Effects of the Combination of Astragalus and Angelica on Cognitive and Memory Functions in Alzheimer's Disease Model Mice

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Abstract: *This study aimed to investigate the effects of the combination of Astragalus and Angelica on cognitive and memory functions in Alzheimer's disease (AD) model mice. Male ICR mice were divided into six groups: normal control, positive control (donepezil hydrochloride), model, and treatment groups (high, medium, and low doses of Astragalus-Angelica decoction), with six mice in each group. The AD model was established by administering D-galactose and sodium nitrite. Starting from the third week of modeling, the treatment groups received intragastric administration of 80, 40, and 20 mg/kg Astragalus-Angelica decoction, respectively, while the positive control group received 0.65 mg/kg donepezil hydrochloride via intragastric administration. On day 46 of the experiment, spontaneous alternation rate, novel object preference index, organ indices (brain, thymus, and spleen), malondialdehyde (MDA) content, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the hippocampus were measured. Results showed that, compared to the model group, the spontaneous alternation rates and novel object preference indices in all treatment groups were significantly increased ($P<0.05$), hippocampal MDA levels were significantly reduced ($P<0.01$), and SOD and GSH-Px activities were elevated ($P<0.05$). However, there were no significant differences in organ indices ($P>0.05$). These findings indicate that the Astragalus-Angelica decoction significantly improves impaired cognitive and memory functions in AD model mice, potentially through mechanisms involving enhanced antioxidant capacity in the hippocampus and mitigation of cellular aging.*

Keywords: *Astragalus-Angelica decoction; Alzheimer's disease; cognitive memory; antioxidant*

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder primarily affecting elderly and pre-elderly populations. It is characterized by gradual neuronal degeneration and synaptic dysfunction in the brain, leading to severe cognitive impairment and posing a significant threat to the physical and mental health of older individuals [1]. Current medications can temporarily control cognitive function and behavioral symptoms but cannot halt or reverse neuronal degeneration and are often accompanied by notable side effects. Therefore, there is an urgent need in the medical field to explore new strategies for the prevention and treatment of AD.

Natural products, particularly traditional Chinese medicine, exhibit anti-inflammatory, immunomodulatory, antioxidant, and neuroprotective effects, providing potential for preventing and improving neurodegeneration while promoting holistic patient care. This study is based on the classic formula Danggui Buxue Decoction, focusing on the effects of the combination of Astragalus and Angelica. By employing a model of AD in mice induced with D-galactose and sodium nitrite, the research aims to investigate the improvement of cognitive and memory impairment and explore the underlying mechanisms, offering new insights and approaches for the clinical treatment of AD using traditional Chinese medicine.

1. Materials and Methods

1.1 Experimental Animals

Thirty-six 4-month-old specific *pathogen*-free (SPF) ICR mice, weighing 18–22 g, were obtained

from the Animal Experiment Center of Ningxia Medical University. The mice were housed under standard laboratory conditions with ad libitum access to food and water. The environment was maintained at 23°C, with a humidity of 40%–70% and a 12-hour light-dark cycle.

1.2 Drugs and Reagents

Astragalus and Angelica slices (supplied by Min Nongren Traditional Chinese Medicine Co., Ltd., Min County). Donepezil hydrochloride tablets (manufactured by Eisai Co., Ltd., China, National Drug Approval Number: *H20050978*) were ground into powder and prepared as a 0.65 mg/kg suspension in sterile water, stored at 4°C. D-galactose (purchased from Tianjin Bohuatong Chemical Sales Center, batch number: 20220509), sodium nitrite (meilunbio, CAS: 59-23-24). Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) assay kits were supplied by Ke Lu Biotechnology Co., Ltd.

1.3 Equipment

Y-maze (Ningxia Medical University, School of Pharmacy), manual grinder (Ningxia Medical University, School of Pharmacy), TGL-16G tabletop high-speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory).

