

Anti-Aging and Enhanced Physique Activities Research of *Astragalus Mongolicus* Water Extract in Mice

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Abstract

The present study aimed to investigate the biological activities of *Astragalus mongolicus* (AM) water extract in mice. Mice intraperitoneally received D-gal 300 mg/kg, and orally administered with AM water extract (6, 12 or 24 g/kg) once daily for 56 days, the results of ethological examination exhibited that D-gal can weaken spatial memory function of mice in the Morris water maze test; further analysis demonstrated that the capacity of oxidative stress mediated by oxidase was enhanced in D-gal-injected mice. The decreased activities of SOD and GSH-Px and the increased activity of CAT could be found in hippocampus induced by D-gal. Based on the results above, we inferred and explored enhanced memory and physique abilities of AM water extract using three mice models (water maze test, swim test and beheaded breathing test), the results exhibited that AM water extract could prolong the swimming time, the breathing time and survival time. This work indicated that AM water extract was very helpful to organism, and the protective effect may relate to oxidative stress.

Keywords: *Astragalus mongolicus* water extract; D-gal; Aging; Physique activity

Introduction

Astragalus membranaceus is also named Huang Qi (HQ), and it is a typical traditional Chinese medicine (TCM) plant, and has presented for many years on the Western market (in Europe and USA) as food supplement. HQ has also been used for thousands of years in China and East Asia for kidney diseases, and in modern Chinese medicine, it seems to have renal protective effect in diabetic nephropathy [1]. The extract of the HQ root is usually used in Western phytotherapy as galenic preparations, containing dried extract standardized in polysaccharides, the substances that are mostly considered to be responsible for the presumed immunostimulant properties [2]; it is in particular used for recurrent respiratory diseases or as therapeutic complement in cancer treatment [3]. HQ injection is a preparation of an extract of Radix Astragali: the major components are astragalosides [4], other pharmacological ingredients include polysaccharides, flavones and amino acids. Modern pharmacological research has shown that HQ injection can enhance myocardial contractility, improve circulation, protect myocardial cells and regulate immunity [5,6].

D-galactose (D-gal) is a dialdehyde sugar that can be metabolized at body's normal concentration as the normal nutrient. However, the oxidase or dehydrogenase galacturonic can be generated at high levels of D-gal, and may be further metabolized to xylulose with generating superoxide anion and oxygen-derived free radicals, excess oxygen free radicals can cause oxidative stress. And advanced glycation end products (AGEs) could be formed by D-galactose readily reacting with the free amines of amino acids in proteins and peptides. Evidence shows that AGEs can significantly cause the accumulation of reactive

oxygen species (ROS), especially superoxide radicals and hydrogen peroxide.

Astragalus mongolicus (AM) derives from the dry root of *Astragalus membranaceus* Bge. var. *mongolicus* (Bge.) Hsiao. Some reports exhibited that AM could protect animals from kinds of injury of brain [7-10]. So in this work, we investigated the protective effects of AM on D-gal induced memory impairment *in vivo* and physique activities of AM using three mice models (water maze test, swim test and beheaded breathing test).

Materials and Methods

Animal experiments

The experiments were carried out on adult KunMing male mice (18–22 g) purchased from Animal Center of Jilin University and adapted to conditions for 7 days. Mice were housed in a quiet, temperature- and humidity-controlled room (22 ± 2°C and 60 ± 5%, respectively) with a 12-h light/dark cycle with food and water continuously available. All experiments were performed at the same time of day during the light period. All procedures in the present study were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adapted by the National Institutes of Health (Washington, DC, USA, 1996). Local ethical committee approval was also obtained. All efforts were made to minimize animal suffering and to reduce the number of animals used.

