Int J Clin Exp Pathol 2024;17(12):469-476
www.ijcep.com /ISSN:1936-2625/IJCEP0157693

Original Article

Neuroprotective effect of sulforaphane on hyperglycemia-induced cognitive dysfunction through the Nrf2/HO-1 pathway

Gengyin Wang[1], Liping Wang[1], Xiaohan Zhang[1], Zifeng Wei[1], Kunpeng Wang[3], Jinhua Wang[2]

[1]*School of Basic Medicine, North China University of Science and Technology, Tangshan 063210, Hebei, China;* [2]*Department of Neurology, Huanggang Central Hospital of Yangtze University, Huanggang 438000, Hubei, China;* [3]*Department of Prevention and Treatment of Infectious Diseases, Fengnan District Center for Disease Control and Prevention, Tangshan 063300, Hebei, China*

Received May 11, 2024; Accepted December 3, 2024; Epub December 15, 2024; Published December 30, 2024

Abstract: Objectives: Sulforaphane (SFN), an isothiocyanate in cruciferous plants, has been reported to be effective in treating central nervous system diseases. However, how SFN protects the central nervous system needs further study. The aim of this study was to investigate the neuroprotective effect of SFN and its possible mechanism of action. Methods: Sprague-Dawley rats were used to develop a cognitive impairment model. The Morris water maze (MWM) was used to evaluate the effect of SFN on learning and memory, and haematoxylin-eosin (H&E) staining and terminal transferase deoxyuridine nick-end labelling (TUNEL) were used to observe morphologic changes in neurons and neuronal apoptosis in the hippocampus and cortex. An oxidative stress marker kit was used to detect the content and activity of SFN, and the expressions of nuclear factor drythroid-2 related Factor 2 (Nrf2), heme oxygenase 1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO-1) were measured by RT-PCR. Results: SFN treatment significantly improved cognition, increased the number of neurons, and suppressed neuronal apoptosis. In addition, SFN significantly decreased the content of malondialdehyde (MDA) and enhanced the antioxidant activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the hippocampus and cortex. Furthermore, SFN elevated the expression of Nrf-2, HO-1, and NQO-1. Conclusions: SFN ameliorated diabetes-induced cognitive dysfunction by activating the Nrf2/HO-1 pathway, providing a new perspective for SFN therapy to delay cognitive impairment in diabetes patients.

Keywords: Type 2 diabetes, sulforaphane, cognitive dysfunction, oxidative stress, Nrf2_

Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder characterized by hyperinsulinaemia, insulin resistance (IR), and hyperglycemia [1]. According to statistics, diabetes has become one of the greatest health problems and its prevalence is increasing due to lifestyle changes such as diet, being overweight, and lack of exercise [2, 3]. In addition, the aging of the population is an important factor contributing to the increasing incidence of diabetes [4]. If blood glucose is not well controlled, complications may occur in multiple systems, including the central nervous system [5]. These complications impose heavy psychological and economic burdens on diabetic patients and their families. Clinical and epidemiologic data suggest that T2DM is positively associated with the risk of cognitive decline and dementia [6]. The findings of studies indicate that rats exhibit cognitive dysfunction eight weeks after the onset [7]. Epidemiological data suggest a strong association between diabetes and dementia. However, chronic diabetes causes varying degrees of cognitive decline, which is a little-studied topic; although it is generally accepted that diabetes can induce cognitive decline, its pathogenesis is unclear. Defects in insulin signaling have been reported to be associated with cognitive impairment in patients with Alzheimer's disease (AD) [8].

https://doi.org/10.62347/CHBJ5517

Insulin resistance and oxidative stress (OS) are risk factors for T2DM, which can lead to cognitive impairment [9]. An imbalance between the production and clearance of reactive oxygen species (ROS) leads to OS. Overall, OS is an important determinant of AD pathogenesis and the development of insulin resistance and diabetic complications. In diabetic encephalopathy, OS is a major contributor to the initiation of amyloid precursor protein (APP) cleavage at the β-site, or β-amyloid (Aβ) peptides affecting neurotransmission in the brain, leading to memory deficits and dementia [10]. Currently, in clinical practice, a combination therapy approach is employed to mitigate cognitive impairment caused by diabetes, wherein hypoglycemic drugs and cognitive impairment-delaying medications are administered concurrently. Therefore, our objective is to identify a solution that effectively manages blood sugar levels while simultaneously reducing cognitive impairment.

