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Neuroprotection of Rhizoma Polygonati on Mice with Cognitive Dysfunction Induced by D-Galactose

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Abstract

Cognitive dysfunction (CD) is manifested as forgetfulness and memory loss, accompanied by language, mental, motor dysfunctions and etc. Rhizoma Polygonati (RP) is a traditional Chinese medicine for both medicinal and edible uses in China, which have the functions of improving learning and memory abilities, but neuroprotection of Rhizoma Polygonati (RP) on Mice with Cognitive Dysfunction is unclear. In present study, the ability of processed RP improving CD mice induced by D-galactose (D-gal) was investigated. The results showed that gavage administration of RP could significantly increase the new object recognition index and improve behavioral ability of CD mice through Morris water maze and step-down tests. RP recovered the impaired cells in the hippocampus caused by D-gal. RP enhanced the anti-oxidative activity by increasing the T-AOC and decreasing the MDA content, and significantly inhibited the inflammation in the nervous system by declining the contents of TNF-α and IL-6 in the hippocampus of mice. These results indicated that RP had a significant protective effect on D-gal induced cognitive dysfunction mice.

Keywords

Rhizoma Polygonati, Cognitive Dysfunction, Neuroprotection

1. Introduction

Cognitive dysfunction (CD) is a state of cognitive impairment between normal aging and dementia, symbolized by loss of cognitive functions, such as language, motor, as well as memory (Pei, 2020). CD is considered as an early signal of

Alzheimer's Disease (AD), which is the most common dementia in the world (Wang et al., 2020). AD is a common neurodegenerative disease in the elderly and the major clinical manifestations are memory loss, learning disability and cognitive impairment (Zhang et al., 2019a). At present, the cognitive function of experimental animals is mainly evaluated by the behavioral tests including Morris water maze (Anderson, 2013), new object recognition (Cole, 2020), step-down (Xie, 2006).

There are mainly five types of drugs approved by the Food and Drug Administration (FDA) for the treatment of AD, including Donepezil, tacrine, Ristimine, Galantamine and memantine (Wang, 2009). However, the side effects are obvious, and not recommended for long-term administration. Therefore, there is an urgent need to develop new drugs and treatments. Whereas traditional Chinese medicine and health care products, with multi-targeted mechanisms, has brought additional opportunities for cognitive improvement to patients with AD.

Rhizoma Polygonati (RP) is the dried rhizome of *Polygonatum kingianum* Coll. et Hemsl., *Polygonatum sibiricum* Red. or *Polygonatum cyrtonema* Hua, which is effective in improving memory, anti-aging, anti-oxidation, regulating immunity, anti-tumor, antibacterial and so on (Tao, 2021; Zhang et al., 2019b). RP is a traditional Chinese medicine for both edible and medicinal uses in China, and its effects can be enhanced by the traditional steamed and sun-dried process for alternative nine-times. The purpose of this study was to evaluate the protective effects of processed RP on D-gal-induced CD mice by behavioral observation via water maze, platform jumping, et al. and brain morphological changes.

2. Materials and Methods

2.1. Animals

Male KM mice (6-week-old) were purchased from Hunan Slake Jingda Experimental Animal Co., Ltd (Approval No. SCKX (2019-0004)). The experimental animal quality Certificate was No.43072710100478915, All animal experiments were raised at Laboratory Animal Center, College of Pharmaceutical Sciences, Southwest University, and carried out under the approval of the Ethical Committee for Animal of Southwest University and in accordance with the National Institutes of Health (NIH) (No. IACUC-20210225-01). All mice were accommodated under a 12 h/12h dark/light cycle at 22˚C ± 1˚C and 40% - 60% of relative humidity.

2.2. Animal Groups and Dosage

CD mice were randomly divided into 5 groups with 10 mice in each group after one week of adaptive feeding, including the normal control group (NC), model control group (MC), donepezil group (positive drug group, PC) (100 mg/kg), Low dosage of Rhizoma polygonati (RPL) (0.56 g/kg) and High dosage of Rhizoma polygonati (RPH) (2.25 g/kg).

