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Protective Effect of SGLT2 Inhibitor on D-Galactose-Induced Senescence in Mice and Its Mechanism

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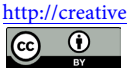

Abstract

Objective: To observe the cerebral protective effect of dagliflozin, a sodium-glucose co-transport protein 2 (SGLT2) inhibitor, in aging mice and to explore its molecular mechanism. **Methods:** 1. 66 male C57BL/6 mice were divided into control group (13) and model group (53), and the model group was moulded by subcutaneous injection of D-galactose into the back of the neck, while the control group was treated with equal amount of saline for 8 weeks. The weight of each group of mice was observed and recorded every 7 days, and two groups of mice were randomly selected for frozen sections of brain tissue at the end of the modelling period to verify the aging model. 2. After the aging model was successfully established, the aging groups were divided into 5 groups: model group, dagliflozin-treated group (high and low dose), and dagliflozin + ex527-inhibited group (high and low dose). Fasting blood glucose was measured in each group every 2 weeks for 8 weeks. At the end of treatment, Morris water maze was performed at the end of the treatment. After execution of the mice, the organ indices of heart, brain, liver, kidney and spleen were measured; the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in serum were determined. **Results:** After the successful establishment of the aging model, it was found that during the treatment phase of dagliflozin. 1) The organ indices of mice in the aging group were significantly lower than those of other groups, and no significant hypoglycemia was observed throughout the treatment process. 2) In the water maze test, mice in the aging group had a significantly longer latency in the plateau phase compared to the control and treatment groups, while the number of times the mice crossed the original plateau and the percentage of time spent exploring the original plateau quadrant were reduced after the plateau was removed. 3) The nerve cells in the aging mice were disorganized and the

nuclei of the mice were deeply stained; the dagliflozin group improved the morphological changes in the brain of aging mice. 4) In addition, compared with the control mice, the serum MDA level was significantly increased and the antioxidant enzyme SOD activity was significantly decreased in the aging group, while compared with the aging group, dagliflozin significantly decreased the MDA level and increased the SOD activity. 5) The expression of SIRT1 and PGC-1*α* was significantly upregulated in the low and high doses of dagliflozin compared to the aging group. **Conclusion:** The present study suggests that dagliflozin can delay organ aging, improve the learning and memory ability of aging mice, and exert antioxidant effects, probably through upregulating the SIRT1/PGC-1*α* signaling pathway.

Keywords

Aging, Neurodegenerative Diseases, SGLT2 Inhibitors, Oxidative Stress, SIRT1 Signalling Pathway

1. Introduction

Ageing is the degeneration of an organism with age, accompanied by a decrease in the body's adaptability to the environment and immunity, as well as the development of age-related syndromes and age-related frailty with minor clinical pathologie [1]. Cognitive impairment is one of the key signs of ageing, and numerous studies have shown that memory function slowly deteriorates with the onset of ageing. In addition, neurological ageing is considered a major risk factor for most neurodegenerative diseases (such as Alzheimer's and Parkinson's diseases) [2], which seriously affects the quality of life and physical and mental health of the elderly. Sodium-glucose cotransport protein 2 (SGLT2) inhibitors are hypoglycemic agents designed to reduce plasma glucose concentrations by inhibiting NA+-glucose coupled transport in the proximal tubule, and their cardioprotective and renoprotective effects independent of hypoglycemic effects have been progressively demonstrated [3]. Meanwhile, in recent years, SGLT2 inhibitors have been postulated to have beneficial effects in delaying ageing and improving cognitive impairment in ageing-related diseases due to their antioxidant and anti-inflammatory effects and calorie restriction mimetic properties [4] [5]. Another study found that dagliflozin could enhance AMPK signaling pathway to improve GABAergic and neurotrophic mechanisms in the amygdala to alleviate anxiety symptoms in rats with dementia [6]. However, there is not enough evidence on the delayed aging and cerebroprotective effects of SGLT2 inhibitors. In this study, we aimed to investigate the multiple protective effects of SGLT2 inhibitors on aging mice in terms of cognitive function improvement, antioxidant and aging signaling pathways in a D-galactose-induced aging mouse model, with the aim of providing a theoretical and experimental basis for the delayed aging and neuroprotective effects of SGLT2 inhibitors.

2. Materials and Methods

2.1. Main Materials

Sixty-six clean C57BL/6 male mice, 8 weeks old, body mass 28 - 32 g, were purchased from the Experimental Animal Center of Hubei Medical College (License No. SCXK(E)2019-0008). Superoxide dismutase (SOD) and cellular senescence β-galactosidase staining kits were purchased from Biyuntian Reagent Company; malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Company; SIRT1, PGC1-α and β-actin antibodies were purchased from Wuhan Sanying Company; blood glucose test strips were purchased from Sanno Biological Company; Dagliflozin is purchased from AstraZeneca Pharmaceuticals Ltd; The water maze equipment is provided by Shanghai Xinweida Company; Electronic balance (Ohaus).

2.2. Animal Modelling and Grouping

After one week of adaptive feeding, 66 mice were divided into two groups: 13 mice in the control group and 53 mice in the senescence group. The mice were weighed on the day the experiment started and every 7 days thereafter until the end of the senescence model. During the experiment, except for the normal control group, mice in the aging group were injected with D-gal subcutaneously on the back of the neck at 400 mg/kg/d [7] and mice in the control group were injected with saline subcutaneously on the back of the neck at the same dose for 8 weeks. At week 8, three mice from each group were randomly selected for the validation of the senescence model. After the senescence model was successfully established, starting from the ninth week, the senescence groups were divided into five groups: model group, dagliflozin-treated group (high and low dose), and dagliflozin + ex527-inhibited group (high and low dose). The dagliflozin high and low dose groups were given gavage at 1 mg/kg/d and 10 mg/kg/d respectively; the ex527 inhibition group was given intraperitoneal injection at 10 mg/kg/d and ex527 intraperitoneal injection was given 20 min after the end of gavage. The control group and model group were gavaged with saline at 100 mg/kg/d daily for 8 weeks. The experimental procedure was grouped as shown in **Figure 1**.

