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Exploring the effect of Polygonatum cyrtonema polysaccharide DPC1 on D-galactose-induced mouse aging model based on AMPK/SIRT1 pathway

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Abstract: Objective: The aim of this study was to elucidate the ameliorating mechanism of Polygonatum cyrtonema polysaccharide (DPC1) on D-galactose (D-gal)-induced oxidative stress-induced senescence in mice and to study the mechanism. Design: A model of senescence was established using D-gal (0.12 g/kg at 8 weeks) induced in ICR mice with concomitant gavage administration (0.15, 0.30, or 0.60 g/kg) of DPC1. Measurement of antioxidant indices in mouse tissues and plasma. Observation of pathological changes in mouse brain tissue. The mitochondrial adenosine triphosphate level and mitochondrial membrane potential of the mouse brain tissue samples were then determined. Molecular biotechnologies such as Western blot was used to investigate the AMPK/SIRT1 signaling pathway and related proteins. Results: Oral administration of DPC1 alleviated D-gal-induced oxidative damage in mice, such as the reduction of malondialdehyde (MDA) content and monoamine oxidase (MAO) activity level, and the increase of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) activity levels. At the same time, DPC1 improved the pathological changes of mouse brain tissue. In addition, DPC1 had a restorative effect on both mitochondrial membrane potential (MMP) and ATP content in mouse brain tissues. DPC1-treated group was able to AMP-activated protein kinase/silent information regulator 1 (AMPK/SIRT1) pathway and regulate oxidative enzymes by inhibiting the ratio of Acety-Forkhead Box O1/Forkhead Box O1 (Ac-FoxO1/FoxO1), thereby restoring the pro-oxidant/antioxidant balance. It also reduced P53 and P21 expression. Conclusions: Our results suggest that DPC1 inhibits oxidative stress-induced senescence by activating the AMPK/SIRT1 pathway.

Keywords: Polygonatum cyrtonema polysaccharide; senescence; oxidative; AMPK; SIRT1

1. Introduction

Aging is a complex natural phenomenon that progresses over time and is characterized by degenerative changes in the organism's structure and a decline in physical functions. In the twenty-first century, the global population has en-

tered an aging phase, which has affected all areas of society, making aging is a major worldwide challenge. To date, nearly 100 aging theories have been proposed, including the telomere theory^[1], oxidative stress theory^[2], free radical theory^[3], mitochondrial damage theory^[4], and genetic ageing theory^[5]. This indicates the urgency of human research on aging.

The rhizomes of *Polygonatum kingianum*, *P. sibiricum*, or *P. cyrtoneuma* make up *Polygonatum*^[6]. It is a traditional Chinese food and medicine that is a member of the asparagus family. *Polygonatum* polysaccharides are a major bioactive component of *P. cyrtoneuma*^[7]. Studies have reported that *P. cyrtoneuma* polysaccharides have a number of biological activities such as antioxidant, anti-aging, immune-enhancing, antimicrobial, and anti-inflammatory^[8] but are widely recognized primarily for their antiaging activity. Modern medicine has confirmed that *Polygonatum* polysaccharides can exert antiaging effects by inducing anti-oxidative stress, thereby improving mitochondrial function and promoting telomerase activity^[9-11]. In addition, *Polygonatum* polysaccharides play ameliorating roles in many aging-related diseases, including the prevention of Alzheimer's disease, osteoporosis, and the treatment of diabetes mellitus^[12-14].

AMPK (AMP-activated protein kinase) is a cellular energy sensor that controls a number of processes, such as autophagy, mitochondrial homeostasis, and the metabolism of glucose, proteins, and fats.^[15] AMPK activates SIRT1, which has been shown to prolong lifespan and influence the onset and course of aging^[16,17]. Therefore, the activation pair of AMPK/SIRT1 could be a therapeutic approach to slow down the aging process.

Our group has previously isolated and purified DPC1 from dried *P. cyrtoneuma*^[18]. The structure indicates that DPC1 is a novel fructan similar to agavin-type fructans^[19]. Moreover, DPC1 has a wide range of applications, including the creation of novel medications and biologics, and can be highly homogeneously prepared and purified. However, studies on the pharmacological effects of DPC1 are still limited, its effects on senescence and aging have not been investigated, and its aging-related mechanisms remain to be clarified.