2.3. Animal Model of Cognitive Dysfunction Induced with D-Galactose (D-Gal) and Behavioral Observation

CD mice in the groups of MC, PC, RPL and RPH were intraperitoneally injected D-gal (150 mg/kg) between 9: 00-11: 00 in the morning every day to induce the model of cognitive dysfunction, and the NC group was intraperitoneally injected with the same amount of normal saline. For PC, RPL and RPH groups, they were intragastrically given donepezil (100 mg/kg), RPL (0.56 g/kg) and RPH (2.25 g/kg) at 16:00-17 in the afternoon every day, respectively. Those of NC and MC groups were intragastrically given the same volume of normal saline. One week after modeling, prophylactic administration was started, and continuous administration was performed for 10 weeks. At the fourth week of modeling, the success of the D-Gal-induced cognitive dysfunction model was tested by the novel object recognition experiment in mice (according to whether the discrimination index decreased significantly). CD mice were weighed weekly and recorded. Then, the behavioral experiments for novel object recognition, Morris water maze test, jumping-platform test and histopathological examinations would be carried out.

2.4. Histopathological Examinations

For H & E staining, samples of mouse hippocampus were resected and fixed with 4% paraformaldehyde, embedded in paraffin, sectioned at 4 μm thickness, stained with hematoxylin/eosin (H & E) to observe the morphological changes. Sections were reviewed and images were captured by light microscopy (Nikon ECLIPSE Ci).

2.5. Determination of Biochemical Indexes and Inflammatory Factors in Mice

Malondialdehyde (MDA) and total antioxidant capacity (T-AOC) were measured according to the kit instructions of Beijing Solaibao Biotechnology Co., LTD. Tumor necrosis factor α (TNF-α) and mouse interleukin-6 (IL-6) were determined according to the ELISA kit instructions of Shanghai Enzyme Linked Biotechnology Co., LTD.

2.6. Statistical Analysis

The experimental data were analyzed by Microsoft Excel 2016 and GraphPad Prism 8 software. All data were presented as the mean ± standard deviation (SD)/standard error (SE). $P < 0.05$ meant significant difference, and $P < 0.01$ meant extremely significant difference.

3. Results

3.1. Effects of RP on Object Recognition Experiments in D-Gal-Induced CD Mice

The mice model of cognitive dysfunction (CD) induced by D-gal was obtained according to the methods shown in **Figure 1**.

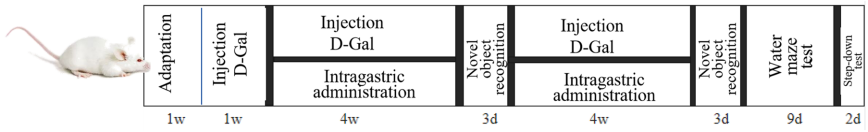


Figure 1. Experimental procedures for D-gal induced CD mice model.

As shown in **Figure 2(a)**, at week 4, compared with the NC group, CD mice in the MC group showed lower discrimination index and weaker memory. The experimental results showed that the D-gal induced cognitive dysfunction model was successful. As shown in **Figure 2(b)**, at the 10th week, compared with the MC group, after training and adaptation, the discrimination index of each drug group increased in varying degrees and memory was enhanced, the discrimination index of the RP high-dose group (RPH) increased, and the difference was significant ($P < 0.05$). The results showed that RPH can significantly improve the object recognition and memory ability of D-gal-induced CD mice.