Sulforaphane (SFN) is mainly found in cruciferous vegetables, especially broccoli and cauliflower. With further research, the biological activities of SFN such as its anti-inflammatory, hypoglycemic, and antioxidative effects, have been widely explored [11]. Oxidative stress and inflammation are related to neurodegenerative diseases; therefore, neuroscientists are very interested in the biological activity of SFN. SFN has been used to study neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS). To demonstrate the protective effect of SFN on cognitive dysfunction induced by diabetes, we used Sprague-Dawley (SD) rats to establish a diabetic cognitive impairment model, and tested the cognitive function of these rats and related indicators of oxidative stress and expression of oxidative stress-related genes to verify the neuroprotective mechanism of SFN.

Materials and methods

Animals

Forty-five male SD rats (8 weeks old) were provided by North China University of Science and Technology and maintained at a room temperature of 22±2°C for 12 hours of light per day. All animal manipulations were performed in accordance with the provisions of the Guide for the Care and Use of Laboratory Animals of North China University of Science and Technology, and this experiment was approved by the Animal Ethics Committee of North China University of Science and Technology (Approval No: LAEC-NCST-20200002). Fifteen rats were randomly selected as the normal control (NC) group (n=15) and fed with a normal diet. The remaining 30 rats were fed with a high-fat and high-sugar diet for 3 weeks and then intraperitoneally injected with STZ (45 mg/kg b.wt) [12]. Fasting blood glucose (FPG) was measured on days 3 and 7 after STZ injection. Rats with FPG above 16.7 mmol/L were considered as T2DM models. Then the T2DM rats were divided into a diabetic group (DM group, n=15) and a SFN treatment group (SFN group, n=15). Rats in the SFN group were injected intraperitoneally (IP) with SFN (5 mg/kg b.wt) [13], and other animals in the NC and DM groups were given an equal volume of vehicle with phosphate buffer. After eight weeks, the related indices of the rats were detected and compared with those before SFN treatment to determine the neuroprotective effect of SFN on diabetic rats (**Figure 1A**).

Morris water maze (MWM) test

The MWM test was carried out to assess spatial learning and memory. The rats were placed in a cylinder filled with water with a diameter of 120 cm and a depth of 50 cm. During the five days, the rats were allowed to swim freely in the cylinder and given 60 s time to find and climb on the platform hidden 1 cm below the water platform, and this time was recorded as the escape latency. If the rat did not successfully find the platform within 60 s, the escape latency was recorded as 60 s. On the sixth day of the experiment, the underwater platform was removed, and the time spent in the quadrant where the platform was located and the number of platform crossings were both recorded.

H&E staining

After the MWM test, all the experimental animals were decapitated following anesthesia by pentobarbital (60 mg/kg, i.p.), and the hippocampus and cortex were isolated and embedded in paraffin. The embedded paraffin tissue was cut into 5 μm thick sections and set aside. Next, the slices were dehydrated in various concentrations of alcohol and then immersed in xylene twice for 10 minutes each. The sections

