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Salidroside prevents diabetes-induced cognitive impairment via regulating the Rho pathway

XIUZHEN HAO[1*], JIE YUAN[2*] and HUIXIAO DONG[1]

[1]Department of Neurosurgery, Jining No. 1 People's Hospital, Jining, Shandong 272000;
[2]Institute of Mental Health, North China University of Science and Technology, Tangshan, Hebei 063009, P.R. China

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Abstract. In previous years, it has been found that Rhodiola has a wide range of pharmacological effects in diseases of the cardiovascular system, as it can remove superoxide anions and hydroxyl radicals in chemical reactions. Behavioral assessment was used to measure cognitive impairment. Inflammation, oxidative stress and caspase-3 activity were measured using commercial kits. Western blot analysis was used to measure Rho/Rho-associated kinase (ROCK)/sirtuin 1 (SIRT1)/nuclear factor (NF)-κB protein expression. The objective of the present study was to investigate the protective effect of salidroside on diabetes and diabetes-induced cognitive impairment. The results of the study demonstrated that salidroside prevented cognitive impairment, decreased serum blood glucose levels and increased body weight, reduced fasting blood glucose levels and blood lipid levels, and inhibited oxidative stress, inflammation and nerve cell apoptosis in the diabetic rat model. Salidroside suppressed ROCK/ SIRT1 NF-κB pathway and protein expression in the diabetic rats. These data showed that salidroside prevented diabetes-induced cognitive impairment by regulating the Rho/ROCK/SIRT1/NF-κB pathway.

Introduction

Diabetes and its complications have become health problems of public concern, as the number of patients with diabetes has increased more than expected in the world, with the developments in society and economy in recent years (1). According to the statistics reported by the International Diabetes Union, the number of patients with diabetes reached 370,000,000 in 2011, 80% of whom were in developing countries and diabetic patients aged >20 years old accounting for 9.7% of cases in China, which is the country with the highest number of diabetic patients (2,3).

Diabetes is caused by disorder of the metabolism of sugar, fat and protein, and causes injury to multiple body systems, tissues and organs, resulting in complications that involve the eye, kidney, brain and other organ diseases, among which neurological complication is the most common, including diabetic neuropathy, ischemic cerebrovascular disease and atherosclerosis (4). At present, there have been several reports on diabetes-induced peripheral neuropathy, also known as diabetes-induced cognitive impairment or diabetic encephalopathy, within and outside China (5).

Diabetes-induced cognitive impairment is a diabetes-induced complication, characterized by cognitive disorders, and accompanied by structural and pathophysiological changes in the brain (6). This has been referred to as diabetic encephalopathy and diabetes-associated cognitive decline, but no agreement has been reached (7). Its pathogenesis and pathophysiological changes remain to be fully elucidated, and it has been investigated from the perspective of glucose toxicity, membrane signal disorder, homeostasis imbalance, inflammation, oxidative stress injury and vascular diseases (7,8).

Rho-associated kinase (ROCK) is a Rho downstream target molecule, which has been investigated thoroughly (9). The Rho/ROCK signaling pathway induces cytoskeletal reconstruction, cell migration and the formation of stress fibers, which are associated with endothelial permeability, tissue contraction and growth, in addition to other physiological functions involved in the incidence of diabetic nephropathy, eye diseases, cancer, heart disease, nerve injury diseases, hypertension, radiation damage and leukemia; therefore, it has attracted increasing attention as a target drug (10).

As an original signal activating factor, nuclear factor (NF)-κB can lead to cell dysfunction and activate other pathways (11). Under high glucose and oxidative stress, the expression of NF-κB increases and alters the expression of nitric oxide synthase, matrix metalloproteinase-9 and tumor necrosis factor (TNF)-α, which leads to a series of pathological changes and to the apoptosis of hippocampal neurons (12).