2.3. Dosage Design

The standard daily dose of Dagliflozin for adults is 5 - 10 mg/60 kg. Based on the extensive literature review, the designed dose for this study was 1 mg/kg for the low dose group and 10 mg/kg for the high dose group. SIRT1 inhibitor ex527 administered at a dose of 10 mg/kg/d by intraperitoneal injection [1] [7] [8] [9] [10] [11].

2.4. Body Weight, Blood Glucose Recording and Organ Coefficient Determination in Mice

The mice were weighed on days 1, 7, 14, 21...56, *i.e.* every other week, from the first day of the experiment until the aging model was established at week 8, and

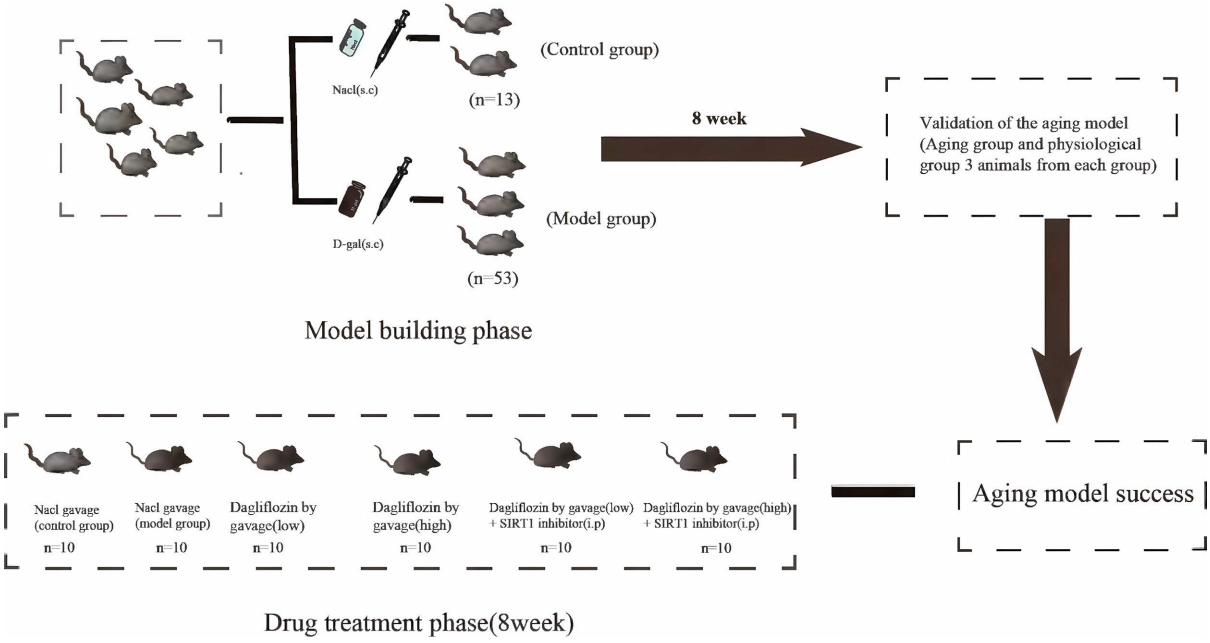

Figure 1. Schematic diagram of the animal model and grouping of administered treatments.

the changes in body weight were observed and plotted. Fasting blood glucose levels were measured during the dagliflozin administration phase, while the mice and their organs were weighed at the end of the behavioural experiment.

2.5. β-Galactosidase Staining of Brain Tissues

At the end of the senescence model, three mice were taken from each of the physiological control and senescence groups, anesthetized and fixed, and the whole brains were rapidly isolated. The brain tissue was fixed in 4% paraformaldehyde for 48h and then frozen sectioned according to the steps of sampling—rinsing—dehydration—embedding—sectioning—fixation. The sections were then washed with PBS to remove the fixative, the PBS was aspirated and the staining solution was prepared according to the kit instructions. Microscopic observation of β-gal staining of brain tissue.

2.6. Effect of Dagliflozin on Cognitive Learning in Mice

The water maze test was started at the end of the moulding period to train the mice in spatial learning and memory. The mouse is grabbed so that its head is facing the wall of the pool to prevent it from seeing the reference object and is quickly placed in the water and the image acquisition system is activated. A camera is set up at about 1.5 m above the centre of the circular pool to record the path of the mouse and is connected to the computer processing and analysis system to record and analyse the experimental data of the water maze.

2.7. Determination of Serum Superoxide Dismutase (SOD) Activity and Malondialdehyde (MDA) Content in Mice

After completing the water maze and behavioral cognition test, the mice were

anesthetized using a general anesthesia method. The limbs of the mice were quickly fixed, the chest was exposed, and the heart beat point was found. The needle was inserted parallel to the heart with a 1 ml syringe and aspirated immediately after a slight breakthrough sensation, followed by the collection of blood from the mice in anticoagulated centrifuge tubes. The collected blood was placed at room temperature for 20 - 30 min and centrifuged in a room temperature centrifuge for 13 min to separate the serum. The serum was stored in a refrigerator at −20˚C and used for the antioxidant assay.

The control and standard groups were set up and the standard curves were measured in strict accordance with the instructions of the Total SOD Activity Assay Kit (Biyuntian) and Malondialdehyde Kit (Nanjing Jiancheng), and the assays were carried out according to the operation procedures. The SOD activity and MDA content were calculated by using a multifunctional enzyme marker to measure the absorbance of the samples according to the different absorbance at the corresponding wavelengths.

2.8. Histopathological Changes in Mouse Brain Tissue

The mice in each group were executed after completion of the behavioural tests using HE staining, brain tissue was removed, and some brain tissue samples were collected, fixed overnight in 4% paraformaldehyde, dehydrated, paraffin-embedded and cut into 3 um sections. After dewaxing, brain tissue sections were rehydrated in different concentrations of ethanol solution, stained with hematoxylin and eosin, and the histopathological changes were observed under light microscopy.

