12 cm × 12 cm × 30 cm plastic boxes. The floors of these boxes were made of parallel bronze bars. The five boxes were separated by opaque black plastic. A high and diameter of 4.5 cm insulating platform as a safety platform to avoid electric shock for mice was placed at the right rear in each box.

In the training session, each mouse was put into the box for adapting to 3 min and then was put on the grid floor with its back against the platform. A continuous electric shock (36 V) was delivered to the grid floor by an isolated stimulator. When the electric shock was delivered, mice escaped from the grid floor back onto the platform. In this session, the number of repeated step-down in 300 s was counted as errors. At 24 h after training, mice were placed on the platform for the retention test. Step-down latency (time of mice stepping from platform to grid floor for the first time) and the number of errors were recorded with improved retention, as reflected by a long latency and a reduction in errors. The electric shocks were still delivered for 300 s.

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