As a perennial herb or subshrub, *Rhodiola* belongs to the *Rhodiola L.* genus with creeping rhizomes (13). There are ~90 types of *Rhodiola* in the world, distributed in East Asia, Central Asia, Siberia and North America (13). There are

Correspondence to: Mr. Huixiao Dong, Department of Neurosurgery, Jining No. 1 People's Hospital, 6 Jiankang Road, Jining, Shandong 272000, P.R. China
E-mail: dovlbm13964@126.com

[*]Contributed equally

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~73 species, two subspecies and seven varieties of *Rhodiola* in China, accounting for >80% of all *Rhodiola* in the world, mainly distributed in north and northeastern China, in Gansu province, Xinjiang province, Sichuan province, Tibet province and Guizhou province (14). As one of the rare medicinal herbs, the whole plant of *Rhodiola* can be used as a medicine with notable antihypoxic, cold-relieving, antifatigue and antiviral effects, in addition to delaying aging and preventing geriatric disease (15). Salidroside (Fig. 1), the main active ingredient of *Rhodiola*, can scavenge oxygen free radicals and protect cell membranes, and inhibit the hypoxia-induced expression of proto oncogenes, being widely used in the treatment of altitude sickness, anti-aging, as a nutrition supplement and in clinic (14). Therefore, the objective of the present study was to investigate the protective effect of salidroside on diabetes, and on diabetes-induced cognitive impairment, and examine changes in the sirtuin 1 (SIRT1)/NF-κB pathway and Rho/ROCK pathway.

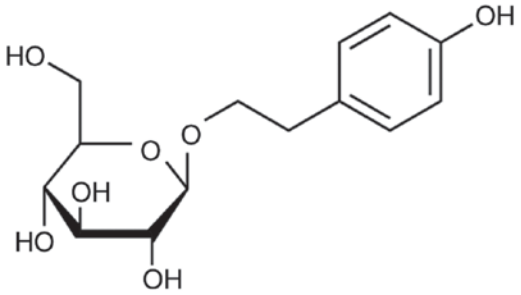

Figure 1. Constitutional formula of salidroside.

Materials and methods

Animals and induction of diabetes. Male Sprague-Dawley rats (200-230 g, 8-10 weeks) were purchased from the Animal Testing Center of Shandong University and housed in cages (22-24˚C and 55-60% humidity), maintained on a 12-12 h light-dark cycle, and allowed free access to food and water. All rats were randomly assigned into three groups: Control, diabetes (DM) model group, salidroside treatment group. Streptozotocin (60 mg/kg; Sigma; EMD Millipore, Billerica, MA, USA) was injected intraperitoneally to induce diabetes in the DM model group. In the salidroside treatment group, the DM rats were gavaged with 200 mg/kg/day of salidroside (Sigma; EMD Millipore) for 4 weeks. Fasting blood glucose (FBG) was measured using a glucose oxidase peroxidase diagnostic enzyme kit at 72 h post-streptozotocin injection, and an FBG level >200 mg/dl was considered to be diabetic.

Behavioral assessment. The rats were placed in a circular water tank (150 cm in diameter and 50 cm high, 25-26˚C) containing a platform (17 cm in diameter and 31 cm high), and the path of an animal swimming in a large pool of water was analyzed. The tank was maintained at 32x10 cm and the platform was placed inside, in the middle of a certain quadrant at a distance of ~22 cm. A test was performed to enable the rat to get used to the pool, and to assess swimming ability and vision on day 0 of visible platform training. The subsequent tasks were performed to evaluate the extent of learning on days 1-4 of the hidden platform training. The platform with no flag remained in the same location but was submerged at 1 cm, and the rat trials were performed (four trials per day with a 1-h interval between trials). The rats swum until they climbed onto the platform for 60 sec. The rat was allowed to remain for 15 sec on reaching the platform. If the rat failed to locate the platform within 60 sec, the test was ended. On day 5, the platform was removed, and a probe trial was conducted to assess memory.

Measurement of inflammation, oxidative stress and caspase-3 activity. Follow treatment with salidroside, mice were sacrificed via decapitation under 35 mg/kg pentobarbital sodium, and hippocampal tissue samples were collected and homogenized using RIPA assay for 30 min for 4˚C. The extracted protein was used to analyze the levels of TNF-α, interleukin (IL)-6, glutathione peroxidase (GSH-PX), glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Caspase-3 activity was measured using an ELISA kit (Beyotime Institute of Biotechnology, Haimen, China).

