

## Pretreatment with *Rhodiola Rosea* Extract Reduces Cognitive Impairment Induced by Intracerebroventricular Streptozotocin in Rats: Implication of Anti-oxidative and Neuroprotective Effects

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**Objective** To investigate the pretreatment effects of *Rhodiola rosea* (*R. rosea*) extract on cognitive dysfunction, oxidative stress in hippocampus and hippocampal neuron injury in a rat model of Alzheimer's disease (AD). **Methods** Male Sprague-Dawley rats were pretreated with *R. rosea* extract at doses of 1.5, 3.0, and 6.0 g/kg for 3 weeks, followed by bilateral intracerebroventricular injection with streptozotocin (1.5 mg/kg) on days 1 and 3. Behavioral alterations were monitored after 2 weeks from the lesion using Morris water maze task. Three weeks after the lesion, the rats were sacrificed for measuring the malondialdehyde (MDA), glutathione reductase (GR) and reduced glutathione (GSH) levels in hippocampus and histopathology of hippocampal neurons. **Results** The MDA level was significantly increased while the GR and GSH levels were significantly decreased with striking impairments in spatial learning and memory and severe damage to hippocampal neurons in the model rat induced by intracerebroventricular injection of streptozotocin. These abnormalities were significantly improved by pretreatment with *R. rosea* extract (3.0 g/kg). **Conclusion** *R. rosea* extract can protect rats against cognitive deficits, neuronal injury and oxidative stress induced by intracerebroventricular injection of streptozotocin, and may be used as a potential agent in treatment of neurodegenerative diseases such as AD.

**Key words:** *Rhodiola rosea*; Oxidative stress; Neuroprotective effect; Learning and memory; Alzheimer's disease; Intracerebroventricular streptozotocin

### INTRODUCTION

*Rhodiola rosea* (*R. rosea*), also known as a golden or rose or arctic root, belongs to the plant family of *Crassulaceae*, subfamily of *sedoideae* and genus *Rhodiola*<sup>[1]</sup> and is widely distributed in the Arctic and mountainous regions throughout Europe and Asia. It is a popular plant in traditional medical systems in Eastern Europe and Asia, and can be used to stimulate the nervous system, decrease depression, enhance work performance, eliminate fatigue, and prevent high altitude sickness<sup>[2]</sup>. Of the *Rhodiola* species, *R. rosea* has been extensively studied on its phytochemical and toxicological properties<sup>[3]</sup>. Modern pharmacological studies indicate that its extracts can increase neurotransmitter level, central nervous system activity and cardiovascular function. It was reported that *R. rosea* ingestion can improve

cognitive function<sup>[4]</sup>, reduce mental fatigue<sup>[5-6]</sup>, promote free radical mitigation, and has anti-oxidative effect<sup>[7-12]</sup> and neuroprotective effect<sup>[13-16]</sup>, and increase endurance performance<sup>[4,17-18]</sup>, and learning and memory<sup>[2,19]</sup>.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and widespread loss of neurons and their synapses in the cerebral cortex and hippocampus<sup>[20]</sup>. Hippocampal neurons are especially vulnerable to injury induced by AD.

$\beta$ -amyloid peptide, one of the most notable neuropathologic hallmarks in AD, contributes to progressive neuronal degeneration and death in various brain regions, especially in hippocampus, an important area for memory and cognition<sup>[21]</sup>. Oxidative stress, an imbalance between free radicals and antioxidant system, plays a critical role in the

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pathogenesis of AD, and antioxidants have been used in treatment of various neurodegenerative diseases, including AD<sup>[22-23]</sup>.

Streptozotocin (STZ), a glucosamine-nitrosourea compound, generates a cytotoxic product that preferentially destroys  $\beta$  cells in pancreatic islet and produces diabetes mellitus when it is metabolized. It has been found that intracerebroventricular injection of STZ at a sub-diabetogenic dose in rats causes prolonged impairment of brain glucose and energy metabolism by desensitizing neuronal insulin receptors<sup>[24]</sup>, which is accompanied with impairment in learning and memory<sup>[25-29]</sup> in addition to decreased choline acetyltransferase levels<sup>[27,30]</sup> and increased oxidative stress in hippocampus<sup>[27-29]</sup>. However, STZ has no effect on blood glucose when given via intracerebroventricular (ICV) injection<sup>[31-32]</sup>, implicating that its action is independent of inducing hyperglycemia. Impairment of memory can be reduced by chronic treatment with antioxidants, melatonin, and rosvaterol<sup>[29,33]</sup>, supporting that ROS plays a role in its etiology. It was recently reported that a rat model of ICV injection of STZ (ICV STZ) can mimic the cellular and molecular abnormalities (including hyperphosphorylation of tau protein and senile plaque like deposits) of sporadic AD<sup>[32,34-35]</sup>. ICV injection of STZ has been used in animal models of neurodegenerative diseases, including AD<sup>[27,29,36]</sup>.

