

Original Research Article

Memory-enhancing effect of *Rhodiola rosea* L extract on aged mice

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Abstract

Purpose: The memory-enhancing effects of *Rhodiola rosea* L. extract (RRLE) on normal aged mice were assessed.

Methods: In the open-field test, the effect of RRLE (150 and 300 mg/kg) on mouse locomotive activities was evaluated by investigating the extract's influence on CAT and AChE activities in the brain tissue of mice.

Results: Compared with aged group, high dose of RRLE reduced the total distance (3212.4 ± 123.1 cm, $p < 0.05$) significantly, increased catalase (CAT) activity (101.4 ± 12.2 U/mg pro, $p < 0.05$), and inhibited acetyl cholinesterase (AChE) activity (0.94 ± 0.12 U/mg pro, $p < 0.05$) in the brain tissue of aged mice.

Conclusion: The results show that RRLE improves the memory functions of aged mice probably by increasing CAT activity while decreasing AChE activity.

Keywords: *Rhodiola rosea*, Memory function, Catalase, Acetyl cholinesterase, Open-field test

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INTRODUCTION

As a result of increased human life expectancy, age-related learning and memory disorders have become prevalent in the aging population, even in the absence of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. To tackle such a major global healthcare issue, it is vital to develop effective prophylactic and therapeutic agents for enhancing and maintaining memory functions before the onset of memory impairments [1-3].

Traditional Chinese Medicine (TCM) has been frequently used for treating memory and cognitive deficits for thousands of years. *Rhodiola rosea* L., a well-known medicinal plant in TCM, has been commonly used as memory enhancer in China, and it is also included in some traditional prescriptions treating central nervous system disturbances [4-6].

In the present study, the aim is to investigate the effects of *Rhodiola rosea* L. extract (RRLE) on learning and memory impairments in normal aged mice. Learning and memory parameters

were evaluated by using open-field test. In addition, potential mechanisms were also examined.

EXPERIMENTAL

Plant material and extraction

Samples of *Rhodiola rosea* L. were collected from Bozhou City, Anhui Province in China in September 2015. Taxonomic identification of the plant was performed by Professor Zhi Li of ShanDong University in China. A voucher specimen (no. RRL 20150908) was deposited in the herbarium of College of Pharmacy, ShanDong University, China for future reference.

The whole plant of *Rhodiola rosea* L. was dried in a drying oven at 100 °C for 12 h. Aqueous extract of RRL was obtained by steeping the dried *Rhodiola rosea* L. in water at 60 °C for three times, each for one hour. Then it was dried in an oven and then the last extract was freeze-dried to obtain the powder. One gram powder was obtained from about 1.8 g dried sample, i.e., a yield of 55.6 %.

Animals and groups

Female C57BL/6J mice (sixty 15-month-old mice and twelve 3-month-old mice) were purchased from Experimental Animal Center of ShanDong Province (Certificate no. SYXK 2006-0001). They were housed in groups of five animals per cage under a 12:12 h light-dark cycle at constant temperature (23 ± 2 °C) and humidity (50 ± 10 %). The animals had free access to standard chow diet and sterilized drinking water in the SPF Animal House. The 3-month-old mice were used as the normal control group. The 15-month-old mice were randomly assigned to six groups, including the normal control group, the aged control group, galantamine (3 mg/kg) group, and the various concentrations of RRLE (75, 150, and 300 mg/kg, respectively) groups. Drug administration and behavioral assays were carried out using a double-blind method. The rat experiment was approved by the Animal Care and Use Committee of ShanDong University (approval ref no. 20101005) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

Animal studies

After 2 days of feeding, mice received orally water (the normal control group and the aged control group), galantamine (3 mg/kg), or various concentrations of RRLE (75, 150 and 300 mg/kg)

for a period of 4 weeks before behavioral measurement was assessed. Locomotive activities of mice were tested in open-field on the first day of behavioral test. Then, the mice were killed by cervical vertebrae, and the brain tissues were dissected quickly on ice for detection of the content of CAT and AChE.