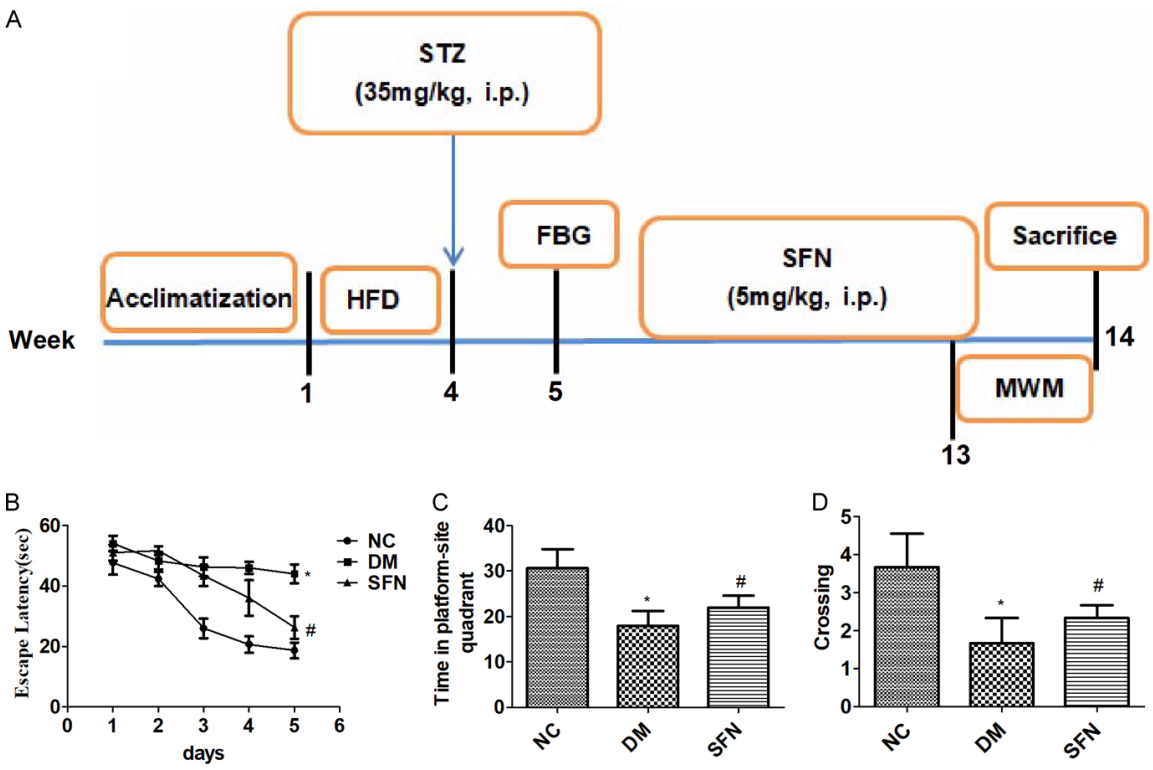

Figure 1. Changes in the learning and memory ability of diabetic rats. A. Experimental design for all rats; B. Escape latency; C. Time in platform-site quadrant; D. Crossing. *P < 0.05 vs NC group, #P < 0.05 vs DM group.

were subjected to hematoxylin eosinstained (Hematoxylin and Eosin Staining Kit, C0105M, Beyotime, China). staining. Finally, the sections were closed with PermountTM mounting medium, and the cell morphology was observed under a light microscope and photographed.

Oxidative stress injury in rat brain tissue

The cortex and hippocampus were separated on ice, cut into pieces and transferred to phosphate solution for homogenization. The supernatant was centrifuged at 4°C and the centrifugation speed was set at 15,000 g×g for 30 min. Superoxide dismutase (SOD, A001-3-2, Nanjing, China), glutathione peroxidase (GSH-PX, A005-1-2, Nanjing, China) activity detection kits, and malondialdehyde (MDA, A003-1-1, Nanjing, China) content detection kits were purchased from Jiancheng Bio Company in Nanjing, China.

TUNEL assay

An in situ apoptosis kit (C1098, Beyotime Institute of Biotechnology, China) was used to detect apoptosis in rat hippocampal and cortical neurons, and the experiment was performed strictly according to the instructions of the kit. After dehydration, the paraffin brain slices were incubated with proteinase K for 15-30 min at room temperature after which 50 μ of the TUNEL reaction mixture was added. The mixture was incubated at 37°C for 60 min in a wet chamber, 50 μl of transforming agent was added to the wet chamber, and the nuclei were counterstained with haematoxylin.

Quantitative real-time PCR analysis

Total RNA was extracted from rats from NC group, DM group and SFN group using Trizol (cat. no. R0016; Beyotime Institute of Biotechnology, China), as reported previously. RNA was qualitatively and quantitatively analysed and then reverse transcribed into cDNA, and specific primers were added to 50 μl of cDNA reaction solution (reaction system: DAN, 2 μL; Buffer, 5 μL; primers, 2 μL; dNTPs, 4 μL; water, 34.6 μL; Tap plus DNA polymerase, 0.3 μL). The reaction cycles for RT-PCR were as follows: 95°C for 30 s, 95°C for 5 s for 40 cycles and 60°C for 30 s. The β-actin gene was used as an internal control. The $2^{-\Delta\Delta CT}$ method was used to

Table 1. Primers for real-time PCR

Nrf-2 forward primer	GGCTCAGTCACTCGATAGCTC
Nrf-2 reverse primer	CCTGCTGCTTGTTTTCCGTA
HO-1 forward primer	CGAGGAAAATCCCAGATCAGC
HO-1 reverse primer	TAAATTCCCACTGCCACGGTC
NQO-1 forward primer	CATCTCTGGCGTATAAGGAAGG
NQO-1 reverse primer	CAAGCACTCTCTCAAACCAGC
GAPDH forward primer	TATGACTCTACCCACGGCAAG
GAPDH reverse primer	ATACTCAGCACCAGCATCACC

analyze the differences in relative gene expression to determine the amount of target RNA in hippocampus and cortex of each group. The primers used for the Nrf-2, HO-1, NQO-1 and GAPDH are shown in **Table 1**. The primers were designed by Yunsi Biotech.

Statistical analysis

All the data are presented as the means ± SD. One-way ANOVA and LSD test were performed using SPSS 23.0. A *P* value less than 0.05 was considered significant.