*R. rosea* has been categorized as an adaptogen by Russian researchers due to its observed ability to increase resistance to a variety of chemical, biological and physical stressors. *R. rosea* root contains about 28 compounds, of which, salidroside (rhodiolide), rosavins, and p-tyrosol are thought to have the most critical therapeutic activity<sup>[37]</sup>. Alcohol and water extracts from *R. rosea* have significant free-radical scavenging activity and contain a variety of antioxidant compounds<sup>[38-39]</sup>. It has been shown that P-tyrosol is absorbed in a dose-dependent manner and appears to produce a significant antioxidant<sup>[40]</sup>. Salidroside increases the scavenging superoxide radical anion activity in a concentration and time-dependent manner<sup>[8]</sup>, and protects SH-SY5Y human neuroblastoma cells against hydrogen peroxide-induced apoptosis<sup>[9]</sup>.

*R. rosea* may play a role in amelioration of cognitive deficits induced by neurodegenerative diseases, such as AD via its antioxidant potential and neuroprotective properties. The present study was to investigate the pretreatment effects of *R. rosea* extract on cognitive dysfunction and oxidative stress in hippocampus and hippocampal neuron injury in a rat model of ICV STZ.

## MATERIALS AND METHODS

### Drugs

*R. rosea* extract was obtained from Hong Kong Holistal International Ltd. Sodium carboxymethylcellulose (CMC-Na) was purchased from Tianjin Fuchen Chemical Reagent Factory (Jiangsu province, China). Glutathione reductase (GR), reduced glutathione (GSH) and malondialdehyde (MDA) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu province, China). Coomassie brilliant blue protein assay kit used for protein assay in GR, GSH, and MDA were also purchased from Nanjing Jiancheng Bioengineering Institute. Sodium pentobarbital and STZ were purchased from Sigma Chemical Co. (St. Louis, USA).

### Experimental Animals

Male Sprague-Dawley rats, weighing 240-260 g, were purchased from the Experimental Animal Center of Sun Yat-Sen University, and fed with standard chow diet and tap water ad libitum. The animals were housed in cages at 24 $\pm$ 2 °C with a 50%-60% relative humidity in a 12 h light/dark cycle for at least 1 week before the experiment.

### Grouping and Drug Administration

The animals were randomly divided into normal control group, model group, low/medium/high *R. rosea* extract dose groups (L-RR, M-RR and H-RR groups) ( $n=12$ ). The rats were pretreated with *R. rosea* extract or CMC-Na for 3 weeks before injury induced by ICV STZ. *R. rosea* extract was diluted with a 0.5% CMC-Na water solution. Rats in the L-RR, M-RR and H-RR groups orally administered 1.5, 3.0, and 6.0 g/kg of *R. rosea* extract, respectively, by gavage, twice a day, for 21 days. Rats in the normal control and model groups received an equal volume of 0.5% CMC-Na solution for 21 days.

### Surgical Procedures

STZ was injected through ICV as previously described<sup>[27]</sup>. Briefly, rats were anesthetized with sodium pentobarbital at a dose of 40 mg/kg, i.p. The head was positioned in a stereotactic frame and skull was exposed. Burr holes were drilled in the skull on both sides over the lateral ventricle using the following coordinates<sup>[41]</sup>: 0.8 mm posterior to bregma, 1.8 mm lateral to sagittal suture, and 4.0 mm beneath the surface of skull. Rats in the model group and three *R. rosea* extract pretreatment groups were given a bilateral ICV injection of STZ (1.5 mg/kg). STZ was dissolved in an artificial cerebrospinal fluid (CSF)

containing 147 mmol/L NaCl, 2.9 mmol/L KCl, 1.6 mmol/L MgCl<sub>2</sub>, 1.7 mmol/L CaCl<sub>2</sub>, and 2.2 mmol/L dextrose, to make a 25 mg/mL solution on ice just before the injection. The injection was repeated on the third day. Rats in the normal control group underwent the same surgical procedure and injected with the same volume of artificial CSF instead of STZ.