D-gal induced memory impairment

60 animals were randomly divided into five groups with twelve each group. Group I was as control group: mice were treated with distilled water (5 ml/kg) orally. Group II was as model group: mice were

treated with distilled water (5 ml/kg) orally and D-galactose (300 mg/kg) by ip. Group III was as low dose of AM group: mice were treated with AM water extract (6 g/kg) orally and D-galactose (300 mg/kg) by ip. Group IV was as middle dose AM group: mice were treated with AM water extract (12 g/kg) orally and D-galactose (300 mg/kg) by ip. Group V: mice were treated with AM water extract (24 g/kg) orally and D-galactose (300 mg/kg) by ip. All the mice were treated twice daily for 56 days and given normal diet. On the last day of experiment, the mice were deprived of food overnight, behavior evaluation was performed the next day. At last, blood from eyes was collected in polystyrene tubes without the anticoagulant. Serum was immediately separated by centrifugation at 3,000 rpm at room temperature for 10 min and assayed for activities of GSH-PX, SOD, CAT.

Water maze test

The water maze test is a widely accepted method for memory test, thus we performed this test as the method to determine memory impairment. Maze testing was performed by the TaiMeng program and equipment (ChengDu, China). A circular plastic pool (height: 35 cm, diameter: 110 cm) was filled with water (with dark ink) which was kept at 22–25 °C. An escape platform (height: 14.5 cm, diameter: 4.5 cm) was submerged 0.5–1 cm below the surface of the water. Mice were trained twice per day for 5 days. Each trial lasted for 60 s or ended as soon as the mice reached the submerged platform and remained on the platform for 10 s. Mice were allowed to swim until they sought the escape platform. Escape latency, escape distance, swimming speed and swimming pattern of each mouse was monitored by a camera above the center of the pool connected to a SMART-LD program. A quiet environment, consistent lighting, constant water temperature and fixed spatial frame were maintained throughout the period of the experiment.

Antioxidant enzyme activities of serum

Antioxidant enzyme activities were assayed with superoxide dismutase (SOD) activity assay kit (Nanjing, Jiancheng), Glutathione peroxidase (GSH-PX) assay kit (Nanjing, Jiancheng) and Catalase (CAT) assay kit (Nanjing, Jiancheng). The assay was in accordance with the manufacturer's instructions.

Physique activities experiment

80 male mice were randomly divided into four groups with 20 mice of each group. Group I: normal control (NC), mice were treated with distilled water (5 ml/kg). Group II: mice were treated with 6 g/kg AM water extract. Group III: mice were treated with 12 g/kg AM water extract. Group IV: mice were treated with 24 g/kg AM water extract. All the mice were treated twice daily for 7 days and given normal diet. Behavior evaluation was at the end of administration.

Swim test

10 mice were selected randomly from the four groups respectively for swimming experiments. After 1 h of the last administration with AM water extract, animals were put into the swimming tank (the depth of the water was not less than 30 cm, the water temperature was controlled at 25 ± 0.5 °C and 5% body weight load was added to root of mice tails), time period was recorded from putting animals into water to death (mice stayed on the bottom for 5 seconds).

Beheaded breathing test

10 mice were selected randomly from the four groups respectively for beheaded breathing test. After 1.5 h of the last administration with AM water extract, mice were beheaded, time period was recorded from beheaded to death (mice stay mouth close for 5 seconds).

Statistical analysis

Data were shown as mean with standard deviation. Values of $p < 0.05$ were considered as significant difference. Behavioral data were analyzed with one-way analysis of variance (ANOVA).

Results

To examine the effect of AM on memory impairment induced by D-gal using the Morris water maze, the mice were continuously administered with AM water extract at a dose of 6, 12 and 24 g/kg by oral and D-gal 300 mg/kg by ip for 8 weeks respectively, and then the animals were trained 10 times (2 twice/5 days) by the water maze test. Mice in Group II slowly arrived at the location of the platform compared to group I, and impairment by D-gal in all the mice of three groups treated with AM water extract (group III, IV and V) were improved on ability of escape latency (Figure 1). The mice exhibited shorter and shorter escape latency by the end of the training trial.

