Antiiaging Effect of *Cordyceps sinensis* Extract

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This experiment studied the effect of *Cordyceps sinensis* extract (CSE) on mice aged by α-galactose and castrated rats to analyse its antiaging effect. Water maze and step-down type avoidance tests were used to examine the effect of CSE on learning and memory. CSE shortened escape latency, prolonged step-down latency and decreased the number of errors in mice aged by α-galactose. The effect of CSE on the sexual function of castrated rats was evaluated by measuring the penis erection latency, mount latency and ejaculation latency. CSE appeared to shorten penis erection latency and mount latency in castrated rats. The study also measured the effect of CSE on the activity of age-related enzymes. The results showed that CSE improved the activity of superoxide dismutase, glutathione peroxidase and catalase and lowered the level of lipid peroxidation and monoamine oxidase activity in the aged mice. The study demonstrated that CSE can improve the brain function and antioxidative enzyme activity in mice with α-galactose-induced senescence and promote sexual function in castrated rats. All of these findings suggest that CSE has an antiaging effect.

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Keywords: *Cordyceps sinensis* extract; antiaging; antioxidation.

INTRODUCTION

*Cordyceps sinensis* is a fungal parasite that grows on the larvae of *Lepidoptera*, it has been used as a tonic herb in Chinese traditional medicine for centuries. In Chinese medicine, *Cordyceps sinensis* has long been used to ameliorate conditions associated with aging and senescence; it is principally applied to treat weakness in the loins and knees, impotence and seminal emission, hyposexuality, fatigue, night sweating and other conditions related to aging. Research shows that *Cordyceps sinensis* contains crude protein, β-mannitol (Zhang et al., 1991), cordycepin (Cunningham et al., 1951; Kredich and Guarino, 1960) and cordyceps polysaccharide (Miyazaki et al., 1997; Yamada et al., 1984). Recent studies have demonstrated its multiple pharmacological actions, including modulation of immune response (Wu et al., 2006), inhibition of tumor growth (Yoshida et al., 1989; Yoshikawa et al., 2007) and antihypertensive and antiarrhythmic effects (Manabe et al., 1996; Chiou et al., 2000). However, little research has been conducted on its antiaging effects. In the present experiment, the antiaging effects of *Cordyceps sinensis* on aged mice and castrated rats were studied and the antioxidative effects investigated.

MATERIALS AND METHODS

**Materials.** The following materials were used: the dried fruit bodies of cultured *Cordyceps sinensis* (Noevir Co. Ltd, Kobe, Japan), authenticated by Professor Shao-Qing Cai, Department of Nature Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, China; vitamins E and C (Beijing Double-Crane Pharmaceutical Co., Beijing, P.R. China); α-galactose (Shanghai Hengxin Chemical Reagent Co., Shanghai, P.R. China); urethane, testosterone, estradiol benzoate and progesterone (Shanghai General Pharmaceutical CO., Shanghai, P.R. China); pyrogallol and assay kits for protein (Nanjing Jiancheng Bioengineering Institute, Nanjing P.R. China) and assay kits for glutathione peroxidase (GSH-Px) (Genetimes Technology Inc., Beijing, P.R. China).

**Preparation of CSE.** A hot water extract of *Cordyceps sinensis* (CSE) was prepared by autoclaving 20 g of *Cordyceps sinensis* in 400 g of water at approximately 120 °C for 20 min, followed by filtration and freeze-drying. The yield of CSE was approximately 30%. CSE was a pale grey powder and was dissolved in distilled water and administered in a volume of 10 mL/kg. In this experiment, the animal dose of CSE was the dose of the crude drug.

**Animals.** Male and female Sprague-Dawley rats weighing 140–160 g and male ICR mice weighing 16–18 g were provided by the Experimental Animal Center of Peking University, China. The animals were group-housed in a regulated environment (22 ± 1 °C, humidity 60% ± 5%) with a 12 h light and 12 h dark cycle (08:00–20:00, light). Food and water were given *ad libitum*, except during behavioral experiments. The laboratory animal protocol used for this study was approved by the Peking University Committee for Control and Supervision of Experimental Animals.