### *Behavioral Testing*

Morris water maze (MWM) task was used to investigate the effects of *R. rosea* extract on the behavioral alterations in rats after ICV STZ.

MWM test was carried out 2 weeks after ICV STZ to test the spatial learning and memory of rats as previously described<sup>[27]</sup>. The test device consists of a circular water tank (152 cm in diameter and 60 cm in height) partially filled with water (25±2 °C). A non-toxic paint was used to render the water opaque. The pool was divided into four equal quadrants (labeled I - II -III-IV). An escape platform (10 cm in diameter) was hidden 2 cm below the surface of water on a fixed location in quadrant II of the pool. Rats were trained to escape from water by swimming to the platform. A video monitor and data analysis software were used to record the trials of rats and analyze the data online. The training procedures consisted of the following 3 phases<sup>[42]</sup>.

*Adaptation training/swimming ability test* To exclude the effects of various swimming abilities on the performance of MWM task, adaptation training was carried out just prior to the following acquisition training. Rats were allowed to swim freely in the pool for 120 s without platform. The swimming distances were calculated to measure the swimming ability of rats. Rats swimming extremely long or short distance were excluded from MWM task.

*Hidden-platform acquisition training* The acquisition training phase was designed to study the ability of rats to acquire memory, including blocks of four swimming trials starting randomly from four positions. Rats were given two blocks of training in the morning and afternoon per day for 4 days. Each trial had a ceiling time of 60 s and a trial interval of approximately 30 s. If the rats failed to reach the escape platform within the maximum time of 60 s, they were gently placed on the platform and allowed to remain there for 10 s. The time to reach the platform (escape latency in second) was calculated. The lower the latency value, the better the performance.

*Spatial probe trial* After the acquisition phase, a probe test was conducted by removing the platform on the last day of MWM task. Rats were allowed to swim freely in the pool for 120 s. The time spent in the target quadrant previously containing the

hidden platform, was recorded, which indicated the degree of memory consolidation after learning.

### *Estimation of Oxidative Stress Parameters in Hippocampus*

Rats were sacrificed on day 21 after ICV STZ. Whole brain was taken out quickly to dissect hippocampus on ice. The hippocampus was weighed and homogenized in cold normal saline (NS) to get a 10% homogenate solution. According to the instructions of test kits, the homogenate was centrifuged at 2000×g for 8 min at 4 °C. Supernatant was collected. Aliquots were stored at -80 °C for detection of MDA, GR, and GSH and protein assay.

*Protein assay with Coomassie brilliant blue method* The sample was diluted with NS to 1% concentration. Protein assay was conducted with Coomassie brilliant blue method. The absorbance was measured at 595 nm using a spectrophotometer.

*Detection of lipid peroxidation* MDA, a measure of lipid peroxidation, was detected spectrophotometrically with a testing kit following its manufacturer's instructions using the TBA method<sup>[43]</sup>. The concentration of MDA was expressed as nmol/mg protein.

*Detection of GSH* Glutathione was detected with a testing kit following its manufacturer's instructions as previously described<sup>[44]</sup>. The concentration of GSH was expressed as GSH/mg protein.

*Detection of GR activity* According to the testing kit instruction, GR activity was detected with a testing kit following its manufacturer's instructions as previously described<sup>[45]</sup>. The enzyme activity was quantified at 25 °C by measuring the disappearance of NADPH at 340 nm. One U of GR was defined as the amount of GR in 1 g tissue protein catalyzing 1 mmol NADPH oxidation per minute.

### *Histopathological Examination*

Three weeks after ICV STZ, rats were deeply anaesthetized with pentobarbital sodium. The brains were fixed by transcardial perfusion, first with 150 mL of NS containing heparin (5 U/mL), then with 250 mL of 4% paraformaldehyde in 100 mmol/L phosphate buffer (PB, pH 7.4), dissected, postfixed in the same fixative overnight and transferred to a 30% sucrose solution for cryoprotection. Coronal sections (15 µm) were cut in a cryostat. Neutral red staining was used to examine the general morphology. Sections were rinsed with 0.01 mol/L PBS for 5 min, incubated with a 1% neutral red staining solution for 20 min at room temperature, washed with deionized water, dehydrated in ascending series of ethanol, cleared in xylene and mounted by neutral gum. For

quantitative analysis, the number of neurons in five regions (CA1-1, CA1-2, CA3, DG-1, and DG-2. See Fig. 1) of the bilateral hippocampus was calculated. The number of neurons in hippocampal region was

counted using the Leica IM50 Image Plus computer-assisted image analysis system attached to a light microscope (Leica Microsystem AG, Switzerland).