2.9. Effect of Dagliflozin on the Expression of SIRT1 and PGC-1 Proteins

Western blotting method was used. Brain tissues from each group of mice were extracted with lysis buffer, and the total protein concentration was determined by BCA method. The proteins were separated by SDS-PAGE and transferred to PVDF membrane. Add SIRT1 and β-actin (1:1500) primary antibodies respectively and incubate the membrane with HRP-labelled goat anti-mouse secondary antibody (1:2500) overnight. The bands were detected using an imaging system, and the protein was analyzed semi-quantitatively using β-actin as an internal reference, and the relative expression of the target protein was determined as the grey-scale value of the target and control bands.

2.10. Statistical Analysis

SPSS version 25.0 software was used for data analysis. The measurement data were expressed as mean ± standard deviation (Mean ± SEM). One-way ANOVA was used to analyse differences between groups when two comparisons between groups were made; two-sample t-tests were used to analyse differences between two groups when two different samples were compared. $P < 0.05$ was defined as statistically significant.

3. Results

3.1. Successful Aging Modeling

3.1.1. Effect of D-Gal on Body Weight Changes in Aging Mice

Compared with the physiological group, the body weight of mice in the aging group was basically the same at the beginning of the experiment, and there was no significant difference between the groups ($p > 0.05$). At week 8, the mice in the model group showed a significant weight loss compared to the physiological group. The results are shown in Figure 2.

3.1.2. Effect of D-Gal on β-Galactosidase in Aging Mice

As can be observed in Figure 3, the percentage of positive β-galactosidase staining area in the frozen sections of the brains of mice after 8 weeks of D-galactose injection (*i.e.* the aging group) was higher and significantly different ($p < 0.05$) compared to the physiological control group.

3.2. Effect of Dagliflozin on Aging Mice

3.2.1. Effect of Dagliflozin on Organ Indices in Aging Mice

As shown in Table 1, the organ indices of heart, brain, spleen and liver were significantly reduced in the aging group compared to the control group ($p < 0.05$). The organ indices of brain, spleen and liver were significantly increased in the low dose group compared to the aging group ($p < 0.05$). However, the difference between the high and low dose inhibitor group and the treatment group was not significant compared to the dagliflozin intervention group ($p > 0.05$).

3.2.2. Effect of Dagliflozin on Blood Glucose in Aging Mice

We measured fasting blood glucose in each group of mice at 2, 4, 6 and 8 weeks,

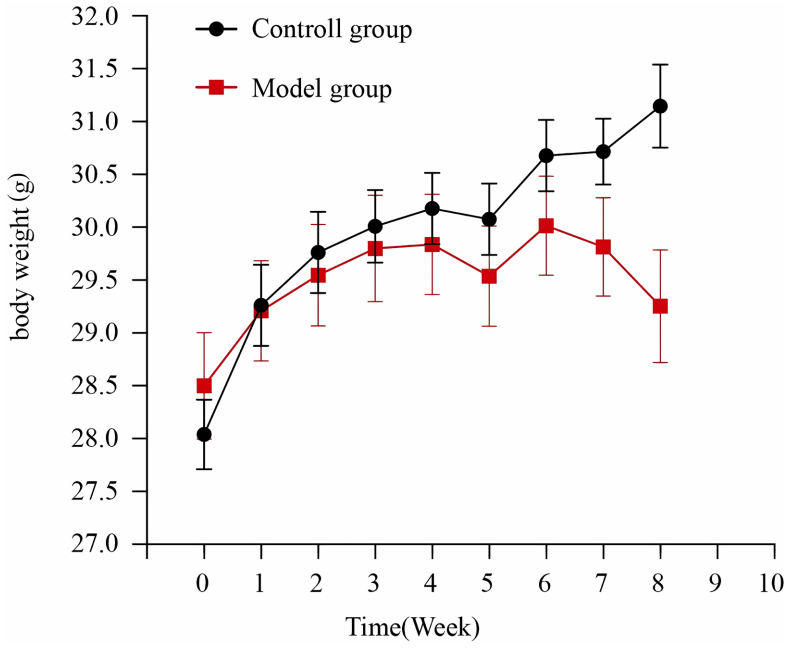

Figure 2. Effect of D-gal on body weight changes in aging mice.

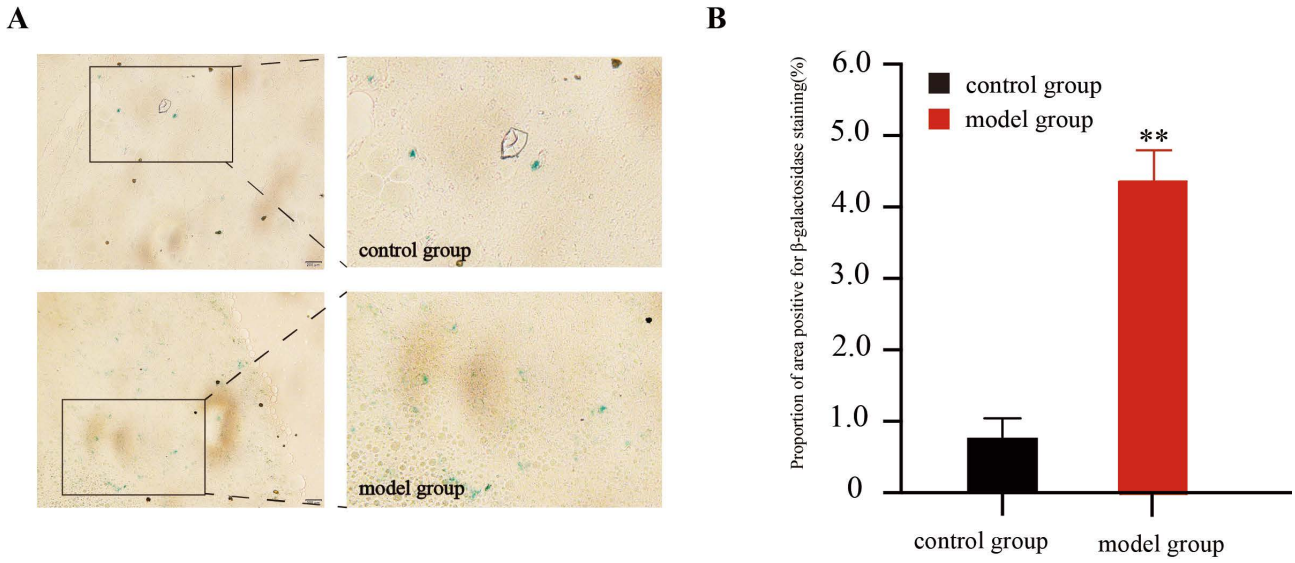

Figure 3. Effect of D-gal on β-galactosidase in senescent mice. Note: A, β-galactosidase staining (SA-β-gal) for detection of aging in mice (200×); B, effect of D-gal on the proportion of positive area of β-galactosidase staining in frozen sections of aging mouse brains. Compared with the control group, ** indicates $p < 0.05$.