1.4 Experimental Methods

1.4.1 Preparation of Astragalus-Angelica Decoction

Thirty grams of Astragalus and 6 grams of Angelica were soaked in eight times the volume of purified water for *30* minutes. The mixture was decocted for 1.5 hours; the second decoction used eight times the volume of purified water for 1 hour. The decoctions were combined, concentrated to 100 mL, and stored at 4°C.

1.4.2 Grouping, Modeling, and Administration

The mice were randomly divided into six groups: normal control, model, treatment (high, medium, and low doses of Astragalus-Angelica Decoction), and positive control (donepezil hydrochloride), with six mice in each group. Except for the normal control group, all mice received 150 mg/kg D-galactose subcutaneously and 90 mg/kg sodium nitrite intraperitoneally daily for 46 days to establish the AD model. The normal control group received physiological saline instead.

Starting from the third week of modeling, the positive control group was intragastrically administered donepezil hydrochloride at 0.65 mg/kg. The treatment groups received Astragalus-Angelica Decoction at doses of 80, 40, and 20 mg/kg, respectively, once daily. The normal control and model groups received an equivalent volume of physiological saline. Administration continued alongside modeling.

1.4.3 Y-Maze Test

The Y-maze test was used to measure spontaneous alternation rates, assessing spatial memory in mice. Each mouse was placed at the center of the Y-maze, and the sequence of arm entries was recorded for 8 minutes. *An* alternation (e.g., ABC, CBA, or BAC) was defined as consecutive entries into all three arms. The total arm entries and alternations were recorded.

The formula for calculating the spontaneous alternation rate is:
Spontaneous Alternation Rate (%) = [Total Alternations / (Total Arm Entries - 2)] × 100%.

1.4.4 Novel Object Recognition Test

The novel object recognition test assessed short-term cognitive memory in mice. Five open experimental cages were prepared, numbered 1 to 5. Black plastic bags were used to cover cages 1, 3, and 5 for shading, reducing external interference, while cages 2 and 4 remained uncovered for adaptation. In cage 3, two identical objects (referred to as B) were placed equidistant from the edges. Cage 5 contained one identical object B and a novel object A (different in shape and color).[2]

The test *procedure* placed each mouse in cages 1–5 sequentially, allowing 5 minutes of free exploration in each cage. The number of explorations of objects A and B in cage 5 was recorded.

Criteria: A mouse touching an object with its nose or paw or displaying exploratory behavior without *contact* was considered an exploration. Continuous exploration of the same object was recorded once

unless interrupted.

Preference Index (%) = [Explorations of A / (Explorations of A + B)] × 100%.

1.4.5 Organ Index Measurement

After completing behavioral tests, mice were euthanized, and the brain, thymus, and spleen were rapidly extracted. These organs were washed with cold physiological saline, dried on filter paper, and weighed using an *analytical* balance to calculate the organ index.

Organ Index = Organ Weight (mg) / Body Weight (g).

1.4.6 Biochemical Index Detection

The hippocampus was dissected from the brain tissue on an ice pack and weighed. Tissue homogenates were prepared by adding nine times the volume of pre-cooled physiological saline and grinding under ice bath conditions. The homogenate was centrifuged at 3000 r/min for 10 minutes, and the supernatant was stored in clean tubes at -20°C for further analysis. The MDA content and activities of GSH-Px and SOD in the supernatant were measured using assay kits.[3]

1.4.7 Statistical Analysis

Data were processed using SPSS 25.0 software and expressed as mean ± standard deviation ($\bar{x} \pm s$). Variance homogeneity tests were followed by one-way ANOVA. LSD tests were conducted for significant differences. Statistical significance was set at $P<0.05$ or $P<0.01$.