Results

Effects of SFN on cognitive function in T2DM rats

The MWM was carried out to investigate the effect of SFN on cognitive function in T2DM rats. Compared with those in the NC group, the rats of the DM group spent more time in the place navigation test. After SFN treatment, the rats in the SFN group exhibited shorter escape latencies than did those in the DM group (**Figure 1B**). In the spatial probe test, the DM group exhibited a significant in decrease both the time swimming in the target quadrant and the target platform crossings number, while the SFN group stayed more time in the target quadrant and crossed more numbers of target platforms than those in the DM group (**Figure 1C**, **1D**; $P < 0.05$). The results showed that diabetes-induced cognitive impairment was reduced by SFN administration.

Effects of SFN on the histopathology of the rat brain

To evaluate the protective effect of SFN on neurons, H&E staining was used to compare number of morphologically normal neurons in the hippocampus and cortex of the three groups. As shown in **Figure 2**, in the NC group, staining revealed that the number of neurons in the hippocampus and cortex was large and they were arranged in order; the shape of the nucleus was uniform and regular, and the nucleolus was clearly visible. In the DM group, we found a large number of irregularly arranged neurons in the hippocampus and cortex characterized by cell body shrinkage and deeply stained nuclei. However, after SFN administration, the abnormal neurons in the hippocampus and cortex of diabetic rats were significantly reduced. Our results showed that SFN could significantly protect neurons in the hippocampus and cortical region from damage and loss.

Effects of SFN on apoptotic cell death

To investigate the antiapoptotic effect of SFN, we used *in situ* TUNEL technology in rats. As shown in **Figure 3**, few TUNEL-positive cells in the hippocampal and cortical regions were detected in brain sections from the NC group. In the DM group, a large percentage of TUNEL-positive neurons were observed, while in rats treated with SFN, the percentage of apoptotic cells was significantly decreased in both the hippocampal and cortical regions.

Effect of SFN on oxidative stress induced in DM rats

To investigate the protective effect of SFN against oxidative stress injury induced by hyperglycemia, the lipid peroxides peroxide (MDA) content, SOD and GSH-Px activities were measured. **Figure 4** shows that the content of MDA was markedly elevated in the DM group, while the activities of SOD and GSH-Px were significantly inhibited. However, SFN significantly decreased MDA content and increased SOD and GSH-Px activities in the brain tissue of diabetic rats. From our results, it can be concluded that SFN has the ability to protect against oxidative stress damage.

Effect of SFN on Nrf2, HO-1, and NQO-1 expression

To further investigate the neuroprotective mechanism of SFN, the expression of Nrf2, HO-1, and NQO-1 was detected by PCR (**Figure 5**). The results revealed that the expression levels of Nrf2, HO-1, and NQO-1 in the hippocampus and cortex of DM rats were significantly lower than those in the hippocampus and cor-