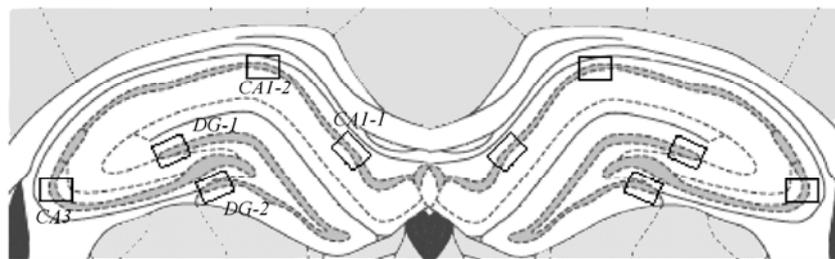


FIG. 1. Diagram of hippocampus coronal section showing the regions (CA1-1, CA1-2, CA3, DG-1, and DG-2) of hippocampus in which the number of neurons was counted.

### Statistical Analysis

Data were expressed as mean $\pm$ SEM. All statistical analyses were performed using SPSS software Version 10.0 (SPSS Inc., Chicago, IL, USA). The statistical difference in escape latency data of MWM test was evaluated by repeated measures and multivariate analysis of variance. Statistical analysis of the other index values was performed by one-way analysis of variance (ANOVA).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Behavioral Observation

No significant ( $P > 0.05$ ) difference was found in the swimming distance of rats in the 5 experimental groups (Fig. 2A).

The effects of *R. rosea* extract on performance of the hidden-platform acquisition training are shown in Fig. 2B. Although the latency to reach the submerged platform decreased gradually in all groups during the 4-day training period, the descent ranges were much less in model and H-RR groups than in normal control, M-RR and L-RR groups. On day 1, the latency level remained unchanged in three *R. rosea* extract administration groups (43.42 $\pm$ 4.51 s, 40.25 $\pm$ 1.99 s, and 45.40 $\pm$ 7.49 s in L-RR, M-RR, and H-RR groups, respectively), but decreased significantly ( $P < 0.05$ ) in normal control group (37.69 $\pm$ 4.14 s) compared with model group (56.78 $\pm$ 1.77 s). However, the latency level was significantly decreased in M-RR group on day 2 (20.22 $\pm$ 6.63 s) and in L-RR group on day 3 (23.51 $\pm$ 5.33 s) ( $P < 0.05$ ) compared with model group

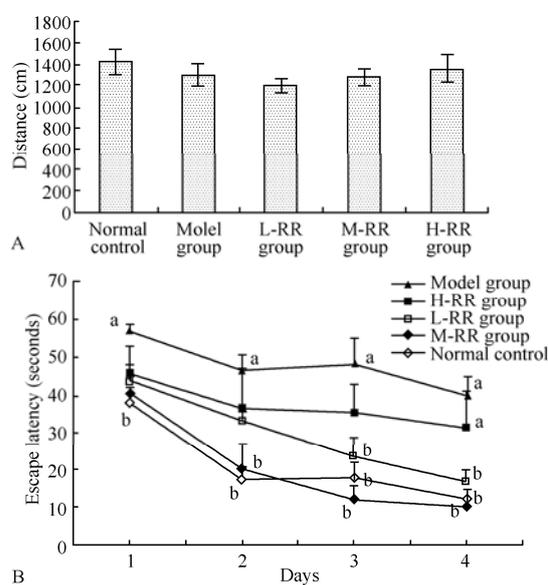


FIG. 2. A: Swimming distances in adaptation training/swimming ability test of rats. No significant difference was found in all groups, indicating that the swimming abilities of rats in five experimental group were similar. B: Effects of *R. rosea* extract on the hidden-platform training of MWM for acquisition of spatial learning in rats after ICV STZ. The average escape latency of eight trials per day for 4 days in each group is shown. Values are expressed as mean $\pm$ SEM of eight animals. (a)  $P < 0.05$  vs normal control group; (b)  $P < 0.05$  vs model group. L-RR: low dose of *R. rosea* extract, M-RR: medium dose of *R. rosea* extract, H-RR: high dose of *R. rosea* extract.