Table 1. Organ indices (mg/g).

Group	Heart Index	Brain Index	Spleen Index	Renal Index	Hepatic Index
Control	5.29 ± 0.14	17.06 ± 0.45	3.06 ± 0.12	6.75 ± 0.05	54.70 ± 2.66
Aging model	4.70 ± 0.14**	15.47 ± 0.48**	2.20 ± 0.22**	6.44 ± 0.41	42.99 ± 1.97**
Low dose	5.19 ± 0.20	16.51 ± 0.26##	2.89 ± 0.21##	7.23 ± 0.45	43.32 ± 1.99##
High dose	4.92 ± 0.24	15.45 ± 0.47	2.66 ± 0.13	6.26 ± 0.20	36.96 ± 1.43
Low dose inhibitors	5.06 ± 0.53	16.27 ± 0.48	2.74 ± 0.13##	6.67 ± 0.23	40.71 ± 1.31
High dose inhibitors	4.96 ± 0.22	15.41 ± 0.56	2.56 ± 0.20	6.24 ± 0.13	41.00 ± 1.06

Note: Compared with the control group: ** indicates significant difference ($p < 0.05$), compared with the model group, ## indicates significant difference ($p < 0.05$).

and in this test, hypoglycaemia was defined as a blood glucose measurement of less than 2.30 mmol/L after 10 - 12 hours of fasting without water. During the experiment, no mice were found to be unconscious or died due to hypoglycemia. The mean blood glucose values of the mice in each group and the changes in blood glucose during the treatment are shown in Table 2.

3.2.3. Effect of Dagliflozin on Learning Memory and Cognitive Exploration in Aging Mice

On days 1, 3 and 7 of the water maze, mice in the model group had a significantly longer escape latency compared to the physiological group ($p < 0.05$), whereas the escape latency of the high-dose dagliflozin group was significantly shorter on days 1, 3 and 7 of the water maze compared to the model group (($p < 0.05$); there was no significant difference between the low-dose treated mice and the model group at the beginning of the training period ($p > 0.05$), As training

Table 2. Effect of dagliflozin on blood glucose in aging mice (mmol/L).

Group (n = 10)	2 Week	4 Week	6 Week	8 Week
Control	6.12 ± 0.32	5.85 ± 0.40	5.04 ± 0.53	6.91 ± 0.53
Aging model	6.89 ± 0.49	6.75 ± 0.64	5.59 ± 0.62	7.58 ± 0.41
Low dose	7.28 ± 0.47	6.21 ± 0.47	4.65 ± 0.63	7.34 ± 0.35
High dose	3.46 ± 0.16	7.26 ± 0.35	6.80 ± 0.51	6.93 ± 0.46
Low dose inhibitors	6.53 ± 0.46	5.69 ± 0.68	4.74 ± 0.64	4.99 ± 0.66
High dose inhibitors	6.53 ± 0.46	5.69 ± 0.68	4.74 ± 0.64	4.99 ± 0.66

progressed, a significant shortening of the escape latency was observed in the low-dose group only on the seventh day ($p < 0.05$). In addition, although the mice in the high and low dose inhibitor groups of dagliflozin showed an increase in escape latency compared to the dagliflozin treated group, the difference was not significant ($p > 0.05$).

During the platform concealment period, the mice in the aging group showed a significant decrease in the number of times they crossed the original platform area, a longer time to reach the original platform for the first time and a significant decrease in the exploration time in the original safe platform quadrant after the withdrawal of the platform, with statistically significant differences ($p < 0.05$). At the same time, mice in the high and low dose treatment groups showed the opposite trend compared to the aging group, *i.e.*, an increase in the number of crossing the original plateau area, a shortening of the latency to first reach the original plateau position and a significant increase in the proportion of time spent exploring the original plateau quadrant ($p < 0.05$). In addition, a decrease in the proportion of time spent exploring in the home platform quadrant was observed in the inhibitor group (low and high doses) compared to the treatment group ($p < 0.05$), while no significant differences were observed in the remaining indicators, as shown in **Figure 4**.

3.2.4. Effect of Dagliflozin on the Antioxidant Level of Brain Tissue in Aging Mice

The results of the experiment are shown in **Figure 5**. Compared with the physiological group, the CuZn-SOD activity in the model group showed a significant decrease ($p < 0.05$), while the MDA content showed a differential increase ($p < 0.05$), which indicated that the level of oxidative stress in the model group mice was significantly higher than that in the control group. On the other hand, compared with the model group, CuZn-SOD activity was significantly higher in the dagliflozin treatment group (low and high dose) as well as the dagliflozin high dose inhibitor group ($p < 0.05$), while MDA content was significantly lower in the dagliflozin high dose group ($p < 0.05$). In addition, there was a decrease in SOD activity and an increase in MDA content in the inhibitor group (low and high dose groups) compared to the treatment group, but the difference was not significant.