**Learning and memory tests**

**Induction of senescence by α-galactose.** ICR mice were randomly divided into a normal group, an aged group,
three CSE groups and a positive control group. The normal group received sodium chloride s.c. and water orally. The aged group received α-galactose (1.25 g/kg, s.c) and water orally. The CSE group received α-galactose (1.25 g/kg, s.c) and CSE (1.0, 2.0 or 4.0 g/kg orally). The positive control group received α-galactose (1.25 g/kg, s.c) and vitamins E and C (50 mg/kg, orally). All drugs were administered for 6 weeks (Shen et al., 2002).

Water maze performance. The water maze performance test was performed according to the method of Zhang (2006). The water maze apparatus (Institute of Materia Medica, Chinese Academy of Medical Sciences, P.R. China) was a double-layer opaque plastic box that included three start points, a terminal platform and four non-exits. The maze was filled with water to a depth of 12 cm, and the temperature was kept at 25 ± 1 °C. Each mouse was trained to find the terminal platform within 3 min. There was one non-exit on days 1 and 2, two on days 3 and 4, and three on the days 5 and 6. On day 7, performance was evaluated by the time the mice took to find the terminal platform from among four non-exits.

Step-down type passive avoidance test. The passive avoidance test was conducted with an acrylic box with a stainless-steel grid floor. A platform was fixed in the end of the box. Electric shocks (36 V) were delivered to the grid floor for 3 s with an isolated pulse stimulator. At the beginning of training, mice were placed in the box to adapt for 5 min. When electric shocks were delivered, mice jumped to the platform. Twenty-four hours after training, the mice were placed on the platform for testing, and the step-down latency and the number of errors within 3 min were recorded (Joshi and Parle, 2006).

Histological studies. For electron microscopy, mice were decapitated, and the brains removed. The region of the brain that contained the hippocampus was sectioned into 2 mm slabs and placed in a fixative solution of glutaraldehyde (4%) in phosphate buffer (0.1 M; pH 7.4) for 24 h. Samples were then washed in phosphate-buffered saline (pH 7.4) before being post-fixed in osmium tetroxide 2% in 0.1 M phosphate buffer. Samples were dehydrated with acetone and then coated with epoxy resin. The semi-thin sections, obtained with an ultramicrotome, were stained with uranyl acetate (4%) in 70% ethanol and then lead nitrate. Sections were observed under the electron microscope.

Sexual functional tests

The Sprague-Dawley rats were divided into a sham group, a castrated group, a castrated testosterone group, and three castrated CSE groups. The sham and castrated groups were supplemented with water orally for 21 days, and the testosterone group received testosterone (2 mg/kg s.c.) for 21 days. The CSE groups were supplemented with 0.5, 1.0 or 2.0 g/kg CSE orally for 21 days.

Sexual functional deficiency induced by castration. Rats were either castrated or sham castrated under urethane anesthesia (20%, 7 mL/kg). Animals assigned to castration received a small incision at the posterior tip of the scrotum, through which each testicle was removed and ligated. The incision was sutured and antibiotics were applied. The control (sham castrated) rats received the same incision, but their testicles were not removed.

Measurement of the penis erection latency. For this experiment, a BT87-3 electric stimulus thrombus forming apparatus (BaoTou Medical University, BaoTou, P.R. China) was used. After 21 days of administration of water (sham and castrated groups), testosterone or CSE, the rat penises were scraped with sodium chloride to make the skin moist. Then an electrode was placed on the penis and continuous electric shocks (4 mA) delivered until the penis became erect. The time from shock to erection (the penis erection latency) was recorded (Luo et al., 2006).

Sexual activity testing. The sexual activity of male rats was estimated by copulation behavior after a female rat in estrus was placed into the male’s cage. All females were ovariectomized under urethane anesthesia. Two weeks after the ovariectomy, they were brought into estrus with injections of estradiol benzoate (20 μg/rat s.c.) and progesterone (500 μg/rat s.c.), 48 h and 4 h, respectively, before tests. The following behavioral measures of copulation were used: mount latency, time from introduction of the stimulus female to the first attempt to mount the female, and ejaculation latency (Sachs and Bialy, 2000). This experiment was conducted during the dark phase in a room with low levels of red light.