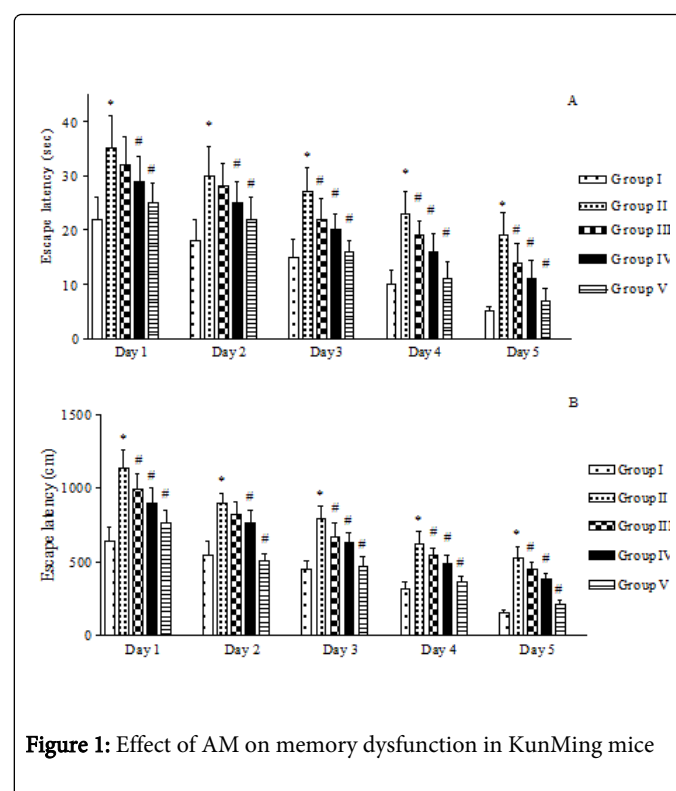


Figure 1: Effect of AM on memory dysfunction in KunMing mice

Training trials were performed twice per day for 5 days. (A) Swimming time and (B) swimming distance to arrive at the platform. Values are presented as means ± SD from 12 mice, * $p < 0.05$, different from group I, and # $p < 0.05$, different from group II.

The levels of T-SOD, GSH-Px and CAT in the serum of mice were measured. SOD level of serum in AM treated mice was significantly decreased compared to that in the CN group, and the concentration of SOD was significantly increased with the increasing dose of AM Figure

2A. GSH-Px level of serum in AM treated mice was significantly decreased compared to that in the CN group, and the concentration of SOD was significantly increased with the increasing dose of AM Figure 2B. CAT level of serum in AM-treated mice was significantly increased compared to that in the CN group, and with the increasing dose of AM, the concentration of SOD was significantly decreased Figure 2C.