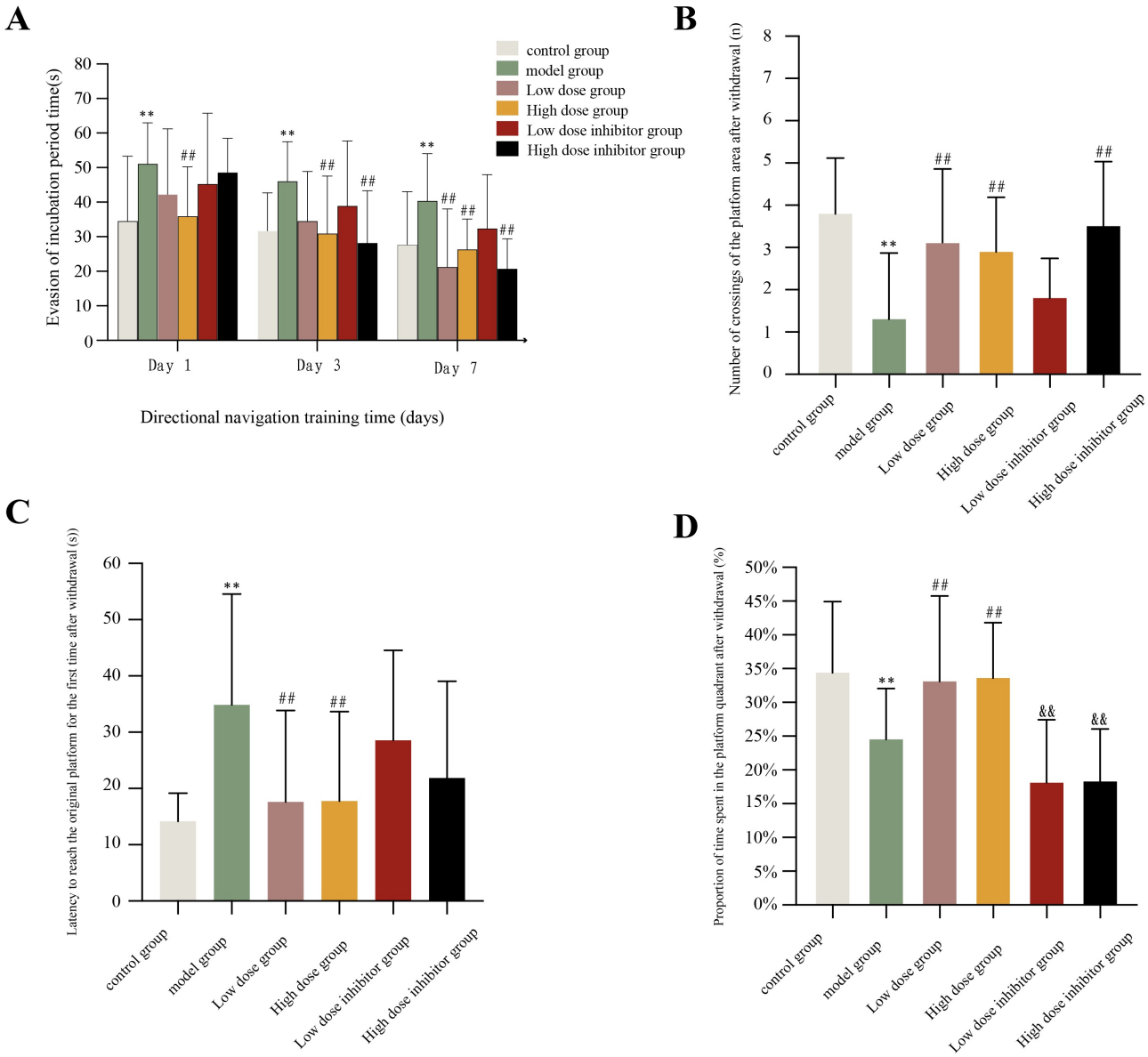

Figure 4. Effect of dagliflozin on the learning memory ability of aging mice (n = 10). Note: A, latency to escape before withdrawal; B, number of times crossing the platform area after withdrawal; C, latency to reach the original platform for the first time after withdrawal; D, proportion of time spent in the original platform quadrant after withdrawal. Compared with the control group, ** indicates $p < 0.05$; compared with the aging group, ## indicates $p < 0.05$; compared with the treatment group, && indicates $p < 0.05$.

3.2.5. Effect of Dagliflozin on Neurons in the Hippocampal Region of Brain Tissue in Aging Mice

As can be observed from **Figure 6**, compared with the control group, the neurons in the model group were of different sizes, disorganized and loosely arranged, and even some neurons had irregular nuclei, and most of the neurons showed nuclear consolidation and deep staining. In the treated group (low and high) mice, the neurons were relatively neatly arranged compared to the aging group, and a few cells showed nuclear consolidation and darker staining. In contrast to the treated group, the inhibitor group showed a decrease in the