Western blot analysis. The tissue samples were collected and homogenized using RIPA assay for 30 min for 4˚C. The protein concentrations were determined using a BCA protein assay kit. The protein was loaded (50 μg per well) and separated on a 8-12% sodium dodecyl sulfate polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% skim milk powder in Tris-buffered saline containing 0.05% Tween-20 (TBST) at room temperature for 1 h, and then incubated with the following primary antibodies overnight: B-cell lymphoma 2-associated X protein (Bax; 1:4,000, ab32503, Abcam, Cambridge, UK), Rho (1:2,000, ab40673, Abcam), ROCK (1:2,000, ab45171, Abcam), SIRT1 (1:2,000, ab110304, Abcam,), NF-kB (1:2,000, ab16502, Abcam) and GAPDH (1:5,000, ab8245, Abcam). The membrane was washed with TBST followed by incubation with the peroxidase-conjugated secondary antibodies (1:2,000, sc-2004 or sc-2005, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at room temperature for 1 h and detected using ECL detection reagents. The protein levels were analyzed using an analysis system (ImageJ 1.37 software, NIH, Bethesda, MD, USA).

Statistical analysis. All results are expressed as the mean ± standard deviation. Repeated measures and one-way analysis of variance, followed by Tukey's test were used to assess treatment. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Salidroside prevents changes in body weight and serum glucose in diabetic rats. The present study examined whether salidroside had effects on the body weight and serum glucose levels of diabetic rats. As shown in Fig. 2A, the body weight of the DM model group was lower, compared with that of the control group. The serum blood glucose level of the DM model group was higher, compared with that of the control group

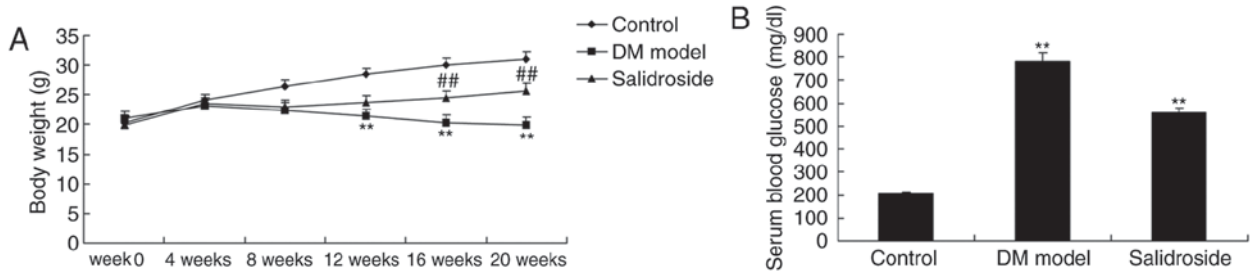

Figure 2. Salidroside prevents body weight and serum glucose changes in diabetic rats. Salidroside prevented (A) body weight and (B) serum glucose in the rat model of diabetes. **P<0.01, compared with the control group; ##P<0.01 compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group.