Age-related enzyme assays

To address some of the antiaging mechanisms of CSE, the effect of CSE on age-related enzymes was investigated. Blood samples were collected from the orbital plexuses of the ICR mice that were used in the learning and memory test. Mice were decapitated, and the brains and livers removed rapidly and homogenized (10%) in cold saline, centrifuged at 3000 × g at 4 °C for 20 min, and the supernatant was used for assay. Superoxide dismutase activity was determined by measuring the inhibition of pyrogallol autoxidation (Marklund and Marklund, 1974). The activity of GSH-Px was determined by quantifying the catalysed reaction rate of H2O2 and GSH spectrophotometrically at 37 °C and 412 nm according to Lawrence and Burk (1976). Catalase activity was determined by monitoring the absorbance of H2O2 at 230 nm (Aebi, 1987). Monoamine oxidase B (MAO-B) activity was determined by detecting the amount of benzylaldehyde at 242 nm (Kan and Benedetti, 1981). Lipid peroxidation was assayed by the measurement of malondialdehyde level on the basis of malondialdehyde reacting with thiobarbituric acid (detected at 532 nm) (Ohkawa et al., 1979).

Statistical analysis

The data are expressed as mean ± SD. Dunnett’s t-test was used to compare the differences between the treated groups and control groups, and differences were considered significant at p < 0.05.
RESULTS

Learning and memory test

Effects of CSE on learning and memory in mice aged by D-galactose. The study examined whether CSE affected working or reference memory with the water maze test and the step-down passive avoidance test, which can evaluate spatial memory and learning memory. Mice aged by D-galactose had a significantly longer escape latency than did normal control mice in the water maze test ($p < 0.01$). Vitamins E and C (50 mg/kg) shortened the escape latency significantly ($p < 0.01$). CSE (1.0, 2.0 and 4.0 g/kg) also significantly shortened the escape latency, compared with the aged mice ($p < 0.01/p < 0.05$), and the effect was dose-dependent (Fig. 1A).

Aged mice had a shorter step-down latency and made more errors than did normal control mice ($p < 0.05/$ $p < 0.01$) in the step-down passive avoidance test. Vitamins E and C (50 mg/kg) prolonged the step-down latency and decreased the number of errors ($p < 0.01$). Administration of CSE (2.0, 4.0 g/kg) prolonged the step-down latency ($p < 0.05/p < 0.01$) and decreased the number of errors ($p < 0.01$) markedly (Fig. 1B, 1C).

Effect of CSE on ultrastructure of hippocampus. The effects of CSE on the ultrastructure of the hippocampus in aged mice were observed with an electron microscope. In normal mice, neurons were well arranged; their organelles and nuclear structures were intact and clear. The neuraxis and myelin sheath structure could be seen clearly. There was no change in glial cells and no vacuolar degeneration of perivascular astrocytes and vascellum (Fig. 2A). In aged mice, a slight pyknosis in the hippocampus neurons was observed, with enriching of cytoplasm, swelling of organelles, lipofuscin deposits, axon and glial cell matrix raritas and degeneration, perivascular oedema and degeneration of endothelial cells (Fig. 2B). In mice treated with CSE (4.0 g/kg), the cellular structure appeared to be well conserved, with intact organelles and nuclear structure; no swelling was observed and less neuron pyknosis and cytoplasmic enriching (Fig. 2C).

Sexual functional test

Effect of CSE on the penis erection latency in castrated rats. The effect of CSE on the penis erection latency in castrated rats was investigated. The penis erection latency of castrated rats was significantly longer than among the sham castrated rats ($p < 0.01$). Testosterone (2 mg/kg) shortened the penis erection latency for castrated rats ($p < 0.01$). Administration of CSE (1.0, 2.0 g/kg) also shortened the penis erection latency for castrated rats ($p < 0.01/p < 0.05$) in a dose-dependent manner (Fig. 3).

Effect of CSE on the sexual activity in castrated rats. The effect of CSE on the sexual activity in castrated rats was investigated. The mount latency and the ejaculation latency were longer than among the sham rats ($p < 0.05$). Testosterone (2 mg/kg) shortened the mount latency ($p < 0.01$) and ejaculation latency ($p < 0.05$). CSE (2.0 g/kg) also shortened the

Figure 1. Effect of CSE on the aged mice induced by D-galactose in the water maze test and in the step-down test. (A) Escape latency; (B) Step-down latency; (C) Error number. The normal group received sodium chloride s.c. and water orally. The aged mice group received D-galactose (1.25 g/kg, s.c) and water orally. The CSE group received D-galactose (1.25 g/kg, s.c) and CSE (1.0, 2.0, 4.0 g/kg) orally. The positive control group received D-galactose (1.25 g/kg, s.c) and VE-C (50 mg/kg) orally. All drugs were administered for 6 weeks. The values shown are the mean ± SD of 12 mice. * $p < 0.05$, ** $p < 0.01$ vs aged mice group.
mount latency ($p < 0.05$), although no effect was observed on ejaculation latency (Table 1).