Open-field test

The effect of RRLE on mice locomotor activities was evaluated automatically using an open-field computer-aided controlling system as described in the literature [8,9]. The apparatus consists of four metal tanks (30 cm in diameter and 40 cm in height) with a video camera fixed at the top, and the apparatus was illuminated by a light source of 120 Lux on the ceiling. The experiments were performed in a quiet room; four mice were tested simultaneously. Thirty minutes after drug administration, each mouse was placed at the center of the metal tank and allowed to explore freely for 5 min. Thereafter, the distance was measured for 10 min, which was recorded to evaluate the locomotive activity of the mouse.

Preparation of brain tissue samples and biochemical evaluation

After behavioral measurements, all the mice were sacrificed by decapitation; the brain tissues were quickly removed, washed with cold saline solution, followed by 50 mM Tris-HCl buffer (pH 7.4), and weighed. They were then placed in a glass bottle, labeled, and stored in a deep freezer (-25 °C) until processing (maximum 10 h). The tissues were homogenized in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Elektrocrafts, Mumbai) for 2 min at 5000 rpm after cutting it into small pieces. The homogenate was then centrifuged (Remi, India) at 1000 rpm for 10 min to remove the debris. The clear upper supernatant fluid was extracted with an equal volume of ethanol and centrifuged at 17000 rpm for 30 min; the clear upper ethanol layer was taken and used for biochemical assay. All the preparations were performed at 4 °C, and then CAT and AChE were estimated using commercial kits according to the manufacturer's protocols.

Determination of catalase activity in the brain tissue of mice

CAT activities were assessed by measuring the disappearance of hydrogen peroxide at 405 nm [10,11]. One unit (U) of CAT corresponds to the amount of the enzyme that hydrolyses 1 mmol of hydrogen peroxide per minute at 25 °C. Catalase

activity was expressed as n moles of H₂O₂ metabolized/mg protein/h.

Determination of AChE activity in the brain tissue of mice

AChE activity was determined as described by Ellman *et al* [12] with some modifications. In brief, 30 µL of diluted homogenate was added to the reaction mixture, which contained 100 mM phosphate buffer (pH 8.0) and 1.0 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 2 mL, and incubated at 37 °C for 6 min.

Hydrolysis was monitored by the formation of the thiolate dianion of DTNB at 412 nm for 3 min with a spectrophotometer. AChE was calculated from the quotient between lymphocyte AChE activity and protein content. One unit (U) of enzyme activity was defined as 1 micromole of ACh hydrolyzed per hour and per mg of brain homogenate or per mL of blood (pH 8.0, 25 °C).

Data analysis

All data were analyzed using Statistical Package SPSS 16.0 (SPSS Inc., Illinois, Chicago, USA), and are expressed as mean ± standard error of mean (SEM), and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. P < 0.05 was considered statistically significant.

RESULTS

Effect of RRLE on mouse locomotive activities in the open-field

As shown in Table 1, significant effect of RRLE (150 and 300 mg/kg) on mouse locomotive activities was observed in the open-field test (p < 0.05). Furthermore, galantamine 3 mg/kg reduced the total distance significantly compared with the aged control group (p < 0.05).

Table 1: Effect of RRLE on locomotive activities of mice (n = 10)

Group	Dosage (mg/kg)	Total distance (cm)
Control	-	3894.5 ± 125.6
Aged	-	3792.3 ± 114.8
Gal	3	3116.7 ± 121.2*
L-RRLE	75	3695.1 ± 124.9
M-RRLE	150	3405.3 ± 115.5*
H-RRLE	300	3212.4 ± 123.1*

*P < 0.05 and **p < 0.01 versus aged group; L-RRLE: low dose of RRLE, M-RRLE: medium dose of RRLE, H-RRLE: high dose of RRLE

Effect of RRLE on CAT activities in the brain tissue of mice

As shown in Table 2, CAT activities of brain tissue in the aged control mice decreased significantly as compared with the normal control mice (p < 0.05). However, mice treated with RRLE (150 and 300 mg/kg) and galantamine (3 mg/kg) showed high CAT activities compared with the aged control mice (p < 0.05).