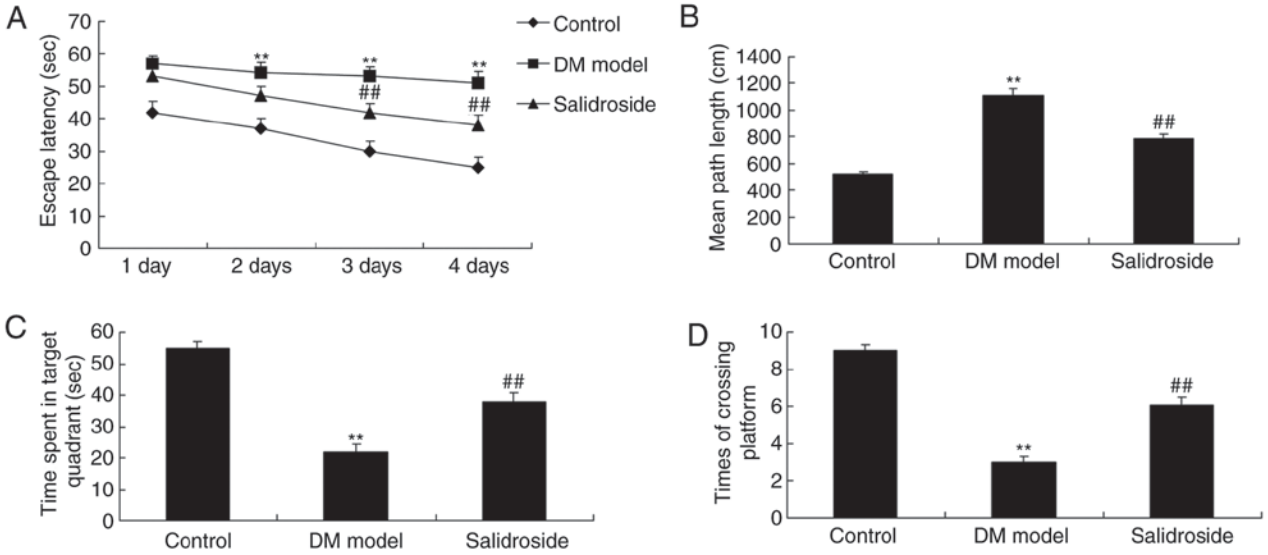

Figure 3. Salidroside prevents cognitive impairment in diabetic rats. (A) Escape latency; (B) mean path length; (C) time spent in target quadrant; (D) times crossing platform. **P<0.01, compared with the control group; ##P<0.01 compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group.

(Fig. 2B). Therefore, treatment with salidroside increased body weight and inhibited serum blood glucose in the DM rats, compared with the rats in the DM model group (Fig. 2).

Salidroside prevents cognitive impairment in diabetic rats. The duration of escape latency and the mean path length in the DM model group were higher, compared with those in the control group (Fig. 3A and B). However, the time of spent in the target quadrant and times crossing the platform in the DM model group were lower, compared with those in the control group (Fig. 3C and D). Treatment of the rats with salidroside significantly prevented cognitive impairment, inhibited the duration of escape latency and mean path length, and increased the time of spent in the target quadrant and crossing the platform in the DM rats, compared with the DM model (Fig. 3A-D).

Salidroside prevents inflammation in diabetic rats. To visualize the effects of salidroside on inflammation in the diabetic rat, the levels of TNF-α and IL-6 were measured using ELISA kits. The results, as shown in Fig. 4A and B, revealed significant increases in the levels of TNF-α and IL-6 in the DM model group, compared with those in the control group. Treatment with salidroside significantly inhibited the levels of TNF-α and IL-6 in the DM rats, compared with those in rats of the DM model group (Fig. 4A and B).

Salidroside prevents oxidative stress in diabetic rats. The levels of GSH-PX, GSH and SOD in the DM group were lower, compared with those in the control group (Fig. 5A-C). The level of MDA was also higher, compared with that in the control group (Fig. 5D). Salidroside significantly prevented oxidative stress, increased the levels of GSH-PX, GSH and SOD, and reduced the level of MDA in the DM rat, compared with the DM model group (Fig. 5A-D).

Salidroside prevents nerve cell apoptosis in diabetic rats. The results of the present study also showed that the activity of caspase-3 and protein expression of Bax were significantly induced in the DM model group, compared with those in the control group (Fig. 6A-C). Treatment with salidroside significantly reduced the activity of caspase-3 and protein expression of Bax in the DM rat, compared with the DM model group (Fig. 6A-C).

Salidroside suppresses the Rho/ROCK pathway in diabetic rats. To investigate the mechanism underlying the effect of

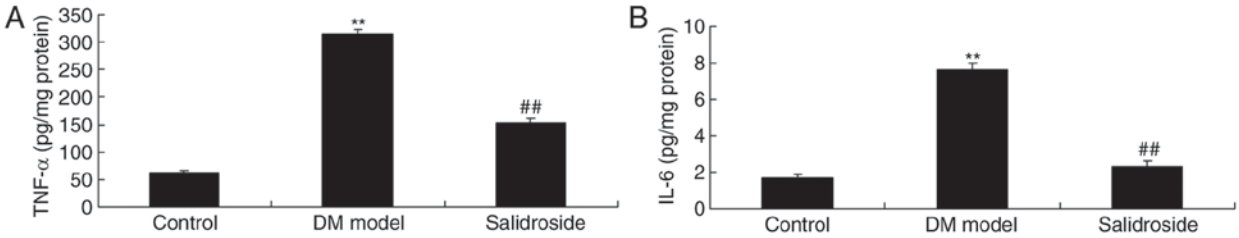

Figure 4. Salidroside prevents inflammation in diabetic rats. Levels of (A) TNF-α and (B) IL-6 are shown. **P<0.01, compared with the control group; ##P<0.01, compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

