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Research report

# Salidroside ameliorates cognitive impairment in a D-galactose-induced rat model of Alzheimer's disease

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## HIGHLIGHTS

- Up-regulation of Txnip and down-regulation of Trx were first detected in the D-gal-induced sub-acute aging model.
- Sal improved cognitive capacity of AD rats.
- Sal significantly inhibited inflammatory actions and depressed apoptosis-related proteins of AD rats.

## ARTICLE INFO

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## ABSTRACT

The purpose of the present study was to investigate possible preventive effects of salidroside (sal) on a rat model of Alzheimer's disease and to explore its possible mechanism. Sub-acute aging was induced in male SD rats by subcutaneous injection of D-gal (120 mg/kg) for 42 days, and the rats were treated with sal (20, 40 mg/kg) or normal saline for 28 days after 14 days of D-gal injection. Morris water maze (MWM) test and step-down passive avoidance test were conducted to evaluate the cognitive function of the rats. The levels of inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) in hippocampus were assayed by enzyme-linked immunosorbent assay (ELISA) to assess the anti-inflammatory effect of sal. Further, we estimated the expression levels of thioredoxin (Trx), thioredoxin interacting protein (Txnip/vitamin D3 up-regulated protein/thioredoxin binding protein-2), Bax, Bcl-2, caspase-9 and related-proteins of nuclear factor kappa B (NF-κB) signaling pathway by western blot assay. It showed that administration of sal significantly attenuated all the D-gal-induced changes in the hippocampus, including cognitive impairment and neuroinflammation. These analytical results provides evidence that sal can improve cognitive capacity by inhibiting neuroinflammation and affecting apoptosis-related proteins in hippocampus.



## 1. Introduction

Alzheimer's disease (AD), the most common form of dementia, clinically characterized by progressive memory dysfunction, diminished cognitive function and behavioral disorder with advancing age. Neurofibrillary tangles (NFTs) which primarily consist of phosphorylated microtubule-associated protein tau and abnormal deposits of amyloid-β (Aβ) are pathological hallmarks of AD [1]. Recently, mounting evidence has indicated that inflammatory cells like astrocytes and microglia are activated by the NFTs and Aβ plaques, then release pro-inflammatory mediators including various cytokines and chemokines which may result in nonspecific inflammatory cell infiltration [2,3]. In addition, it is widely accepted that pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF-α) may lead to neuronal damage and the formation of neuritic plaques in AD [4–6]. Thus, increasing literatures have focused on the roles of anti-inflammatory and immunomodulatory agents in AD.

The thioredoxin (Trx) system, which contains NADPH, thioredoxin reductase (TrxR) and Trx, is a major antioxidant system

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that protects cells from oxidative stress [7]. Many studies have suggested that Trx is an essential anti-inflammatory molecule by modulating both intra- and extra-cellular signaling pathways [8]. Thioredoxin interacting protein (Txnip), also known as vitamin D3-upregulated protein-1 or TRX-binding protein-2, is an endogenous inhibitor of the Trx [7]. Decades of researches have indicated that Trx/Txnip signaling complex plays a partial role in the pathogenesis of various diseases including diabetes, autoimmune disease and cancer [9,10]. Chen et al. demonstrated that up-regulation of Txnip expression can augment the inflammatory actions and apoptosis characterized by activations of TNF-α and ASK1-p38MAPK/JNK pathway in vascular endothelial cells [11]. Furthermore, it was investigated that over-expression of Txnip contributed to the up-regulation of NF-κB in retinal endothelial cells [12]. Based on these findings, we assumed that Trx and Txnip emerged as important indicators in the sub-acute aging model of rats.

Salidroside (Sal), a phenylpropanoid glycoside isolated from a popular traditional Chinese medicinal plant *Rhodiola rosea* L., possesses multiple pharmacological properties including anti-aging, anti-fatigue, anti-oxidative, anti-cancer and anti-inflammatory effects [13–17]. In view of the multifaceted activities of sal, we hypothesized that it held promise as a therapeutic candidate for cognitive deficits associated with AD. Therefore, in this study, we investigated anti-inflammatory and anti-apoptotic effects of sal on Alzheimer's disease and its possible underlying mechanisms.