2. Experimental Results

2.1 Effect of Astragalus-Angelica Decoction on Spatial Memory in Mice

The results of the Y-maze test showed that compared to the normal control group, the spontaneous *alternation* rate in the model group was significantly reduced ($P < 0.01$). However, compared to the model group, the high- and medium-dose treatment groups, as well as the positive control group, demonstrated a significant increase in spontaneous alternation rates ($P < 0.01$), while the low-dose treatment group showed a notable increase ($P < 0.05$). These results indicate that spatial memory in the model group mice was significantly impaired. Treatment with Astragalus-Angelica Decoction at high, medium, and low doses significantly counteracted the spatial memory impairment induced by D-galactose and sodium nitrite, effectively improving cognitive function in the mice.

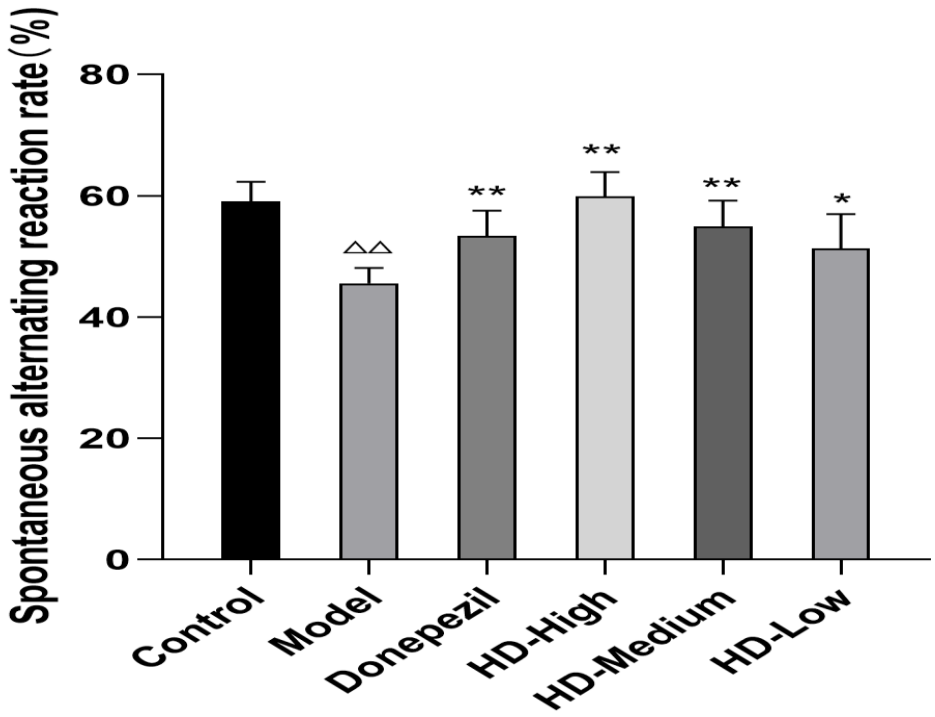

Figure 1 Spontaneous Alternation Rate in Mice Across Different Groups

Note: Compared with the normal control group, △△P < 0.01; compared with the model group, *P < 0.05, **P < 0.01.

2.2 Effect of Astragalus-Angelica Decoction on Cognitive Function in Mice

The results of the novel object recognition test showed that, compared to the normal control group, the preference index for the novel object in the model group was significantly reduced (P < 0.05). In comparison to the model group, the high-dose treatment group and the positive control group exhibited a significant increase in the novel object preference index (P < 0.01), while the medium-dose treatment group showed a notable increase (P < 0.05). These findings indicate that the cognitive function of the model group mice was impaired. Astragalus-Angelica Decoction at high and medium doses effectively improved short-term cognitive memory deficits in AD mice, enhancing their desire to explore novel objects.