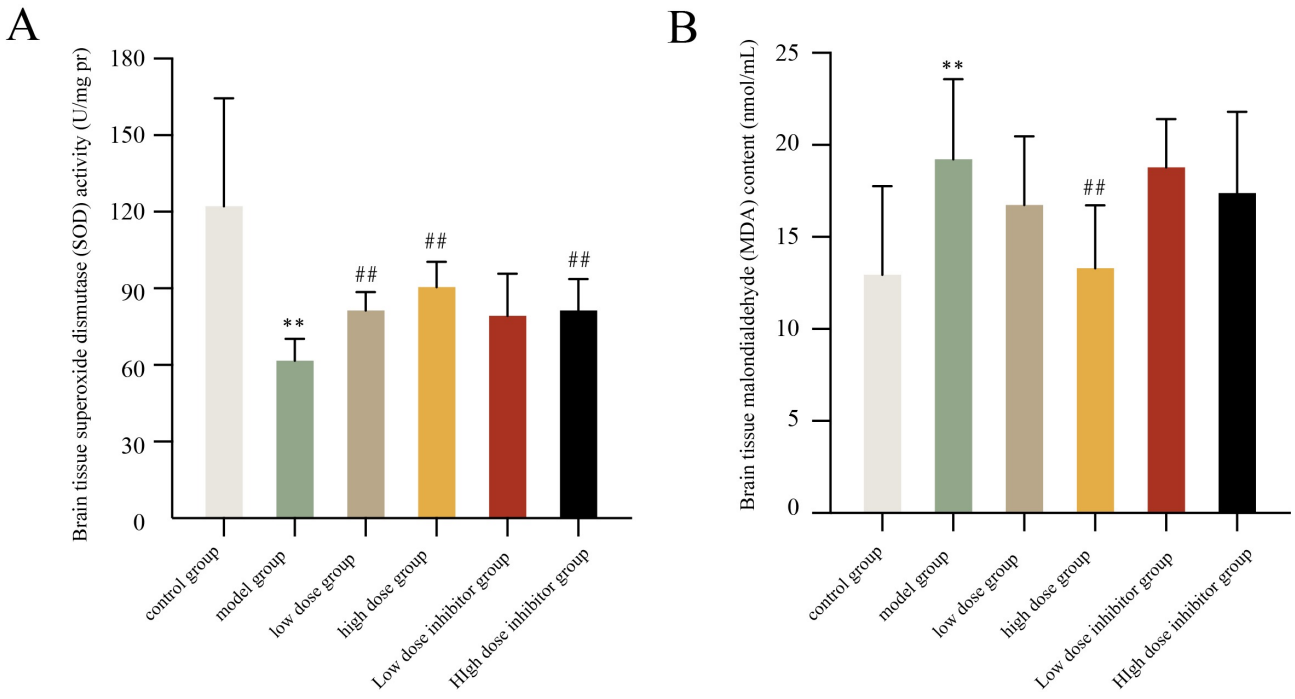

Figure 5. Effect of dagliflozin on antioxidant enzyme activity and MDA content in aging mice (n = 10). Note: Compared with the control group, ** indicates $p < 0.05$; compared with the aging group, ## indicates $p < 0.05$.

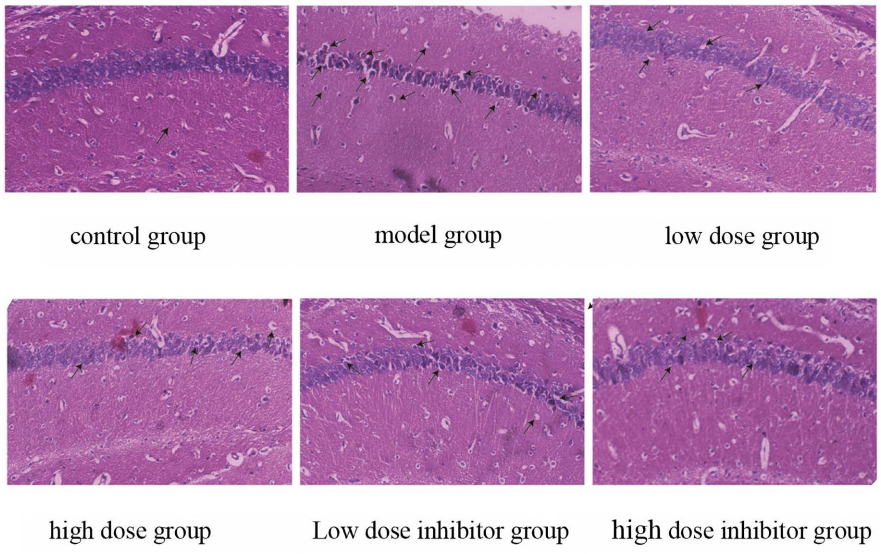

Figure 6. Pathological changes in hippocampus of aging mice by dagliflozin (200×, HE staining).

number of normal neurons and different cell morphology. The nuclei of individual cells were shrunken, but the overall neuronal morphology was slightly neater than that of the aging mice.

3.2.6. Effect of Dagliflozin on the Expression of SIRT1 and PGC-1α Protein in Brain Tissues

Compared with the physiological group, the brain tissues of aging mice showed

a trend of low expression of SIRT1 and PGC-1α protein ($p < 0.05$); meanwhile, compared with aging mice, an increase in SIRT1 and PGC-1α protein expression was observed in both high and low doses of dagliflozin treatment group ($p < 0.05$). In addition, compared with the treatment group, the high and low dose groups of SIRT1 inhibitor showed a trend of low expression of SIRT1 protein, while the difference of PGC-1α protein was only observed in the low dose inhibitor group compared with the treatment group ($p < 0.05$), and no significant difference was observed in the high dose group. The results are shown in **Figure 7**.

4. Discussion

Ageing is usually accompanied by the ageing and decline of the body's organ functions, and changes in brain function, such as memory loss and cognitive decline, can have a serious impact on the physical and mental health of patients [12]. Cognitive dysfunction is often present throughout the aging process, mainly in the form of memory loss and dementia, and the exploration of potential interventions to delay the onset of cognitive dysfunction is a hot topic in geriatric research.