**Effects of CSE on the activity of age-related enzymes in mice aged by D-galactose**

In order to investigate whether the antiaging effect of CSE is related to its antioxidative effects, the effects of CSE on the activity of antioxidant enzymes and the level of lipid peroxidation in blood, brain, and liver tissues of mice aged with D-galactose were observed. In the aged mice, superoxide dismutase activity in the liver, brain and erythrocytes was substantially lower than in the normal control mice ($p < 0.01$), and catalase activity in the blood was significantly lower than that of the normal control mice ($p < 0.05$). GSH-Px, one of the glutathione-depleting enzymes, is a more efficient metabolizer of hydrogen peroxide than catalase. It was found that the activity of GSH-Px was significantly reduced

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**Table 1. Effect of CSE on the latency of mount and ejaculation in castrated rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>$n$</th>
<th>Mount latency (min)</th>
<th>Ejaculation latency (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>–</td>
<td>10</td>
<td>$5.2 \pm 1.9^a$</td>
<td>$7.4 \pm 2.4^a$</td>
</tr>
<tr>
<td>Castrated</td>
<td>–</td>
<td>10</td>
<td>$16.0 \pm 6.2$</td>
<td>$20.0 \pm 0.00$</td>
</tr>
<tr>
<td>Castrated + testosterone</td>
<td>0.002</td>
<td>10</td>
<td>$7.4 \pm 2.0^a$</td>
<td>$14.0 \pm 6.9^a$</td>
</tr>
<tr>
<td>Castrated + CSE</td>
<td>0.5</td>
<td>10</td>
<td>$15.9 \pm 7.3$</td>
<td>$20.0 \pm 0.0$</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>$15.8 \pm 6.9$</td>
<td>$20.0 \pm 0.0$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>$8.7 \pm 4.4^a$</td>
<td>$20.0 \pm 0.0$</td>
</tr>
</tbody>
</table>

All values are expressed as mean $\pm$ SD. $^a p < 0.05$, $^b p < 0.01$ vs castrated group.
in the blood of aged mice when compared with the normal control \((p < 0.05)\). Administration of CSE in aged mice \((2.0, 4.0 \text{ g/kg})\) enhanced the activity of superoxide dismutase in liver, brain and erythrocytes (Fig. 4A, 4B) and the activity of GSH-Px (Fig. 5A) and catalase (Fig. 5B) in blood when compared with the model control mice \((p < 0.01/p < 0.05)\). The lowest dose of CSE \((1.0 \text{ g/kg})\) enhanced GSH-Px and catalase activity \((p < 0.05)\). The effect of CSE on the activity of these enzymes was dose-dependent.

Malondialdehyde as an index of lipid peroxidation is presented in Fig. 6A. The results demonstrate that spontaneous lipid peroxidation in the brains and livers of aged mice increased significantly compared with the normal control mice \((p < 0.01)\). Treatment with CSE \((1.0, 2.0, 4.0 \text{ g/kg})\) significantly decreased the level of lipid peroxidation in the brains and livers of aged mice \((p < 0.01/p < 0.05)\) in a dose-dependent manner.

In the mice aged by D-galactose for 6 weeks, the activity of brain MAO-B was increased markedly \((p < 0.01)\) compared with the activity seen in normal control mice. Administration of CSE \((1.0, 2.0, 4.0 \text{ g/kg})\) led to a significant decline of monoamine oxidase activity \((p < 0.01/p < 0.05)\) in a dose-dependent manner (Fig. 6B).

**DISCUSSION**

It was found that CSE improved learning and memory and increased the activity of antioxidative enzymes in aged mice; it also increased sexual function in castrated rats. Previous studies showed that chronic injections of D-galactose subcutaneously into mice induced changes that resembled accelerated aging. The aging model shows neurological impairment, decreased activity of antioxidative enzymes and poor immune responses. D-Galactose is a reducing sugar that can form advanced glycation end products in vivo, which can not be further metabolized and accumulate in nerve cells; this effect at least partially contributes to the pathological mechanism of this aging model (Song *et al.*, 1999). Therefore, D-galactose-treated mice were used as an experimental senile model to investigate the antiaging effect of CSE.