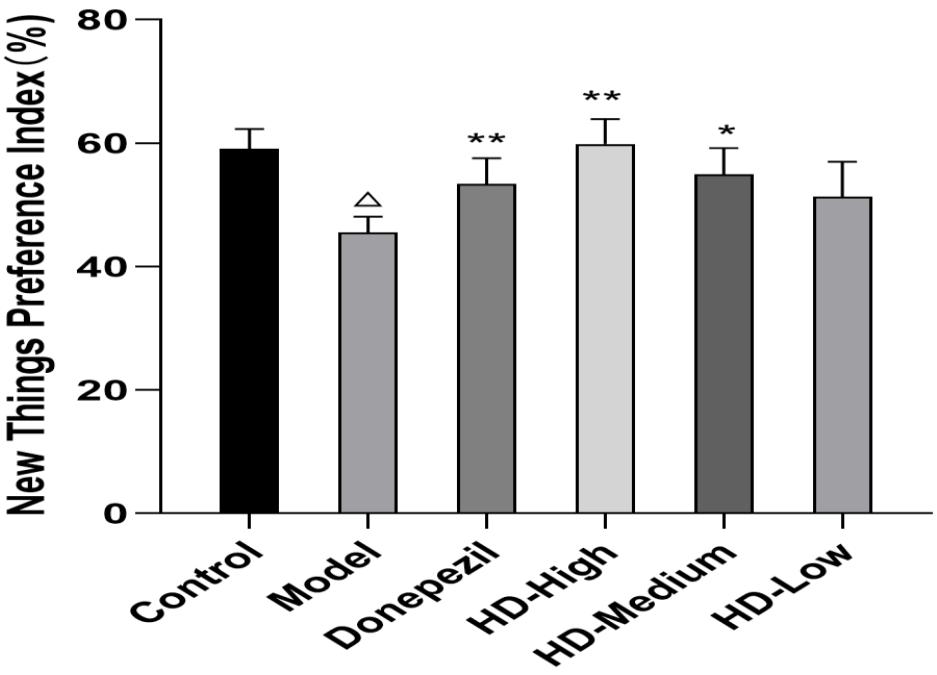

Figure 2 Preference Index for Novel Objects Across Different Groups

Note: Compared with the normal control group, △P < 0.05; compared with the model group, *P < 0.05, **P < 0.01.

2.3 Effect of Astragalus-Angelica Decoction on Organ Indexes in AD Mice

The results of the organ index analysis showed that, compared to the model group, the high-dose Astragalus-Angelica Decoction group exhibited a significant increase in thymus index and brain tissue index (P < 0.05). *However*, there was no significant difference in organ indexes between the model group and the normal control group (P > 0.05). These findings suggest that the effect of Astragalus-Angelica Decoction on organ indexes in AD mice is inconclusive, requiring further experimental investigation.

Table 1 Effects of Astragalus-Angelica Decoction on Organ Indexes in Mice (n=6, mean ± SD)

Group	Thymus Index	Spleen Index	Brain tissue Index
Control	2.98±0.82	5.01±0.68	12.59±0.85
Model	2.40±0.89	5.67±1.39	12.16±0.46
Donepezil	2.32 0.84	4.57±0.81*	12.24±0.68
HD-High	3.33±0.97*	4.81±0.70	13.26±0.58*
HD-Medium	3.01±0.76	4.48±0.77*	12.70±1.45
HD-Low	2.93±0.24	4.49±0.52*	11.82±0.85

Note: Compared with the *normal* control group, △P < 0.05; compared with the model group, *P < 0.05.

2.4 Effect of Astragalus-Angelica Decoction on Antioxidant Capacity in the Hippocampus of AD Mice

*Compa*red with the normal control group, the model group showed significantly decreased SOD and GSH-Px activities (P < 0.05) and significantly increased MDA levels (P < 0.01) in the hippocampus.

Compared with the model group, SOD activity was significantly increased in the positive control group ($P < 0.05$) and markedly elevated in the high- and medium-dose treatment groups ($P < 0.01$). The high-dose treatment group also showed a significant increase in GSH-Px activity ($P < 0.05$). MDA levels were significantly reduced across all treatment groups and the positive control group ($P < 0.01$).