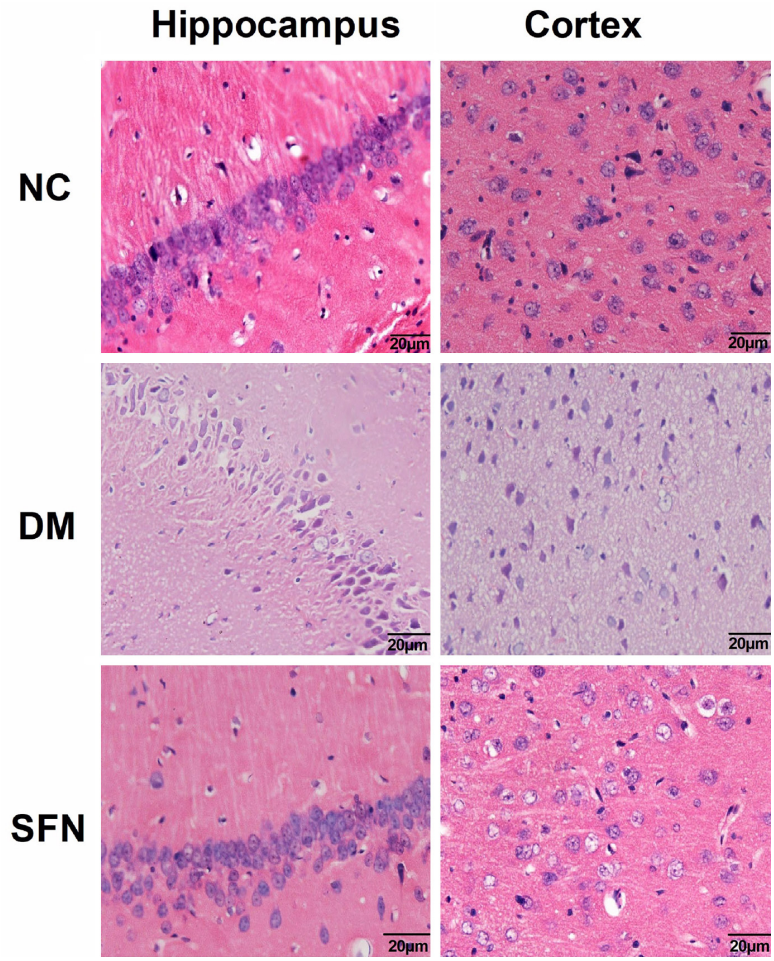

Figure 2. Histologic analysis of hippocampus and cortex.

tex of NC rats ($P < 0.05$). The expression decrease was in response to SFN treatment. These results suggested that the Nrf2/Ho-1 signalling pathway may be involved in the neuroprotective effects of SFN.

Discussion

Diabetes-related cognitive impairment is a prevalent form of dementia with an increasing incidence each year, and the STZ-induced diabetic rat model has been widely accepted and applied. In the present study, the administration of an effective dose of STZ and a high-fat diet caused severe hyperglycaemia. Diabetes causes neuronal loss and accelerates neuronal apoptosis in the hippocampus and cortex. SFN was effective in reducing the severity of cognitive impairment in animals with diabetes.

There is a dynamic balance between the production and clearance of reactive oxygen species (ROS) in the body [14]. Once there is an irreversible imbalance, oxidative stress can occur. Oxidative stress is involved in the occurrence of cognitive impairment related to a variety of neurological diseases. After treatment with oxidative stress markers, SOD and GSH-px in the brain decreased significantly, while MDA, a marker of lipid peroxidation, increased significantly [15]. Other studies have shown that SFN retains the expression of antioxidants in individuals with cognitive impairment caused by central nervous system injury. Moreover, SFN has the property of lowering blood glucose. Oxidative stress is largely responsible for neuronal damage and even death, and the loss of neuronal cells is a decrease in cognitive ability. Therefore, we can hypothesize that treatment with SFN may improve cognitive impairment caused by diabetes by decreasing oxidative stress.

Evidence suggests that excessive ROS production in brain tissue can induce apoptotic cell death and promote neurodegeneration [16]. TUNEL assay data revealed that apoptosis occurred in the hippocampus and cortical regions of dementia model rats. Previous studies have shown that apoptotic cell death is significantly increased in rats with dementia [17]. SFN treatment reduced the number of apoptotic cells, clearly indicating that SFN treatment exerts a neuroprotective effect by reversing programmed cell death in dementia.

In our study, we further linked increased apoptosis with histopathologic changes. H&E staining revealed morphologic changes. In diabetic rats, a large number of nuclei were hyperchromatic and pyknotic, indicating significant neuropathologic changes. It was evident that the number of pyknotic neurons was decreased after SFN administration, which further amelio-