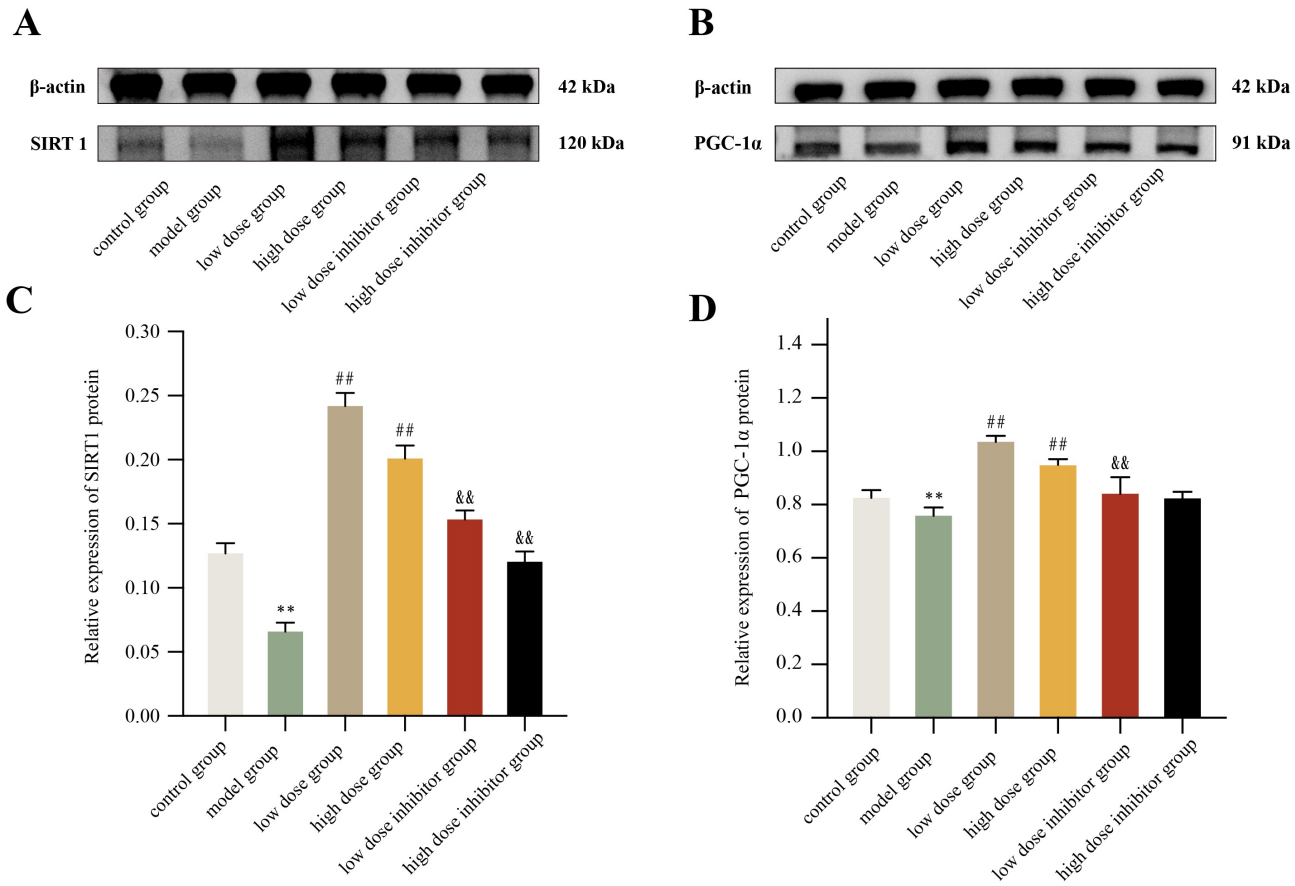

Figure 7. Effect of dagliflozin on SIRT1 and PGC-1α protein expression in brain tissue (each group was repeated 3 times). Note: Compared with the control group, ** indicates $p < 0.05$; compared with the aging group, ## indicates $p < 0.05$; compared with the treatment group, && indicates $p < 0.05$.

The establishment of animal models of ageing is a key prerequisite and basis for the study of ageing and intervention. There are various aging models, among which the D-galactose-induced aging mouse model is widely used because of its stability [13] [14]. In our study, we administered D-galactose subcutaneously to mice to establish a senescence model, and β-galactosidase and mouse body weight changes were used to supplement the success of the model. We found that the aging group showed a significant decrease in body weight compared to the control group. At the same time, we observed a decrease in body weight in both the aging and control groups at week 5, although not as much in the control group as in the aging group. We believe that this may be due to the turning point in weight loss as the mice age. In addition, the percentage of positive β-galactosidase staining in the brain tissue of the aging mice was significantly higher, indicating that the mice were senescent and that a subacute aging mouse model was successfully established.

A key observation of growth and development in mice is the change in body weight, while the degree of cellular senescence can be reflected by the organ index. Numerous studies have observed significant decreases in the organ indices of immune organs in aging mice [15]. In our study, the organ indices of mice in the model group were significantly lower than those of the other groups, and the organ indices of brain, liver and spleen in the dagliflozin intervention group showed significant increases compared to those of the senescent group. This indicates that dagliflozin can promote the health of brain, spleen and liver of aging mice to a certain extent, and can delay the physiological decline and aging of organs caused by ageing, and enhance the function of internal organs and immunity to a certain extent.

As an oral hypoglycemic agent, the side effects of hypoglycemia are the most common concern and prevention in clinical practice, especially in this study where the D-gal model is not a diabetic mouse model, and we should be alert to the risk of hypoglycemia in normal aging mice. During the experiment, we measured the blood glucose of each group of mice every 2 weeks and we found that the dagliflozin intervention group did not show any significant hypoglycemia during the experiment. Although a decrease in blood glucose was observed in the low-dose mice at week 6 and the blood glucose returned to normal at week 8, it was still within the normal glycemic control range, but we believe that more experiments are needed to explore the effect of low-dose dagliflozin on blood glucose in mice. In addition, no mice were found to be unconscious or dead due to hypoglycaemia during the experiment. Takashima M *et al.* found that the cerebral protective effect of SGLT2 inhibitors was not related to their hypoglycemic effect and that luseogliflozin did not cause changes in blood glucose [16]. This is consistent with our findings and indicates the safety and reliability of dagliflozin administration.

The brain is the higher organ for learning, memory and analysis. When functional ageing occurs in the brain, the earliest manifestation of the brain is a significant decline in higher functions such as learning ability and memory. In this

study, the results of the Morris water maze test using a subacute ageing model showed that D-gal-induced impairment of cognitive function in the brain could be alleviated and improved to some extent by dagliflozin.