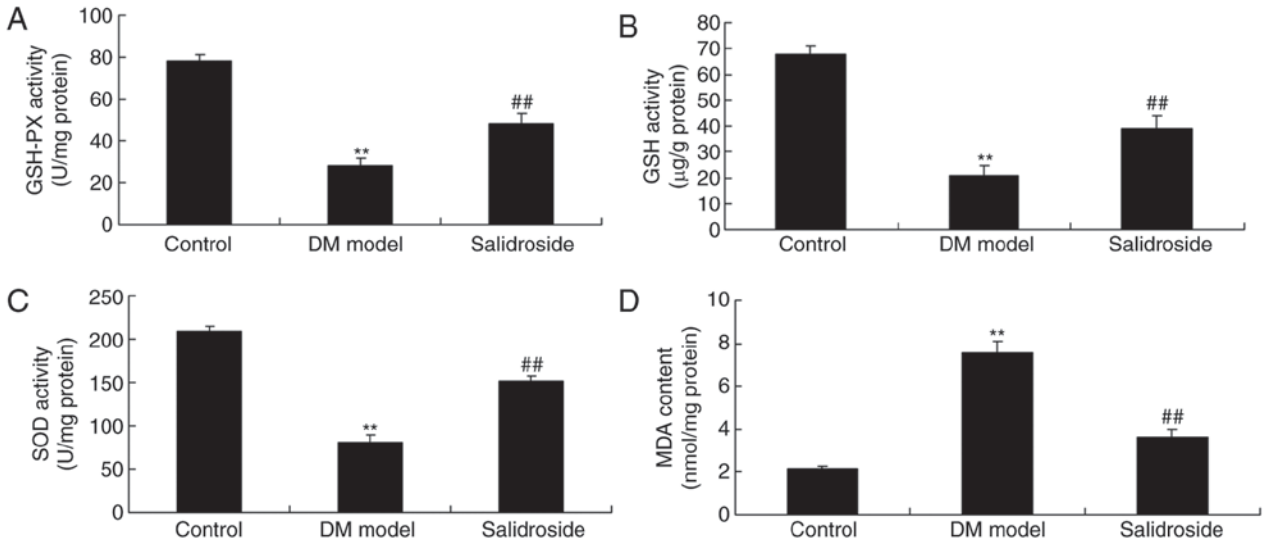

Figure 5. Salidroside prevents oxidative stress in diabetic rats. Levels of (A) GSH-PX, (B) GSH, (C) SOD and (D) MDA are shown. **P<0.01, compared with the control group; ##P<0.01, compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group; GSH-PX, glutathione peroxidase; GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde.

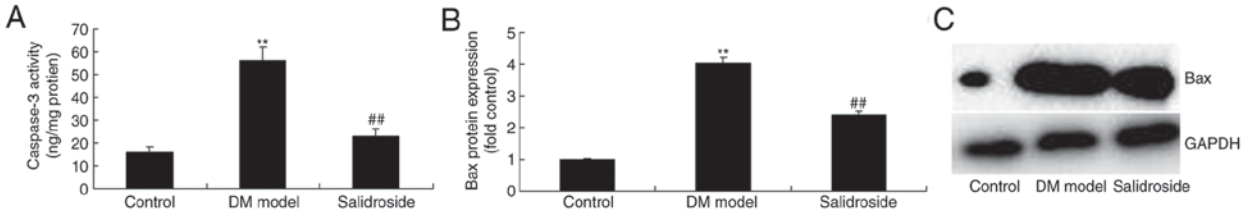

Figure 6. Salidroside prevents nerve cell apoptosis in diabetic rats. (A) Caspase-3 activity. (B) Protein expression of Bax determined by statistics analysis of (C) western blot analysis. **P<0.01, compared with the control group; ##P<0.01, compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group; Bax, B-cell lymphoma 2-associated X protein.

salidroside on cognitive impairment in the diabetic rat, the protein expression levels of Rho and ROCK were measured. As shown in Fig. 7A-C, the protein expression levels of Rho and ROCK in the DM model were higher, compared with those in the control group. Salidroside significantly suppressed the protein expression of Rho and ROCK in the DM rat, compared with the DM model group (Fig. 7A-C).