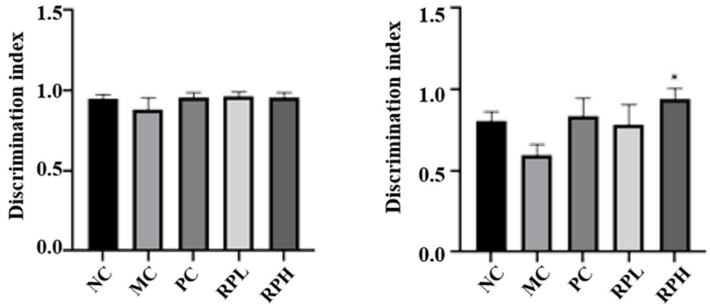
3.2. Effects of RP on Morris Water Maze Experiment in D-Gal-Induced CD Mice

As shown in **Figure 3**, compared with the NC group, the swimming distance of CD mice in the MC group was reduced and the swimming direction was unclear. After adaptation, the swimming distance was increased in the counterpoint exploration experiment, but relatively less. Compared with the NC group, the swimming distance increased in the low and high dose groups. The spatial exploration and counterpoint exploration experiments showed that the direction was clear and the number of crossing the target quadrant increased. Compared with the MC group, the swimming distance increased, and the direction was clearer in the low and high dose groups, and the platform incubation period was long, and the number of crossing the target quadrant increased significantly, with statistical significance.

As shown in **Table 1**, compared with the NC group, the number of times of crossing the target quadrant and the residence time in the target quadrant of CD mice in the MC group were significantly reduced *(*$P < 0.05$). Compared with the MC group, the times of crossing the target quadrant in the low and high dose groups were significantly increased ($P < 0.05$), and the residence time in the target quadrant was increased in different degrees. The results showed that RP could significantly improve the motor and cognitive ability of CD mice in a dose-dependent manner.

3.3. Effects of RP on Jumping Experiment in D-Gal-Induced CD Mice

As shown in **Table 2**, compared with the NC group, CD mice in the MC group spent significantly less time on the platform, and the number of errors that is the time spent on the copper grid under the platform increased significantly ($P < 0.05$). Compared with the MC group, the time spent on the platform increased



(a) (b)

Figure 2. The discrimination index of mice in different modeling periods. A. Fourth week; B. Tenth week; NC: normal control group; MC, model control group; PC: positive control group treated with donepezil; RPL: low dosage group of *Rhizoma Polygonati*; RPH: High dosage group *of Rhizoma Polygonati*. Note: compared with model group, $^{*}P < 0.05$.

Table 1. Results of water maze experiment in mice.

Groups	n	Numbers of crossing target quadrant	Time of staying on target quadrant (s)
NC	10	6.00 ± 1.33*	21.74 ± 5.10*
MC	10	2.14 ± 1.12$^{\#}$	15.30 ± 7.720$^{\#}$
PC	10	5.40 ± 1.43*	22.39 ± 10.54
RPL	10	5.63 ± 1.22*	24.11 ± 7.82
RPH	10	8.00 ± 1.94*	25.35 ± 5.88*

Note: Compared with blank group, $\#P < 0.05$; compared with model group, $^{*}P < 0.05$.

Table 2. Experimental results of step-down in mice.

Groups	n	Time on the platform (s)	Times of mistake (s)
NC	10	292.53 ± 4.80**	2.20 ± 0.45*
MC	10	273.50 ± 11.42$^{\#\#}$	2.83 ± 1.33$^{\#}$
PC	10	291.38 ± 4.05**	2.00 ± 0.71
RPL	10	285.17 ± 7.93**	2.00 ± 0.63
RPH	10	293.87 ± 4.34***	1.50 ± 0.55*

Note: Compared with blank group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. NC: normal control group; MC, model control group; PC: positive control group treated with donepezil; RPL: low dosage group of Rhizoma Polygonati; RPH: High dosage group of Rhizoma Polygonati.

and was extremely significant ($P < 0.01$), and the time spent on the copper grid under the platform decreased in the high-dose group after training and adaptation. The results showed that learning and memory ability of D-gal induced CD mice were significantly improved by RP consumption.