The theory of oxidative stress is now a widely accepted theory of the mechanisms of ageing. The imbalance between the oxidative and antioxidant systems in the body and their tendency to oxidise, and the consequent oxidative stress, leads to a disorder between the accumulation of ROS and resistance to oxidative stress, and an excessive accumulation of free radicals and a decrease in the activity of antioxidant enzymes are closely related to the ageing process. Yang Yang *et al.* showed that the D-gal-induced aging model in rats showed a significant increase in MDA content and decrease in antioxidant enzyme SOD activity compared with the control group, and the expression of the model group was similar to that of the natural aging group [17]. Ling Lingchen *et al.* found that the sesquiterpene compound Albicanol reduced the D-gal-mediated model mice's serum SOD, CAT The sesquiterpene Albicanol was found to reduce the activity of serum SOD, CAT and GSH-Px in D-gal-mediated model mice, inhibit oxidative damage, and reduce oxidative stress-induced damage to delay aging [18]. Our results showed that compared with the control group, the aging mice showed a significant increase in MDA levels and a decrease in SOD activity; whereas, dagliflozin significantly inhibited the level of oxidative stress, reduced the accumulation of peroxidized MDA, and significantly increased SOD activity. This suggests that dagliflozin can reduce the damage caused by oxidative stress in aging mice by reducing the degree of peroxidation and enhancing the antioxidant capacity and balancing the oxidative antioxidant system. In addition, oxidative stress is also an important factor in memory dysfunction and neuronal damage, and we also performed HE staining on mouse brain tissues, which is the most intuitive way to observe the degree of brain function decline. In the present study, the HE staining results showed that the neuronal disorder, nuclei wrinkling and number of neurons were significantly reduced in the aging group compared with the control group, and dagliflozin could effectively improve the neuronal disorder and abnormal morphological changes caused by neuronal aging. This further demonstrated that dagliflozin could reduce the neuronal damage induced by subcutaneous injection of D-gal in mice.

SIRT1 is an NAD+-dependent deacetylase that plays an important role in the body by regulating downstream genes in a variety of important biological processes, such as cellular senescence and metabolic regulation. Previous studies have shown that SIRT1 protein and transcript levels increase with age in animal and human tissues, and that SIRT1 is considered a potential therapeutic target for ageing-related diseases through its protective effects on neurodegeneration and the regulation of oxidative stress [19]. Therefore, SIRT1 is considered to be a longevity factor. In addition, ageing is closely associated with impairment of mitochondrial function and its morphology [20].

Among the downstream factors of SIRT1, PGC-1α is a major regulator of mitochondrial biogenesis. Yang X *et al.* investigated the pharmacological effects

and related mechanisms of kaglegiline in adipocyte and diabetic mice models and found that kaglegiline promotes mitochondrial biosynthesis and function by activating the AMPK/SIRT1/PGC-1α signaling pathway, revealing its new therapeutic function in regulating energy homeostasis [21]. In contrast, dagliflozin was shown to exert neuroprotective effects through activation of SIRT1 signaling in a de-ovalized/D-galactose rat model of Alzheimer's disease [22]. In our study, we found that the expression of SIRT1 and PGC-1α protein was decreased in the aging group compared with the control group, which is consistent with the previous results in the literature, while the expression levels of SIRT1 and PGC-1α protein were significantly increased in the dagliflozin-treated group compared with the aging group. The above results indicated that dagliflozin could effectively reverse the D-gal-induced down-regulation of SIRT1 expression and increase the expression of PGC-1α protein, and may activate the SIRT1/PGC-1α signaling pathway to achieve the cerebral protective effect on aging mice. Regarding the effect of drug dose on aging mice, our study showed that there was no significant difference between high and low dose interventions in terms of learning memory capacity, antioxidant levels and protein expression. However, since the relationship between blood concentration and dose in mice was not measured during this experiment, we believe that more experiments are needed to confirm this conjecture. In addition, in the present study, we administered SIRT1 inhibitors to the group of dagliflozin treatment, and the results showed that a decrease in the proportion of exploration time in the original plateau quadrant could be observed in the inhibitor group compared to the treatment group, and a significant decrease in SIRT1 protein expression was observed in the inhibitor group, Unfortunately, no significant differences were observed in the inhibitor groups in terms of anti-oxidative stress and PGC-1α protein expression, which we speculate may be due to the poor inhibitory effect caused by insufficient time or lag in the administration of the SIRT1 inhibitor EX527, a limitation of this experiment [23]. Overall, the results of the study further support the hypothesis that dagliflozin may achieve cerebral protection and improve memory function in aging mice through the SIRT1/PGC-1α signaling pathway.

In conclusion, this study suggests that dagliflozin can delay organ aging, improve the learning and memory ability of aging mice, and exert antioxidant effects, probably through upregulating the SIRT1/PGC-1α signaling pathway. However, our study still has some limitations and needs further evaluation. Firstly, to verify the cerebral protective effect of SGLT2 inhibitors in aging mice, we used dagliflozin by gavage as the intervention drug of choice, but dagliflozin may not be as effective as chemically pure dagliflozin by intraperitoneal injection in mice, and the use of dagliflozin as a representative of SGLT2 inhibitors is not comprehensive. Secondly, this study only explored the cerebral protective effects and mechanisms of SGLT2 inhibitors in an *in vivo* trial, but it would be more convincing to further validate the results in an *in vitro* trial. In the inhibitor group, we used EX527 intraperitoneally, which may also be ineffective. Thirdly, this experiment was designed to investigate the correlation between dose and ef-

ficacy, but the relationship between blood levels and dose in mice was not measured during the experiment [24]. Fourth, although we explored the protective effects of dagliflozin in terms of behaviour, oxidative stress and pathology, there are other responses involved in the ageing process, including inflammation and apoptosis. We did not perform further evaluation in this study. Therefore, if experimental conditions allow, it would be more ideal to establish a SIRT1 knockout senescence mouse model by gene knockout [25] and to perform the necessary blood levels of SGLT2 inhibitors to further validate the effectiveness of our findings. In addition, other downstream signaling pathways activated by SIRT1 need to be further explored, and the effects of SGLT2 inhibitors on other types of aging phenotypes need to be further explored [26] [27].

5. Conclusion

SGLT2 inhibitor had some delaying effects on aging in the D-gal-induced aging mouse model, including the improvement of aging-induced atrophy of vital organs and the absence of hypoglycemia. At the same time, it exerted a protective effect on the onset of oxidative stress in the brain, and also improved the morphological and structural changes of brain pathology induced by aging mice, which may be related to its ability to reduce oxidative stress and balance the antioxidant system. In addition, we found that the neuroprotective effect of SGLT2 inhibitor on D-gal-induced aging mice may be related to the activation of SIRT1/PGC-1α signaling pathway.

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Conflicts of Interest

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

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