Salidroside suppresses the SIRT1/NF-κB pathway in diabetic rats. Finally, the present study examined the protein expression levels of SIRT1 and NF-κB using western blot analysis. There were significant increases in the protein expression levels of SIRT1 and NF-κB in the DM model, compared with those in the control group (Fig. 8A-C). Salidroside significantly suppressed the protein expression of SIRT1 and NF-κB in the DM rat, compared with the DM model (Fig. 8A-C).

Discussion

As a systemic disease, diabetes leads to the change in the structure and function of a variety of tissues and organs, and its influence on central nervous system has attracted increasing attention (16). The impact on cognitive function by diabetes is an important and unresolved long-term problem. Cognitive disorder, also known as cognitive impairment and cognitive function decline, refers to cognitive impairment of different degrees from mild cognitive

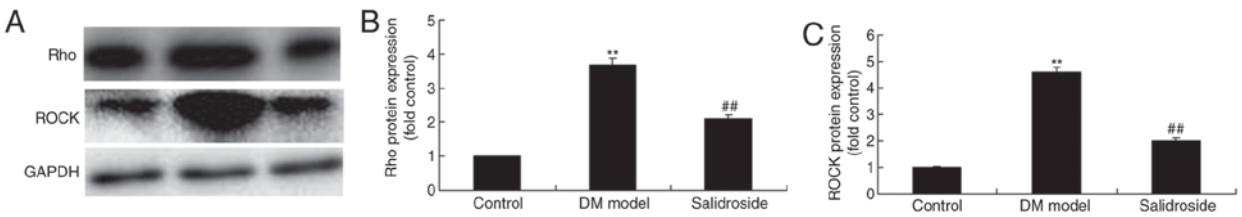

Figure 7. Salidroside suppresses the Rho/ROCK pathway in diabetic rats. (A) Protein expression of Rho and ROCK, detected by western blot analysis. Statistical analysis of protein levels of (B) Rho and (C) ROCK. **P<0.01, compared with the control group; ##P<0.01, compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group; ROCK, Rho-associated kinase.

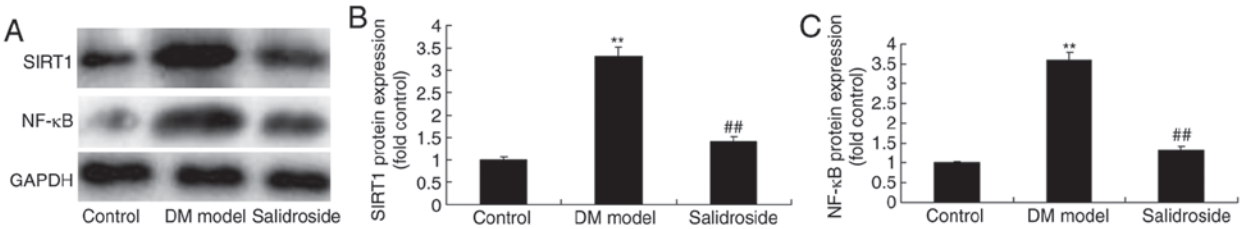

Figure 8. Salidroside suppresses SIRT1/NF-κB pathway in diabetic rats. (A) Protein expression of SIRT1 and NF-κB detected by western blot analysis. (B) Statistical analysis of protein levels of (B) SIRT1 and (C) NF-κB. **P<0.01, compared with the control group; ##P<0.01, compared with the DM model group. Control, control group; DM model, diabetes rat model group; Salidroside, 200 mg/kg salidroside-treated group; SIRT1, sirtuin 1; NF-κB, nuclear factor-κB.

disorder to dementia (17). Therefore, cognitive dysfunction in patients with diabetes is common, and diabetes can induce dementia in the most severe cases. However, due to the population aging and social support, and the lack of primary health care systems, dementia has not received sufficient attention (18). In the present study, it was found that salidroside increased body weight, inhibited serum blood glucose, and prevented cognitive impairment in rats with diabetes.