(46.45 $\pm$ 4.05 s vs 48.21 $\pm$ 6.64 s). On day 4, the latency level was not significantly different in L-RR and

M-RR groups ( $16.67 \pm 3.02$  s vs  $10.45 \pm 2.08$  s) as compared with normal control group ( $12.28 \pm 2.31$  s), but was significantly decreased ( $P < 0.05$ ) compared with model group ( $39.91 \pm 4.82$  s). The latency level was significantly higher in H-RR group ( $P < 0.05$ ) than in normal control group ( $31.03 \pm 9.85$  s vs  $12.28 \pm 2.31$  s), but was not significantly changed compared with model group ( $39.91 \pm 4.82$  s), suggesting that pretreatment with a low (1.5 g/kg) or a medium dose (3.0 g/kg) of *R. rosea* extract could improve the behavioral deficiency in rats after ICV STZ. The medium dose of *R. rosea* extract showed the maximal effect.

The effects of *R. rosea* extract on the performance of spatial probe trial in rats are shown in Fig. 3. Rats in the normal control group ran a large

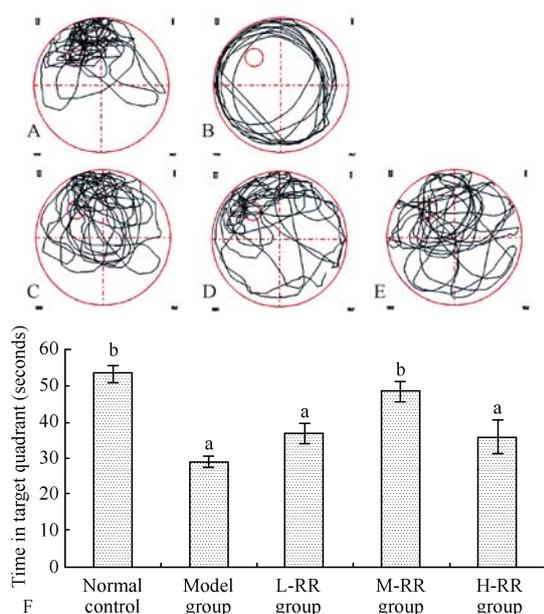


FIG. 3. Effects of *R. rosea* extract on the performance of spacial probe trial in rats of normal control group (A), model group (B), L-RR group (C), M-RR group (D), H-RR group (E), and on the time spent in the target quadrant (F). Typical trail graphs for rats in different groups running in the four quadrants of pool with the platform removed. The rats in STZ model swam around aimlessly during the probe trial and failed to show any focused searching, indicating severe spatial learning impairment. Different doses of *R. rosea* extract improved the performance of rats in probe trial in rats. Values are expressed as mean  $\pm$  SEM of eight animals. A medium dose of *R. rosea* extract (3.0 g/kg) significantly inhibited ICV STZ-induced memory deficits in M-RR group ( $P < 0.05$  vs model group). L-RR: low dose of *R. rosea* extract, M-RR: medium dose of *R. rosea* extract, H-RR: high dose of *R. rosea* extract. (a)  $P < 0.05$  vs normal control group; (b)  $P < 0.05$  vs model group.

part of the distance in the target quadrant (Fig. 3A), while rats in the STZ model group just showed an average distribution curve in four quadrants of the tank (Fig. 3B). Pretreatment with different doses of *R. rosea* extract diminished the behavioral deficiency in rats after ICV STZ (Fig. 3C-E).

The average time spent in the target quadrant for the 5 experimental groups was compared (Fig. 3F). The time was significantly ( $P < 0.05$ ) shorter in model group ( $29.17 \pm 1.52$  s) than in normal control group ( $53.18 \pm 2.37$  s). However, a medium dose of *R. rosea* extract (3.0 g/kg) restored the time value significantly ( $P < 0.05$ ) in M-RR group compared with model group ( $48.52 \pm 2.83$  s vs  $29.17 \pm 1.52$  s). In L-RR and H-RR groups, the time values ( $37.06 \pm 2.71$  s vs  $36.01 \pm 4.74$  s) were not significantly altered in comparison with model group, but were significantly ( $P < 0.05$ ) than in normal control group, suggesting that only pretreatment with a medium dose of *R. rosea* extract has beneficial effects on memory retention deficiency in rats after ICV STZ.

#### Effect of *R. rosea* Extract on Markers of Oxidative Stress in Rats after ICV STZ

**MDA level in hippocampus** The MDA level was significant higher in model group than in normal control group ( $P < 0.05$ ). The MDA level after pretreatment with a low dose (1.5 g/kg) or a medium dose (3.0 g/kg) of *R. rosea* extract was significantly lower in L-RR and M-RR groups than in model group ( $P < 0.05$ ). The MDA level was not significantly different between H-RR group and model group, but was significantly higher than in normal control group ( $P < 0.05$ , Table 1).