This study established a D-galactose (D-gal)-induced aging mouse model using AMPK/SIRT1 as the entry point. The AMPK/SIRT1 signaling pathway and related proteins were investigated using molecular biotechnology techniques such as western blot and quantitative polymerase chain reaction (qPCR) to examine in depth the antiaging pharmacological effects of DPC1 and the associated mechanisms of action. Meanwhile, the mechanism of DPC1 in organismal aging and the effect of DPC1 on mitochondria in brain tissues of aging mice were investigated in depth. This study is of crucial academic value and clinical significance as a reference for further research on the usefulness of DPC1 for delaying organismal aging.

2. Materials and methods

2.1 Chemicals and reagents

P. cyrtoneura rhizomes were sourced from Yibin, Sichuan Province, China and authenticated by Dr. Fei Long from Chengdu University of Traditional Chinese Medicine. In addition, the voucher samples are stored in the resource reserve of the School of Pharmacy of Chengdu University of Traditional Chinese Medicine. Diethylethanolamine (DEAE) was purchased from Beijing Ruida Henghui Biotechnology Co., Ltd. (Beijing, China). D-gal was purchased from Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China). The ether was purchased from Nanjing Reagent Co., Ltd. (Nanjing, China). The superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), monoamine oxidase (MAO), malondialdehyde (MDA), nicotinamide adenine dinucleotide (NAD), mitochondrial membrane potential assay kit (JC-1), adenosine triphosphate (ATP) content assay, and β -galactosidase (β -gal) kit were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). NAMPT (DF6059), SIRT1 (AF3474), p-P53 (AF3075), P21 (AF6290), p-AMPK (AF3423), Ac-FoxO1 (AF2305), FoxO1 (BF0211), and β -actin (AF7018) antibodies were purchased from Affinity Biosciences Group Ltd. (Jiangsu, China).

2.2 Preparation and structural characterization of DPC1

In accordance with our previous research, the DPC1 preparation method was based on Zhang's study^[18]. Dried roots of *P. cyrtoneura* were crushed and extracted twice in boiling water. The polysaccharide was precipitated with graded ethanol, and 80% of the ethanol precipitated crude polysaccharide was taken. The crude polysaccharide was purified on a DEAE-52 fiber column with ultrapure water as mobile phase at a flow rate of 1 ml/min. The elution curve was plotted by phenol-sulfuric acid method, and the absorbance was measured at 490 nm, and the elution curves were combined at the sharp peak (**Figure 1**). Finally, DPC1 was obtained by concentration and freeze-drying. The average molecular weight (Mw) of DPC1 was 3117 Da as determined by high performance gel permeation chromatography (HPGPC).

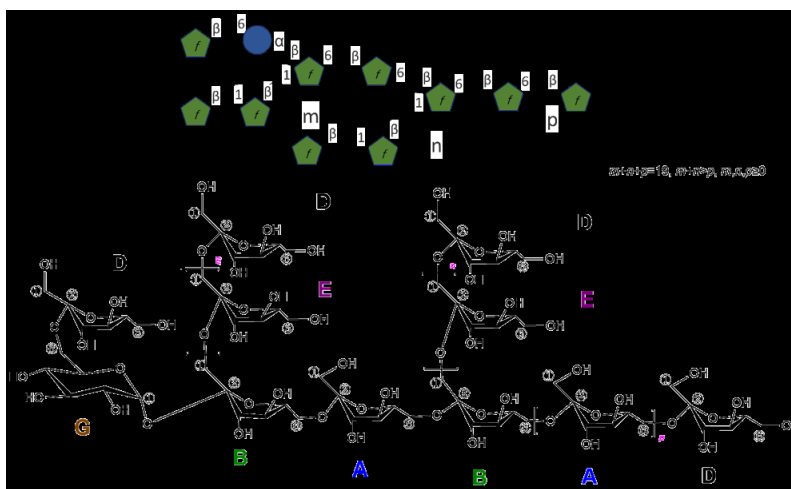


Figure 1. Chemical structure of DPC1.