It has been found that the Rho/ROCK signaling pathway induces cytoskeletal reconstruction, cell migration and the formation of stress fibers, associated with endothelial permeability, tissue contraction and growth, and other physiological functions, being involved in the incidence of diabetic nephropathy, eye diseases, cancer, heart disease, nerve injury diseases, hypertension, radiation damage and leukemia (9). High glucose levels activate the Rho/ROCK signal pathway in mesangial cells, activate transcription factor activator protein-1 and upregulate fibronectin, leading to the accumulation of glomerular matrix proteins (19). The activation of the Rho/ROCK signaling pathway regulates the NF-κB signaling pathway, upregulates inflammatory genes and results in diabetes-induced cognitive impairment (20). ROCK inhibitors can reduce sclerotic cytokines and extracellular matrix, preventing against oxidation and protecting mitochondria, thereby slowing diabetes-induced cognitive impairment (21,22). The present study found that salidroside significantly suppressed the protein expression of Rho and ROCK in DM rats. Zhu *et al* (20) also ascertained that salidroside ameliorates arthritis-induced brain cognition deficits through suppression of the Rho/ROCK/NF-κB pathway.

SIRT1-dependent protein deacetylase can act on histones and a variety of non-histone proteins, to induce anti-inflammatory and anti-apoptotic effects, and inhibit oxidative stress damage, cell aging and other physiological and pathological processes (23,24). Diabetes is a chronic disorder of glucose and lipid metabolism, and its complications can affect all organs of the body; when this complication occurs in the brain, it can cause cognitive disorder, mainly characterized by the decline of learning and memory function (22).

High glucose also induces mitochondrial dysfunction and the mitochondrial release of cytochrome *c* into the cytoplasm, to activate apoptosis-related proteins and thereby induce the apoptosis of nerve cells. Advanced glycation end products accumulate and bind to advanced glycation end products in the cell membrane receptor to stimulate oxidative stress, and then induce the inflammatory response through upregulation of NF-κB transcription factor and target genes, leading to nerve cell injury (25). The apoptotic cells in the cortex and hippocampus of rats with diabetes exceed those in normal rats, indicating that diabetes can induce the apoptosis of nervous cells and lead to cognitive dysfunction (26). Diabetes can also lead to major depression (26). SIRT1 can act on NF-κB and regulate cell apoptosis (26). The results of the present study suggested that salidroside significantly suppressed the protein expression of SIRT1 and NF-κB in the DM rats.

Oxidative stress is mainly caused by the excessive generation of oxygen free radicals, and results in the excessive accumulation of oxygen free radicals and associated metabolites, thereby leading to a variety of toxic effects on cells (27). Oxidative stress can also induce cell senescence. SIRT1 has been confirmed to prevent oxidation by regulating the transcriptional activity of certain key enzymes (28). The present study showed that salidroside significantly prevented oxidative stress, increased the levels of GSH-PX, GSH and SOD, and reduced the level of MDA in the DM rats. Wang *et al* (27) also showed that salidroside inhibited cell proliferation through the inhibition of oxidative stress and expression of phosphorylated p38 in A549 lung cancer cells.

The inflammation described in reports refers to a chronic, sustained, non-specific and low-grade inflammatory response,

with certain differences to autoimmune inflammation and infective inflammation, being free of swelling, fever, redness and pain in certain regions of, or the whole body, mainly characterized by an increase of non-specific inflammatory markers, including IL-β and c-reactive protein (29,30). Inflammation can cause the apoptosis of pancreatic β cells and insulin resistance, and trigger diabetes-induced cognitive impairment (30). The results of the present study showed that salidroside significantly suppressed the protein expression of SIRT1 and NF-κB in the DM rats. Gao *et al* (31) also reported that salidroside suppressed inflammation via the SIRT1/NF-κB pathway in a D-galactose-induced rat model of Alzheimer's disease.

In conclusion, salidroside prevented diabetes-induced cognitive impairment, inflammation and oxidative stress, which likely occurred via suppression of the Rho/ROCK/SIRT1/NF-κB pathway. The present study provides evidence for a promising novel therapeutic strategy or possible drug for diabetes-induced cognitive impairment in clinical applications.

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Availability of data and materials

Not applicable.

Authors' contributions

HD made substantial contributions to the design of the present study; XH and JY performed the experiments; HD and XH analyzed the data; HD wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of North China University of Science and Technology (Tangshan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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