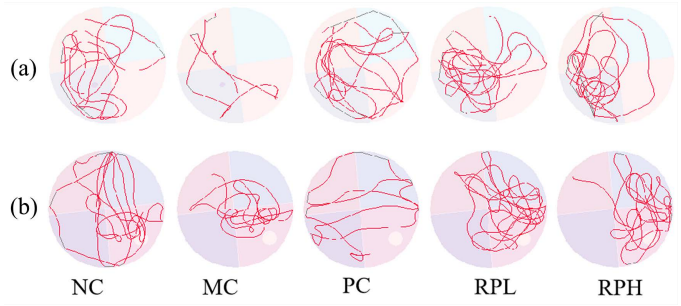


Figure 3. The effect of RP on the swimming trajectory of Cognitive dysfunction model mice. (a): Space exploration; (b): Counterpoint exploration.

3.4. Effects of RP on Pathological Lesions in Hippocampus of D-Gal-Induced CD Mice

The new object recognition experiment has verified the success of the D-gal induced cognitive dysfunction model. In order to further determine whether there is pathological damage in the brain tissue of CD mice, HE staining was performed on the brain hippocampus tissue of CD mice, as shown in **Figure 4**. Compared with the NC group, cells in the MC group were disordered, with serious cavitation, obvious damage and abnormal morphology. Compared with the MC group, the number of CD mice neurons increased significantly in the low-dose and high-dose groups, with compact arrangement, complete cell structure, and no obvious morphological abnormalities, indicating that RP high-dose groups have protective effects on the hippocampus neuron cells of CD mice.

3.5. Effects of RP on Biochemical Indexes and Inflammatory Factors in D-Gal-Induced CD Mice

As shown in **Figure 5**, compared with the NC group, total antioxidant capacity (T-AOC) was significantly decreased while malondialdehyde (MDA), TNF-a and IL-6 were significantly increased in the hippocampus of MC group. Compared with the MC group, RP significantly improved T-AOC, extremely significantly inhibited MDA activity, and significantly reduced the contents of TNF-a, IL-6 and other inflammatory factors in D-gal-induced CD mice.

4. Discussion

Cognitive impairment as an early symptom of AD is often seen to be a warning that AD is developing. Rhizoma Polygonati, a traditional Chinese medicine tonic, showed excellent abilities of renewing brain neuron cells, improving learning and memory, and enhancing cognitive function under modern technique. In our study, CD mice were induced by intraperitoneal injection of D-gal every day for 10 weeks. From the 10th week, through behavioral experiments including new object recognition, Morris water maze (space exploration and counterpoint exploration), and step-down test, the discrimination index and the swimming distance of mice fed high-dose Rhizoma Polygonati were significantly increased

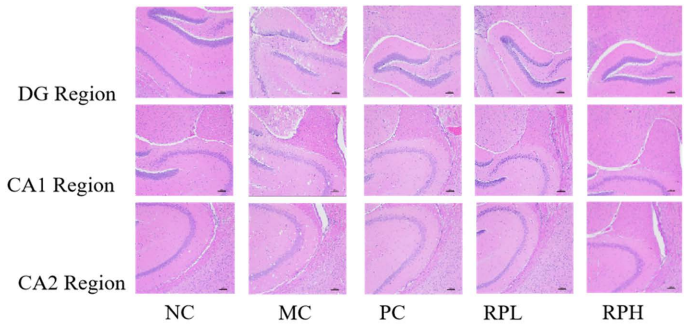


Figure 4. HE staining results of mice hippocampus. (HE staining, ×100). NC: normal control group; MC, model control group; PC: positive control group treated with donepezil; RPL: low dosage group of Rhizoma Polygonati; RPH: high dosage group of Rhizoma Polygonati.