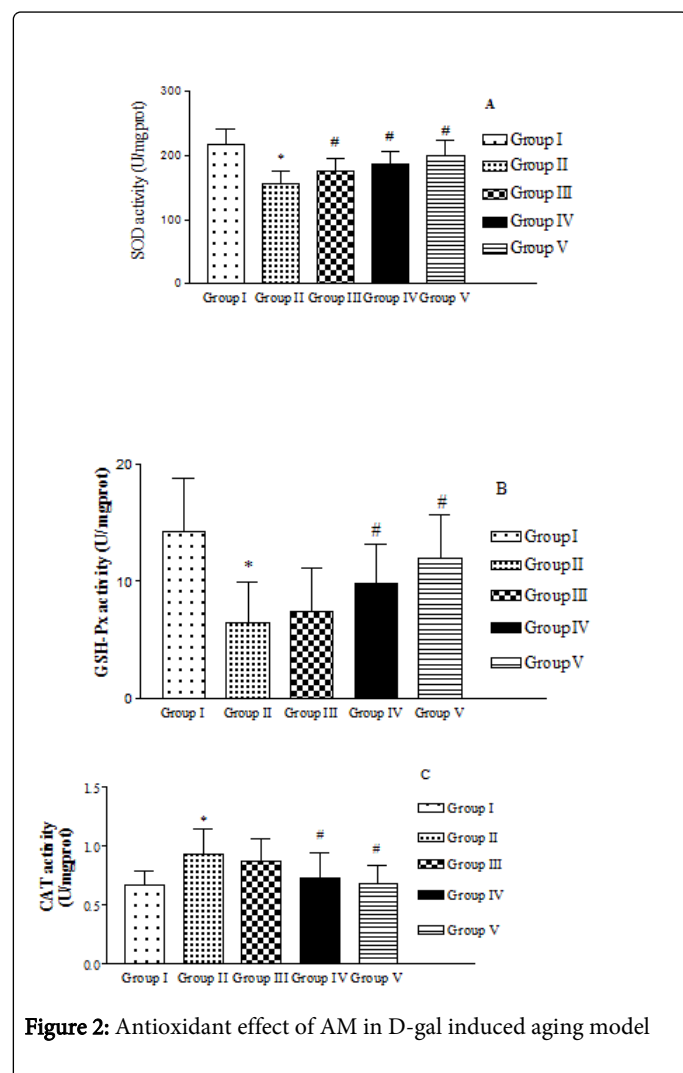


Figure 2: Antioxidant effect of AM in D-gal induced aging model

Based on the results above, we inferred and explored the enhanced physique activities of AM water extract using two mice models (swim test and beheaded breathing test). The results of swimming test and beheaded breathing test were shown in the Figure 3, as is shown in Figure 3A, the swimming time prolonged with the increasing dose of AM water extract, and the breathing time prolonged with the increasing dose of AM water extract too (Figure 3B).

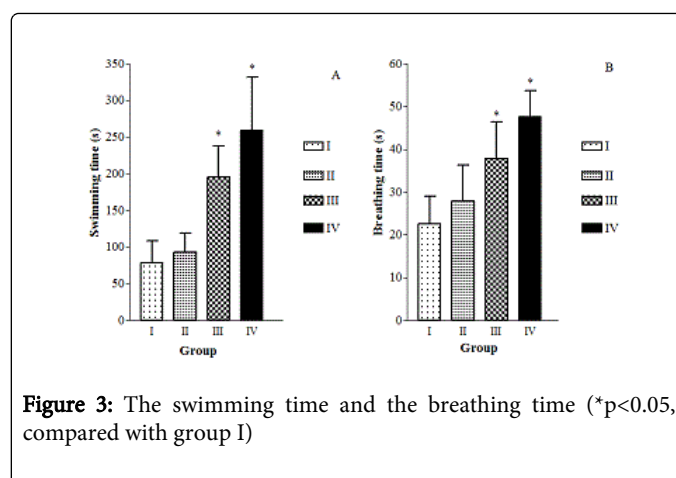


Figure 3: The swimming time and the breathing time (*p<0.05, compared with group I)

Discussion

Alzheimer's disease (AD) is the most common cause of dementia, accounting for 50% to 75% of all cases [11]. Oxidative stress is believed to be a primary factor in the normal process of aging [12]. It is well known that mitochondrial dysfunction and activation of caspases caused by oxidative stress and apoptotic cell death could be increased by exogenous H_2O_2 . Furthermore, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) act by scavenging the superoxide anion and H_2O_2 to prevent reactive-oxygen-species- (ROS-) induced damage [13]. D-galactose (D-gal) induced oxidative stress test was often used to selected medicine with anti-oxidative stress and anti-aging activity [14-17]. The results exhibited that administration of D-gal 300 g/kg daily for 8 weeks prior to training induced a significant increase in memory injury, oxidative stress was also induced by D-gal. Our study demonstrated that AM water extract (more than 12 g/kg p.o.) could reverse all the alterations induced by D-gal. In other word that AM water extract could protect the memory from impairment induced by D-gal in mice, and the more AM water extract was intake, the more protective effects on oxidative stress induced by D-gal in mice would be produced. Thus, the study indicated that AM water extract may have a protective effect against AD via modulating oxidative stress. These results suggest that further study is necessary to evaluate the effect of AM on damage of memory and to determine the molecular mechanisms. D-gal administration sequentially affected multiple-pathways, including protein transport and signal transduction, which could play key roles in maintaining the stability of synaptic structures [18]. The swimming and beheaded breathing tests results exhibited that AM water extract could prolong the swimming time, the breathing time and survival time. All the work results indicated that AM water extract was very helpful to organism, and the protective effect may relate to oxidative stress. In conclusion, D-gal increased ROS production induced by oxidative stress and lipid peroxidation and also maintained endogenous antioxidant enzymatic activities, stabilized mitochondrial function. These results should encourage further studies to explore the potential mechanism of AM effects on aging in neurologic diseases. It is possible that AM acts through several mechanisms, mediating a potential role in memory damage and improved physique activities in mice. This work indicated that AM water extract was very helpful to organism, and the protective effect may relate to oxidative stress.

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