TABLE 1

Effects of *R. rosea* Extract on the Parameters of Oxidative Stress in Hippocampus of Rats ( $\bar{x} \pm s$ ,  $n=7$ )

Groups	MDA (nmol/mg.Pr)	GSH (mg GSH/g.Pr)	GR (U/g.Pr)
Normal Control	$0.32 \pm 0.09^b$	$15.83 \pm 2.43^b$	$30.25 \pm 4.20^b$
Model	$0.86 \pm 0.14^a$	$8.18 \pm 1.79^a$	$18.99 \pm 2.31^a$
L-RR	$0.53 \pm 0.15^b$	$12.96 \pm 1.49^b$	$20.31 \pm 2.03^a$
M-RR	$0.42 \pm 0.16^b$	$14.84 \pm 1.04^b$	$33.28 \pm 5.72^b$
H-RR	$0.68 \pm 0.10^a$	$11.86 \pm 1.34^b$	$14.90 \pm 1.88^a$

Note. <sup>a</sup> $P < 0.05$  vs normal control group; <sup>b</sup> $P < 0.05$  vs model group. L-RR: low dose of *R. rosea* extract, M-RR: medium dose of *R. rosea* extract, H-RR: high dose of *R. rosea* extract.

**GSH level in the hippocampus** The GSH level was significantly lower in hippocampus of model group than in that of normal control group ( $P < 0.05$ ).

*R. rosea* extract reversed the GSH level significantly in three *R. rosea* extract administration groups compared with model group ( $P < 0.05$ , Table 1).

**GR level in hippocampus** The GR level was significantly lower in model group as than in normal control group ( $P < 0.05$ ). The GR level was significantly reversed after pretreatment with 3.0 g/kg *R. rosea* extract in M-RR group compared with model group ( $P < 0.05$ ). The GR level was not significantly changed in L-RR group or H-RR group compared with model group, but was significantly lower than in normal control group ( $P < 0.05$ , Table 1).

#### Histopathological Studies and Neuron Number Quantification

The representative photographs for CA3 in hippocampus are shown in Fig. 4A-E). Histopathological studies revealed striking abnormalities in hippocampus of rats after ICV STZ. The number of neurons in hippocampus was markedly decreased after ICV STZ, and apoptosis of neurons occurred with characteristics such as condensation of chromatin and shrinkage of soma (Fig. 4B, arrows). *R. rosea* extract ameliorated the pathological abnormality in hippocampus (Fig. 4C-E).