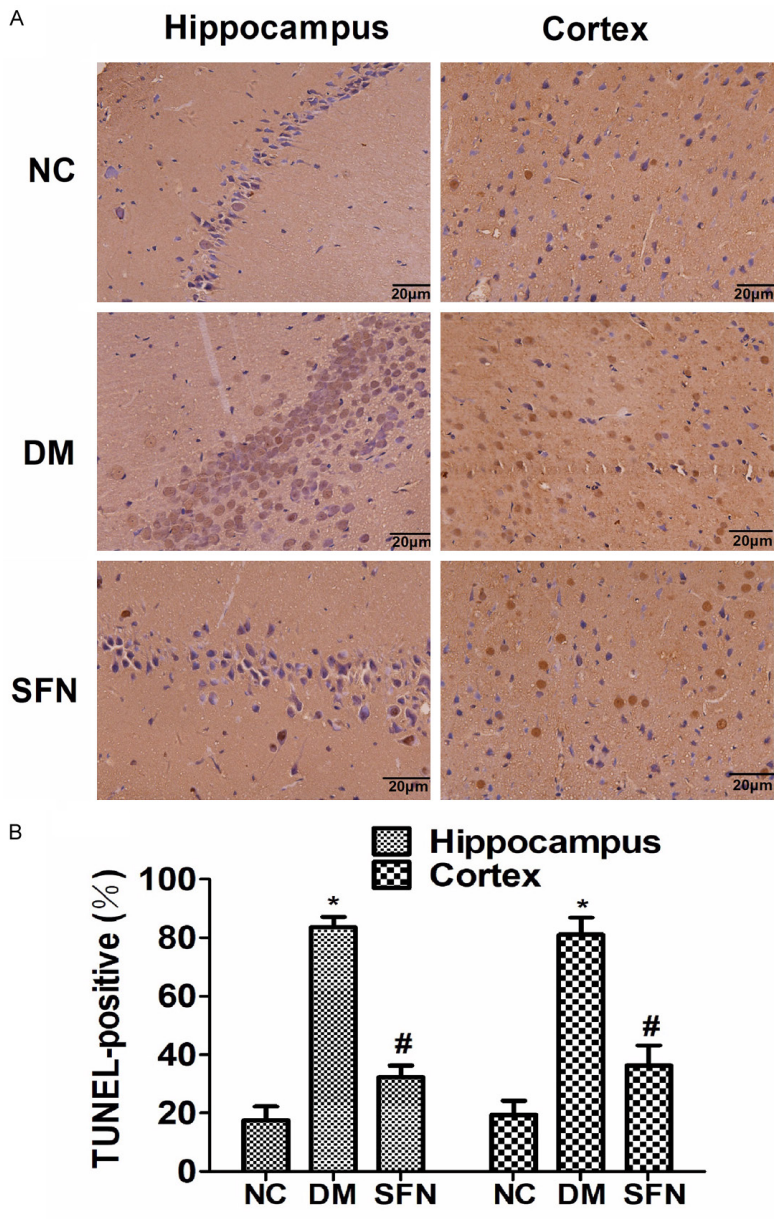

Figure 3. Changes in the number of apoptotic positive neurons. A. Apoptosis of representative neurons in hippocampus and cortex of rats in each group; B. Percentage of TUNEL-positive neurons. *P < 0.05 vs NC group, #P < 0.05 vs DM group.

rated neuronal damage. The RT-PCR results showed that the mRNA levels of Nrf2, HO-1, and NQO-1 were significantly decreased in the cortex and hippocampus of diabetic rats, indicating that the Nrf2/ARE signaling pathway was blocked. Our results are consistent with previous studies showing the downregulation of Nrf2 and ARE-encoding gene expression in dementia models. The transcription factor Nrf2 is a major factor that regulates oxidative stress. Nrf2 binds to Keap1 and functions as an intracellular redox sensor. In response to oxidative stress, Nrf2 dissociates from Keap1 and migrates into the nucleus, where it interacts with the ARE and activates the expression of a range of genes, including HO-1 and NQO-1. Nrf2 knockout mice exhibit increased markers of oxidative stress and deficits in spatial learning and memory [18]. Nrf2 knockout mice exhibit increased markers of oxidative stress and deficits in spatial learning and memory [19]. It has been found that SF has antioxidant biological activity, which can be achieved through the Nrf-ARE signaling pathway [20]. In a study of neurologic impairment after oxidative encephalopathy, it was found that the Nrf2-ARE signaling pathway can be effectively activated after SF treatment, thus delaying the progression of the disease and the therapeutic effect [21-23]. In addition, our results showed that SFN increased the expression of Nrf2, HO-1 and NQO-1, indicating that SFN treatment regulated Nrf2/ARE signaling. A series of studies suggested that the antioxidant activity of SFN may be related to the activation of Nrf2 pathway [24]. Therefore, our findings suggest that SFN may inhibit oxidative stress-mediated diabetes-induced apoptosis by activating the Nrf2/HO-1 signaling pathway. Nrf2 has previously been shown to have neuroprotective effects when overexpressed in animal models of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. In this study, we set only a single dose of SFN to preliminarily confirm the glucose-regulating and neuroprotective effects of SFN. We will use multiple doses of SFN to investigate whether the protective effect increases with increasing concentrations. In