Table 2: Effect of RRLE on the CAT activities in mice brain tissue (n = 10)

Group	Dosage (mg/kg)	CAT activity (U/mg pro)
Control	-	102.8 ± 12.1*
Aged	-	64.3 ± 11.8
Gal	3	96.5 ± 13.7*
L-RRLE	75	71.2 ± 14.3
M-RRLE	150	85.6 ± 11.5*
H-RRLE	300	101.4 ± 12.2*

* p < 0.05 and ** p < 0.01 versus aged group. L-RRLE: low dose of RRLE, M-RRLE: middle dose of RRLE, H-RRLE: high dose of RRLE

Effect of RRLE on AchE activities in the brain tissue of mice

As shown in Table 3, the aged mice showed significant differences in AChE activities as compared with the normal control mice (p < 0.05). On the contrary, treated with RRLE (150 and 300 mg/kg) and galantamine (3 mg/kg), the activities of AchE could markedly decrease (p < 0.05).

Table 3: Effect of RRLE on the AChE activities in mice brain tissue (n = 10)

Group	Dosage (mg/kg)	AChE activity (U/mg pro)
Control	-	1.24 ± 0.21
Aged	-	1.74 ± 0.16
Gal	3	0.88 ± 0.19
L-RRLE	75	1.54 ± 0.17
M-RRLE	150	1.36 ± 0.15*
H-RRLE	300	0.94 ± 0.12

*P < 0.05 and **p < 0.01 versus aged group; L-RRLE: low dose of RRLE, M-RRLE: medium dose of RRLE, H-RRLE: high dose of RRLE

DISCUSSION

Learning and memory abilities are important functions of the brain in humans and rodents. Aging in humans is associated with deterioration of cognitive performance, particularly, learning and memory abilities [13]. Aging animals have

traditionally been used as a model of memory impairments [14]. Behavioral tests are one of the most reliable methods of investigating learning and memory abilities of animals. The extract of *Rhodiola rosea* L. has been used as memory enhancer in Asia for thousands of years. Various animal models have demonstrated that RRLE could improve brain functions [15]. In the present study, the memory-enhancing effects of RRLE on the normal aged mice were investigated by using open-field test.

Aging is accompanied by learning and memory loss. The exact mechanisms responsible for the memory impairments with aging are still unclear, but evidence has accumulated that oxidative stress plays an important role [16]. Oxidative stress occurs when pro-oxidant and anti-oxidant levels become imbalanced. With aging, there is an increased production of reactive oxygen species (ROS) and diminished endogenous antioxidant enzyme levels, leading to an increased oxidizing cellular environment. CAT is the main endogenous antioxidant enzymes, playing an important role in the intracellular antioxidant defense in the brain. Our study results showed that decreasing activities of CAT in the aged mice could be partly reversed by RRLE (150, 300 mg/kg). These findings demonstrate that the memory enhancing effects of EPT on the aged mice may be via antioxidant system.

Aging is often accompanied by some alterations in the neurotransmitter systems such as acetylcholine and monoamine transmitters [17,18]. The transmission of these neurotransmitters in the brain has been long considered an important modulator of synaptic plasticity, memory consolidation, and other cognitive processes [19]. Under normal condition, the metabolic controls, which are responsible for maintaining the levels of ACh and monoamine transmitters, are catalyzed by AChE [20]. Our experimental data suggest that the RRLE-mediated enhancement in spatial and non-spatial learning and memory abilities could be, at least partially, due to the decreasing activity of AChE in aged control mice, which were consistent with multiple behavioral tendencies.

CONCLUSION

These findings of this study reveal that *Rhodiola rosea* L. extract not only ameliorates learning and memory deficits in aged mice, but also exerts antioxidant effect. The plant should be study further ascertain its memory- enhancing effect in humans.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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