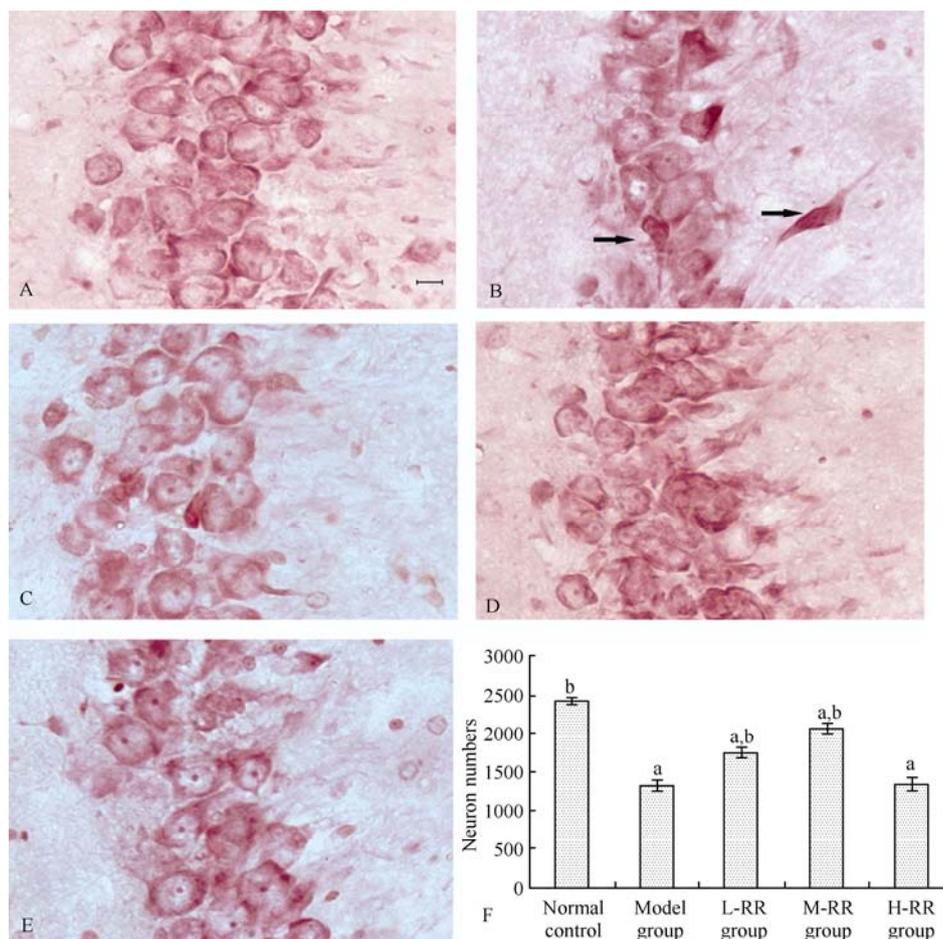


FIG. 4. Representative photographs for neuron Nissl staining in CA3 of hippocampus in rats of normal control group (A), model group (B), L-RR group (C), M-RR group (D), H-RR group (E), and the number of neurons in the hippocampus (F). After behavioral test, rats were sacrificed with their brains processed for histopathology. Coronal sections (15  $\mu$ m thick) were stained with neutral red. The image of model group showed a relatively lower number of neurons than that of other groups, and apoptosis of neurons occurred in model group with characteristics such as condensation of chromatin and shrinkage of soma (B, arrows). *R. rosea* extract ameliorated hippocampal pathological abnormality in rats after ICV STZ (C-E). Calibration bar=10  $\mu$ m. The number of neurons was significantly less in model group than in normal control group ( $P < 0.05$ ). Pretreatment with a low (1.5 g/kg) or a medium dose (3.0 g/kg) of *R. rosea* extract diminished the lesion of neurons in L-RR group or M-RR group. Values are expressed as mean  $\pm$  SEM of five animals.  $P$ -values are indicated over the bar. (a)  $P < 0.05$  vs normal control group; (b)  $P < 0.05$  vs model group. L-RR: low dose of *R. rosea* extract, M-RR: medium dose of *R. rosea* extract, H-RR: high dose of *R. rosea* extract.

To quantify the neuroprotective effects of *R. rosea* extract, the number of neurons in five areas of hippocampus was counted. The number of neurons was significantly decreased in model group compared with normal control group ( $P < 0.05$ ,  $1327.40 \pm 63.57$  vs  $2426.00 \pm 43.21$ ). The number of neurons was reversed significantly after treatment with a low (1.5 g/kg) or a medium dose (3.0 g/kg) of *R. rosea* extract in L-RR or M-RR group compared with model group ( $P < 0.05$ ,  $1751.20 \pm 69.45$  vs  $2078.40 \pm 66.19$ ), but not totally restored compared with normal control group. The number of neurons was not significantly different between H-RR group ( $1354.00 \pm 74.54$ ) and model group, but was significantly lower than in normal control group ( $P < 0.05$ , Fig. 4F).

## DISCUSSION

Although the antioxidant and neuroprotective properties of *R. rosea* have been widely studied, no reports about its effects on neurodegenerative diseases such as AD are currently available.

AD is a progressive and irreversible neurodegenerative disorder of the brain with a poor outcome and unknown etiology. Among various candidates responsible for the pathogenesis of AD, free radicals and oxidative stress can mediate behavioral impairments and memory deficits<sup>[22-23]</sup>. Although how oxidative stress exerts its deleterious effects remains unclear, it may increase lipid and protein peroxidation, DNA oxidation products and deficits in calcium regulatory mechanisms, eventually leading to cell death<sup>[46]</sup>. It has been found that ICV STZ can cause severe oxidative stress in brain of rats with prominent cognitive impairment, and biochemical and physiological abnormalities in AD<sup>[32,34-35,47]</sup>. ICV STZ has been used as animal models of sporadic AD<sup>[27-29,36]</sup>. It was reported that *R. rosea* is a potent antioxidant<sup>[8-11,38-40]</sup>. As an antioxidant, *R. rosea* may protect the nervous system against oxidative damage by free radicals. The endogenous glutathione antioxidant system plays a prominent role in the management of oxidative stress in cells. In conjunction with reductant NADPH, GSH can reduce lipid peroxides and other free radicals, during which, GSH is oxidized to GSSG which is reconverted to GSH by the action of enzyme GR, thus maintaining the pool of GSH. The present study showed that the lipid peroxidation level was significantly increased with striking impairments in learning and memory, which is in accord with the reported data<sup>[27-29,47]</sup>. These abnormalities in rats were significantly ameliorated after pretreatment with *R. rosea* extract. Previous studies indicate that chronic