## 2. Materials and methods

### 2.1. Reagents

Sal (purity 99%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). IL-6, IL-1β and TNF-α kits were produced by Nanjing KeyGEN Biotech. Co. Ltd. (Nanjing, China). All antibodies were purchased from Cell Signaling Technology (Danvers, USA).

All other chemicals and reagents used for study were of analytical grade and were purchased from approved organizations.

### 2.2. Animals

Male Sprague-Dawley rats (approximately 3 months old, weighing 280–300 g) were purchased from Comparative Medical Center of Yangzhou University (Yangzhou, China) and were housed a week to adapt to the environment. They were maintained in a temperature controlled room (25 ± 2 °C) and kept on a 12 h light/12 h dark cycle with food and water available ad libitum. All the experiments and animal care were performed strictly according to the provision and general recommendation of Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Jiangsu Province.

### 2.3. Ethics statement

All experiments were performed in accordance with institutional and national guidelines and regulations and were approved by the China Pharmaceutical University.

### 2.4. Animal treatment

Rats were randomly assigned to 4 groups: (1) control, (2) D-gal-administration (120 mg/kg), (3) D-gal-administration plus sal treatment (20 mg/kg), and (4) D-gal-administration plus sal treatment (40 mg/kg). In the D-gal-administration group and D-gal-administration plus sal treatment group, D-gal (120 mg/kg) was injected subcutaneously daily into rats for 42 days. In the D-gal-administration plus sal treatment group, sal (20, 40 mg/kg) was administrated orally daily for 28 days from day 15th of D-gal injection. All the control animals were given normal saline in the same volume subcutaneously and orally, respectively. After 4 weeks of drug treatment, one part of the rats were submitted to behavioral tests, while the rests were sacrificed by cervical dislocation, and the brain tissues were taken out for biochemical assays. All surgeries were performed under sodium pentobarbital anesthesia, and all the efforts were made to minimize suffering.

### 2.5. Morris water maze test

Spatial memory was assessed by the MWM test, which consisted of 5-day place navigation training and a probe test on day 6. This was carried out as described previously [18]. The maze was a tank (80 cm in radius and 45 cm high) filled with water at approximately 25 °C. The tank was divided into 4 quadrants, one of which contained a circular escape platform (12 cm diameter) placed at a fixed position, 2.5 cm below the surface of the water. There were visual cues around the water maze. Oriented navigation trials were performed 4 times per day, for 5 consecutive days with a constant interval of 1 h. In each trial, the animals were gently placed in water in one of the four quadrants, and the starting quadrant was varied randomly over the trials. Rats were allowed a maximum of 90 s to find the escape platform, where it remained for 30 s. For all training trials, the time that it took the rat to reach the submerged platform (escape latency) was recorded to assess spatial learning ability. On the sixth day, another set of tests consisting of a 120 s trial with the platform removed was conducted. Besides escape latency before reaching the platform, time spent in the target quadrant and the numbers of target crossings over the previous location of the target platform were recorded. The target quadrant was defined as the quadrant that previously contained the platform, the radius of which was limited to 70 cm in this assessment. Data of the escape latency, the percentage of time spent in the target quadrant and the number of platform location crossings were collected by the video tracking equipment and processed by a computer equipped with an analysis-management system (Viewer 2 Tracking Software, Ji Liang Instruments, China).

### 2.6. Step-down type passive avoidance test

One day after MWM test, the step-down type passive avoidance test was performed according to the previously described method [19]. At the beginning of training, rats were placed in the wooden platform to adapt for 2 min. When the rats stepped down from the platform and placed all paws onto the grid floor, electric currents were delivered for 15 s. Then the rats would jump onto the platform to avoid the electric stroke, and the electric currents were maintained for 5 min. After a 24 h interval, the rat were again placed on the platform, and the latency to step down on the grid for the first time and the number of errors subjected to shocks within 3 min were measured as learning performances.

### 2.7. Detection of pro-inflammatory cytokines in the hippocampus by ELISA

Hippocampuses were collected and lysed in ice bath for 30 min. The supernatant was collected after centrifugation (12,000 rpm, 4 °C, 30 min). The levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in the hippocampus in each group were measured by ELISA kit following the manufacturer's instructions.

### 2.8. Western blot analysis

Hippocampuses in each group were collected immediately after the therapy. Total protein was extracted, and the concentrations