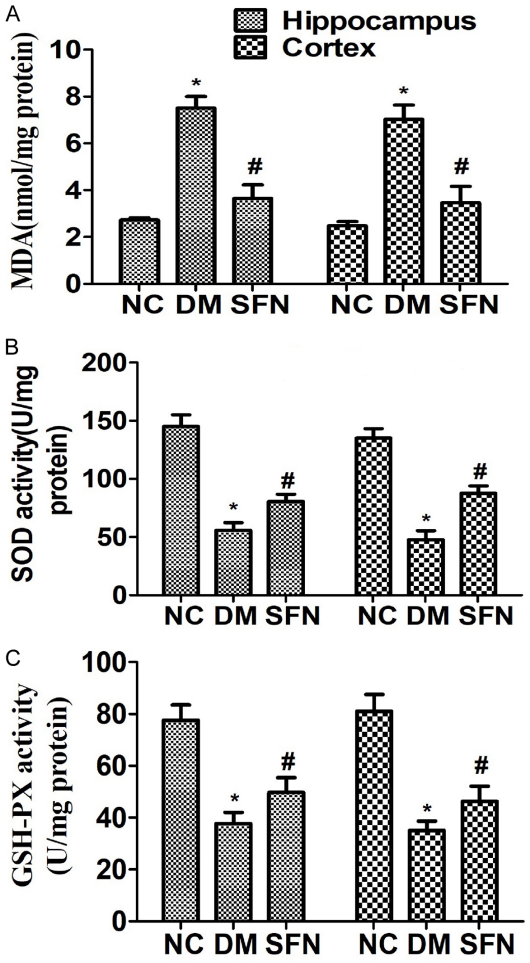

Figure 4. Changes in antioxidant capacity in hippocampus and cortex in rats. A. Relative quantity of MDA; B. SOD activity; C. GSH-PX activity. *P < 0.05 vs NC group, #P < 0.05 vs DM group.

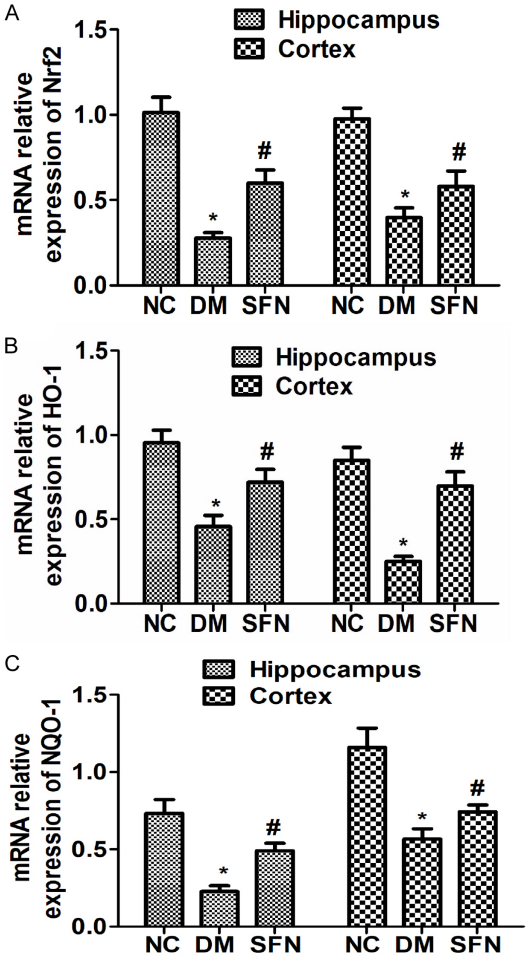

Figure 5. Changes in relative mRNA expression in the hippocampus and cortex in rats. A. Relative mRNA expression of Nrf2; B. Relative mRNA expression of HO-1; C. Relative mRNA expression of NQO-1. *P < 0.05 vs NC group, #P < 0.05 vs DM group.

addition, we will monitor the effects of SFN on cognition in rats through long-term follow-up.

Conclusion

Our findings suggest that SFN may inhibit oxidative stress-mediated diabetes-induced apoptosis by activating the Nrf2/HO-1 signaling pathway. This can be solid evidence for basic research and preclinical studies.

Acknowledgements

The work was supported by Health Commission of Hebei Province (Grant No. 20200130).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jinhua Wang, Department of Neurology, Huanggang Central Hospital of Yangtze University, 6 Qi'an Avenue, Huangzhou District, Huanggang 438000, Hubei, China. Tel: +86-0713-8625054; Fax: +86-0713-8625054; E-mail: wangjh8505@yeah.net

References

[1] Yaribeygi H, Sathyapalan T, Atkin SL and Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev 2020; 2020: 8609213.

[2] Aras M, Tchang BG and Pape J. Obesity and diabetes. Nurs Clin North Am 2021; 56: 527-541.

[3] La Sala L and Pontiroli AE. Prevention of diabetes and cardiovascular disease in obesity. Int J Mol Sci 2020; 21: 8178.