These results suggest that high- and medium-dose Astragalus-Angelica Decoction can significantly *mitigate* oxidative stress-induced cellular damage and aging in the hippocampus of AD mice, with treatment efficacy surpassing that of donepezil hydrochloride.

Table 2 Effects of Astragalus-Angelica Decoction on Hippocampal SOD, GSH-Px Activities, and MDA Content in AD Mice (n=6, mean ± SD)

Group	SOD（U/mgP rot）	GSH-Px（U/mgP rot）	MDA（mmol/mgP rot）
Control	27.86±5.91	20.02±6.20	6.71±3.63
Model	21.35±1.08$^{\triangle\triangle}$	26.73±4.18$^{\triangle}$	13.63±1.43$^{\triangle\triangle}$
Donepezil	26.33±5.01*	23.08±6.03	7.24±2.26**
HD-High	33.48±4.90**	32.48±5.64*	8.95±1.10**
HD-Medium	28.253.63**	24.86±2.10	5.81±0.76**
HD-Low	21.26±1.54	24.42±2.51	11.44±3.87

Note: Compared with the normal control group, $\triangle P < 0.05$; compared with the model group, $*P < 0.05$, $**P<0.01$.

3. Discussion

Alzheimer's Disease (AD) is the most common type of dementia among individuals aged 65 and older. According to Traditional Chinese Medicine (TCM) theory, dementia primarily involves the brain, and deficiency in Qi and blood is its primary pathogenesis. Based on the pathological foundation of Qi and blood deficiency, this study utilized the classic formula *Danggui Buxue Tang* (comprising Astragalus and Angelica at a 5:1 ratio) to develop Astragalus-Angelica Decoction. Astragalus, a prominent Qi-tonifying herb, and Angelica, a key blood-activating and stasis-resolving herb, were combined to investigate their effects on improving cognitive and memory impairment in an AD mouse model.

D-galactose increases free radical levels, damages neurons, and reduces antioxidant enzyme activity. Sodium *nitrite* impairs the oxygen-carrying capacity of hemoglobin, leading to tissue hypoxia, organ damage, and cognitive-behavioral disorders[4]. In this study, the AD mouse model was established using a combination of D-galactose and sodium nitrite. Oxidative stress in tissues was evaluated by measuring MDA levels, while the capacity to remove harmful substances like peroxides and free radicals was assessed through SOD and GSH-Px activity.

Results showed that high- and medium-dose Astragalus-Angelica Decoction significantly enhanced SOD activity and *reduced* MDA levels in the hippocampus. High-dose treatment also significantly increased GSH-Px activity. These findings indicate that Astragalus-Angelica Decoction effectively scavenges free radicals and peroxides, reducing oxidative stress in the brain and combating neuronal degeneration. Its antioxidant and anti-aging effects exhibited a clear dose-dependent relationship.

The Y-maze test assesses spatial memory by recording the spontaneous alternation rate of test animals[5]. Results *revealed* that Astragalus-Angelica Decoction significantly improved the spontaneous alternation rate of mice, indicating its efficacy in mitigating short-term spatial memory impairment in AD mice. This improvement was positively correlated with the dosage.

The novel object recognition test measures cognitive ability[6]. In this study, high- and medium-dose

Astragalus-Angelica Decoction significantly increased the novelty preference index of mice. This result further *confirmed* its effectiveness in improving cognitive and memory deficits in AD mice, with the improvement also being dose-dependent. Therefore, Astragalus-Angelica Decoction shows great potential for enhancing spatial memory and cognitive function in mice.

Conclusion

Astragalus-Angelica Decoction can effectively scavenge free radicals and peroxides, repair damaged *neurons*, improve cognitive and memory dysfunction in AD mice, and delay aging. With its simplified formulation, food-medicine compatibility, and cost-effectiveness, this decoction provides a novel approach and method for AD treatment.

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