treatment with antioxidants, melatonin and rosvaterol<sup>[29,33]</sup> can significantly improve cognitive deficits and oxidative damages in rats after ICV STZ. There is evidence that neuronal defense against  $H_2O_2$ , which is the most toxic molecule to the brain, is mediated primarily by the glutathione system<sup>[48]</sup>, suggesting that the antioxidant property of *R. rosea* can protect rats against cognitive impairment, possibly by enhancing the ability of endogenous glutathione defensive system in brain to combat oxidative stress induced by ICV STZ. The therapeutic effects of *R. rosea* on removal of free radicals or prevention of their formation may be beneficial for diseases like AD.

ICV STZ in rat brain causes severe deficits in learning and memory, implying damaged hippocampal function in brain. However, disrupted spatial memory might be attributed to the impairment of brain glucose and energy metabolism in rats after ICV STZ<sup>[24]</sup>. Shoham *et al.*<sup>[49]</sup> recently pointed out that damage caused by ICV STZ to axons and myelin in the fornix may contribute to spatial memory deficits. In this study, the oxidative damage to hippocampal neurons might contribute to cognitive impairment. It has been well established that the integrity of hippocampal formation is essential for learning and memory. Hippocampal cells are the primary substrate of spatial memory ability<sup>[50]</sup>. Since hippocampus plays a pivotal role in the development of learning and memory, oxidative damage may be particularly critical to this region of brain. In fact, brain tissue contains a large amount of polyunsaturated fatty acids, making the brain particularly vulnerable to free radical attacks<sup>[51]</sup>. In this study, the number of hippocampal neurons was significantly decreased in rats after ICV STZ rats, which was associated with elevated oxidative stress in hippocampus, suggesting that oxidative stress may lead to neuronal apoptosis and/or necrosis in hippocampus, contributing to deficits of cognitive function.

It was reported that salidroside, one of the major active ingredients in *R. rosea*, has neuroprotective and anti-oxidative effects<sup>[8-9,13-16,50]</sup>. However, whether *R. rosea* ingestion produces a similar neuroprotective function in cases referent to oxidative stress is unknown. The results of our preliminary study indicate that *R. rosea* ingestion exerts a beneficial effect on hippocampal neuron damage in depressive rats (data not shown), which prompted us to explore the protective effects of *R. rosea* on hippocampal neuron injuries in rats after ICV STZ. We found that the decreased number of hippocampal neurons was significantly reversed by pretreatment with *R. rosea* extract in rats after ICV STZ, with a substantial

decrease of oxidative stress in hippocampus, suggesting that *R. rosea* can protect hippocampal neurons against injury in rats after ICV STZ, possibly due to its anti-oxidative properties. It is therefore likely that the cognitive protecting effect of *R. rosea* results from the alleviated oxidative damage and subsequent neuronal function improvement. The exact mechanism by which *R. rosea* protects hippocampal neurons against STZ-induced oxidative damage needs to be further investigated.

A bell-shaped dose-response curve for our experimental data was obtained, showing that pretreatment with 3 g/kg *R. rosea* extract was more effective than with 1.5 g/kg *R. rosea* extract, but 6 g/kg *R. rosea* extract was less effective, which is consistent with previous findings<sup>[2,19]</sup>. It was reported that *R. rosea* is possibly safe with no adverse reactions<sup>[52]</sup>. The declined effectiveness of a higher dose of *R. rosea* might be attributed to its sedative effects on the central nervous system<sup>[53]</sup> and/or malnutrition, since a higher dose of *R. rosea* might lead to reduced nurturing behaviors in experimental animals and result in under-nutrition, suggesting that an efficacious dose of *R. rosea* has beneficial physiological effects on oxidative stress.

The results of this study indicate that ICV STZ causes learning and memory deficits and neuron injury in hippocampus of rats probably by generating free radicals. Pretreatment with a medium dose of *R. rosea* extract could significantly improve learning and memory deficits by inhibiting oxidative stress and ameliorating neuron injury in hippocampus. *R. rosea* extract can be used in treatment of cognitive deficits induced by neurodegenerative diseases including AD.

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(Received December 30, 2008 Accepted March 6, 2009)