[4] Kolb H and Martin S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med 2017; 15: 131.
[5] Ferris JK, Inglis JT, Madden KM and Boyd LA. Brain and body: a review of central nervous system contributions to movement impairments in diabetes. Diabetes 2020; 69: 3-11.
[6] Bertram S, Brixius K and Brinkmann C. Exercise for the diabetic brain: how physical training may help prevent dementia and Alzheimer's disease in T2DM patients. Endocrine 2016; 53: 350-63.
[7] Akhtar A and Sah SP. Insulin signaling pathway and related molecules: role in neurodegeneration and Alzheimer's disease. Neurochem Int 2020; 135: 104707.
[8] Michailidis M, Moraitou D, Tata DA, Kalinderi K, Papamitsou T and Papaliagkas V. Alzheimer's disease as type 3 diabetes: common pathophysiological mechanisms between Alzheimer's disease and type 2 diabetes. Int J Mol Sci 2022; 23: 2687.
[9] Ning P, Luo A, Mu X, Xu Y and Li T. Exploring the dual character of metformin in Alzheimer's disease. Neuropharmacology 2022; 207: 108966.
[10] Vanduchova A, Anzenbacher P and Anzenbacherova E. Isothiocyanate from broccoli, sulforaphane, and its properties. J Med Food 2019; 22: 121-126.
[11] Chen R, Zeng Y, Xiao W, Zhang L and Shu Y. LC-MS-based untargeted metabolomics reveals early biomarkers in STZ-induced diabetic rats with cognitive impairment. Front Endocrinol (Lausanne) 2021; 12: 665309.
[12] Rony KA, Ajith TA, Kuttikadan TA, Blaze R and Janardhanan KK. Phellinus rimosus improves mitochondrial energy status and attenuates nephrotoxicity in diabetic rats. J Basic Clin Physiol Pharmacol 2017; 28: 455-461.
[13] Zhou A, Hong Y and Lv Y. Sulforaphane attenuates endometriosis in rat models through inhibiting PI3K/Akt signaling pathway. Dose Response 2019; 17: 1559325819855538.
[14] Chan CM, Huang DY, Sekar P, Hsu SH and Lin WW. Reactive oxygen species-dependent mitochondrial dynamics and autophagy confer protective effects in retinal pigment epithelial cells against sodium iodate-induced cell death. J Biomed Sci 2019; 26: 40.
[15] Wang F, Li R, Tu P, Chen J, Zeng K and Jiang Y. Total glycosides of cistanche deserticola promote neurological function recovery by inducing neurovascular regeneration via Nrf-2/Keap-1 pathway in MCAO/R rats. Front Pharmacol 2020; 11: 236.
[16] Angelova PR and Abramov AY. Role of mitochondrial ROS in the brain: from physiology to neurodegeneration. FEBS Lett 2018; 592: 692-702.
[17] Wang XX, Zhang B, Xia R and Jia QY. Inflammation, apoptosis and autophagy as critical players in vascular dementia. Eur Rev Med Pharmacol Sci 2020; 24: 9601-9614.
[18] Abdel-Rahman RF, Fayed HM, Ogaly HA, Hussein RA and Raslan MA. Phytoconstituents of Sansevieria suffruticosa N.E.Br. Leaves and its hepatoprotective effect via activation of the NRF2/ARE signaling pathway in an experimentally induced liver fibrosis rat model. Chem Biodivers 2022; 19: e202100960.
[19] Cao K, Xiang J, Dong YT, Xu Y and Guan ZZ. Activation of α7 nicotinic acetylcholine receptor by its selective agonist improved learning and memory of amyloid precursor protein/presenilin 1 (APP/PS1) mice via the Nrf2/HO-1 pathway. Med Sci Monit 2022; 28: e933978.
[20] Campbell MR, Karaca M, Adamski KN, Chorley BN, Wang X and Bell DA. Novel hematopoietic target genes in the NRF2-mediated transcriptional pathway. Oxid Med Cell Longev 2013; 2013: 120305.
[21] Wu DM, Zheng ZH, Fan SH, Zhang ZF, Chen GQ and Lu J. Sulforaphane administration alleviates diffuse axonal injury (DAI) via regulation signaling pathway of NRF2 and HO-1. J Cell Biochem 2020; 121: 430-442.
[22] Nowinski WL, Chua BC, Volkau I, Puspitasari F, Marchenko Y, Runge VM and Knopp MV. Simulation and assessment of cerebrovascular damage in deep brain stimulation using a stereotactic atlas of vasculature and structure derived from multiple 3- and 7-tesla scans. J Neurosurg 2010; 113: 1234-41.
[23] Tarozzi A, Angeloni C, Malaguti M, Morroni F, and Hrelia S and Hrelia P. Sulforaphane as a potential protective phytochemical against neurodegenerative diseases. Oxid Med Cell Longev 2013; 2013: 415078.
[24] Alattar A, Alshaman R and Al-Gayyar MMH. Therapeutic effects of sulforaphane in ulcerative colitis: effect on antioxidant activity, mitochondrial biogenesis and DNA polymerization. Redox Rep 2022; 27: 128-138.