Aging has a common symptom – memory dysfunction. Memory declines with age; this effect might be related to the decline of advanced function of the brain (Zhan *et al.*, 1990). In this study, step-down type avoidance and water maze tests were used to examine the long-term memory and spatial working memory, which are an index of senescence of mice. The effects of CSE on learning and memory of mice aged by D-galactose were investigated. CSE increased learning and memory significantly in a dose-dependent manner; it reduced the obstacle of spatial recognition in aged mice. Furthermore,
theories, including the longevity gene. The current view on free radical-induced oxidative damage is that most aging changes are due to molecular and cellular damage caused by free radicals (Harman, 1981; Beckman and Ames, 1998). The aging process is thought to lead to an imbalance between oxidative damage and the antioxidative defense system. Excess free radicals react with cellular lipids, proteins and nucleic acids, leading to local injury and eventual organ dysfunction. The oxidative damage induced by free radicals contributes to several age-associated disorders. To protect against oxidative injuries, the human body has an antioxidative system that consists of antioxidative enzymes: superoxide dismutase, which catalyses dismutation of superoxide anions to hydrogen peroxide; GSH-Px, which catalyses the degradation of \( \text{H}_2\text{O}_2 \) and hydroperoxides originating from unsaturated fatty acids at the expense of reduced

Figure 5. Effect of CSE on blood GSH-Px activity (A) and blood catalase activity (B) in the \( \alpha \)-galactose induced aged mice. The normal group received sodium chloride s.c. and water orally. The aged mice group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and water orally. The CSE group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and CSE (1.0, 2.0, 4.0 g/kg) orally. The positive control group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and VE-C (50 mg/kg) orally. All drugs were administered for 6 weeks. The values shown are the mean ± SD of 12 mice. * \( p < 0.05 \), ** \( p < 0.01 \) vs aged mice group.

Figure 6. Effect of CSE on the content of MDA in the brain and liver (A) and on the MAO activity of brain (B) in the \( \alpha \)-galactose induced aged mice. The normal group received sodium chloride s.c. and water orally. The aged mice group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and water orally. The CSE group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and CSE (1.0, 2.0, 4.0 g/kg) orally. The positive control group received \( \alpha \)-galactose (1.25 g/kg, s.c.) and VE-C (50 mg/kg) orally. All drugs were administered for 6 weeks. The values shown are the mean ± SD of 12 mice. * \( p < 0.05 \), ** \( p < 0.01 \) vs aged mice group.

The brain morphology showed that CSE ameliorated the ultrastructure of the hippocampus, which approximated the structures seen in unaged mice. These findings suggest that CSE can postpone the decline of brain function in aging mice.

During the process of aging, sexual function also declines. The experiment evaluated the effect of CSE on sexual function in castrated rats. CSE appeared to shorten the penis erection latency and mount latency in castrated rats, but it had no effect on ejaculation latency. These results showed that CSE has a mildly beneficial effect on sexual function, although this effect was not as pronounced as the direct actions of sex hormones.

There are several theories of aging: error-catastrophe, protein modification, free radical (oxidative stress), mitochondrial DNA, and some developmental–genetic
glutathione; and catalase, which converts \( \text{H}_2\text{O}_2 \) into molecular oxygen and water (Cunningham et al., 1951). Lipid peroxidation is an autocatalytic process that is a common consequence of cell death. This process may cause peroxidative tissue damage in aging. The MAO-B isoform regulates brain levels of most biogenic amines (e.g. dopamine, serotonin and norepinephrine). Its activity increases with age (Saura et al., 1997). Catecholamine oxidation and MAO activity are considered among the causative factors in increased oxidant stress that occurs during aging (Alper et al., 1999). Some MAO-B inhibitors may improve quality of life in the elderly. The present study investigated the relationship of the antioxidative properties of CSE to its antiaging effect. The experimental results showed that CSE appeared to enhance the activity of the antioxidant enzymes, superoxide dismutase, GSH-Px and catalase; lower the level of lipid peroxidation and the activity of MAO-B in aging mice, and slow the progress of aging.

In conclusion, CSE improved learning and memory in \( \alpha \)-galactose-treated mice and promoted sexual function in castrated rats; moreover, CSE improved the activity of antioxidant enzymes. The findings suggest that CSE has an antiaging effect. The antiaging effect of CSE may be related to its antioxidative effects, and future studies are needed to determine the mechanism of the antiaging effect. The present study may shed light on the pharmacological basis for the use of CSE to prevent the detrimental effects associated with aging.

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