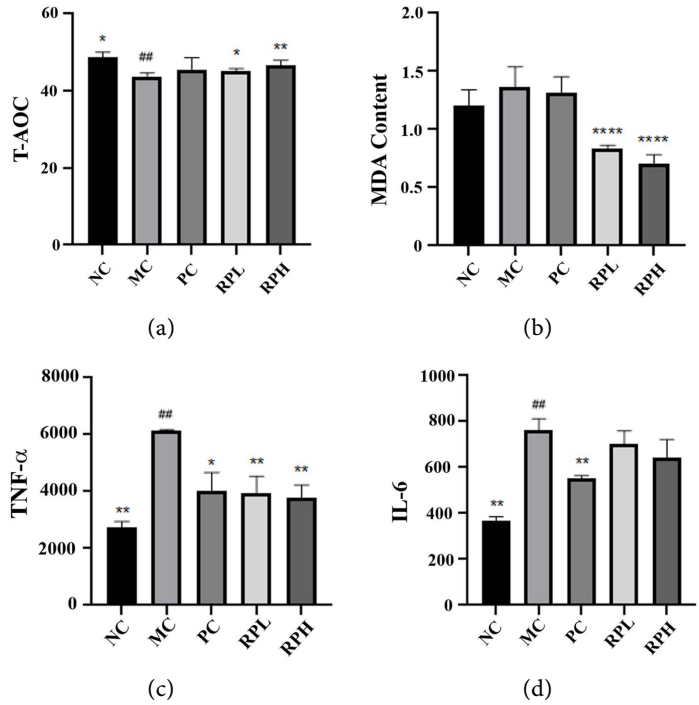


Figure 5. Biochemical indicators and inflammatory factors. (a): T-AOC; (b): MDA; (c): TNF-α; (d): IL-6; NC: normal control group; MC, model control group; PC: positive control group treated with donepezil; RPL: low dosage group of Rhizoma Polygonati; RPH: high dosage group of Rhizoma Polygonati. Note: Compared with blank group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

($P < 0.05$). And mice showed clearer the direction recognition and longer retention time on the platform. Besides, mice in this group entered the water maze indicated quadrant more frequently along with low error rate. These behavioral results demonstrate that Rhizoma Polygonati could significantly improve the learning and memory ability of CD mice with cognitive dysfunction.

HE staining of brain hippocampus showed that, compared with the control group, the model group had obvious abnormality in neuron morphology mani-

fested as disordered cell arrangement and serious vacuolation. Rhizoma Polygonati decreased the number of damaged neuron cells and maintained a compact neuron arrangement. According to Nysch staining, the neuron of the model group was in an abnormal morphology, and the cell membrane was crumpling and breaking. In contrast, Rhizoma Polygonati empowered a normal neuron structure to CD mice and held intact cell morphology and clear outline. Data from these cell staining shows that Rhizoma Polygonati has an encouraging protective effect on hippocampal neurons of CD mice with D-gal induced cognitive dysfunction.

At the molecular level, D-gal significantly decreased T-AOC ($P < 0.05$) and increased MDA. Rhizoma Polygonati revised these adverse changes to physiological levels and even lower, suggesting that Rhizoma Polygonati could improve the antioxidant capacity of cells, and achieve the effect of improving cognitive dysfunction and inhibiting oxidative damage. Studies have shown that inflammatory cytokines damage the cognitive ability of mice. In this study, the levels of TNF-α and IL-6 in the hippocampus of CD mice increased significantly after D-gal treatment ($P < 0.01$). After the intervention of Rhizoma Polygonati, the levels of TNF-α and IL-6 were dramatically reduced, which proposes the inflammation-improving effect of Rhizoma Polygonati in mouse nervous system.

Accordingly, our study shows that Rhizoma Polygonati protects neurons from injure, alleviates inflammation of nervous system, improves cognitive dysfunction in mice, and thus is a natural resource for both drug and food use with potential to treat Alzheimer's disease.

5. Conclusion

The results from the behavioral experiments of novel object recognition, Morris water maze test, jumping-platform test and histopathological examinations showed that RP had obvious protective effect on D-gal induced cognitive dysfunction mice.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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