2.3 Animal experiments

Institute for Cancer Research mice (male, 8 weeks old, 18–22 g, license No. SCXK [Jing] 2019-0010) were purchased from Spivey Biotechnology Co. (Beijing, China). They were housed in 12-h alternating light and dark environments, with the room temperature controlled between 20°C and 22°C, and the relative humidity controlled at 40% to 50%. Before the experiment, they were fed, watered ad libitum, and acclimatized to feeding for 1 week. All animal experiments were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. All study protocols were approved by the Chengdu University of Traditional Chinese Medicine Committee (approval No. 2024127).

The animals were randomly divided into 6 groups, with 10 animals in each group ($n = 10$). The subgroups were as follows: normal control group (Control), D-gal model group (D-gal), Control+DPC1 group (Con-DPC1 0.60 g/kg), D-gal+DPC1 low-dose group (D-gal+DPC1 0.15 g/kg), D-gal+DPC1 moderate-dose group (D-gal+DPC1 0.30 g/kg), and D-gal+DPC1 high-dose group (D-gal+DPC1 0.60 g/kg).

In the normal control group, saline was administered via intraperitoneal injection and oral gavage. In the Control+DPC1 group, saline was injected intraperitoneally, and DPC1 was administered via gavage (0.60 g/kg). Except for the normal control and Control+DPC1 groups, D-gal (5%, 0.9% saline, 0.12 g/kg) was injected intraperitoneally daily to establish an aging model. D-gal+DPC1 was administered via gavage in the D-gal+DPC1 low-, medium-, and high-dose groups (0.15, 0.3, and 0.6 g/kg, respectively).

Each group was treated once daily for 8 weeks, and the reagent doses were adjusted according to the animals' body weights (**Figure 2**).

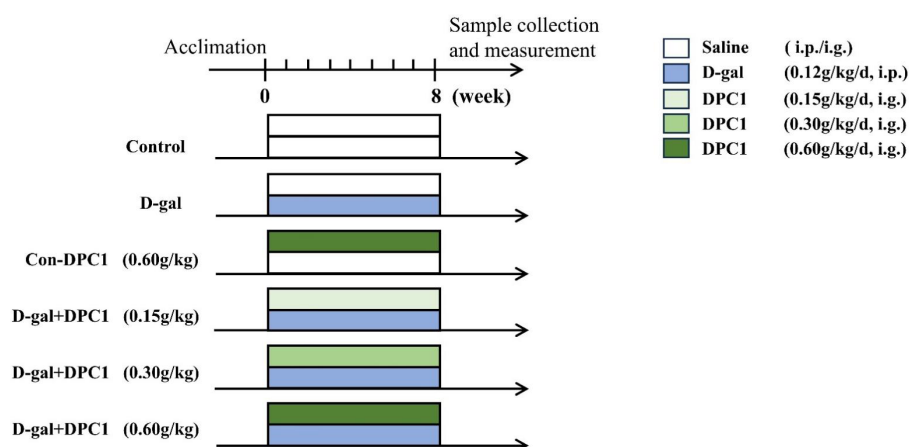


Figure 2. Schematic diagram of the experimental design.

2.4 Antioxidant measurements in serum and brain tissue samples

First, 10 μ L/g of 0.3% pentobarbital sodium was injected intraperitoneally to anesthetize mice, and then blood was collected from the eye. After blood collection, the mice were decapitated quickly in a clean and independent environment after confirming their death. Blood was allowed to stand for 1 hour and then centrifuged for 10 min (4°C and 2000 rpm). The supernatant was also collected. Brain tissue was taken and ground with 9 times homogenizing medium. The ground solution was then centrifuged for 10 min (4°C and 4000 rpm), and the supernatant was collected for biochemical analysis. The MDA, SOD, CAT, and MAO contents, and the GSH-PX activities in the serum and brain tissue samples were determined using the respective assay kits, in accordance with the manufacturer's instructions.

2.5 Hematoxylin and eosin staining

Fresh brain tissue specimens were obtained from the mice by routine surgery, fixed in 4% paraformaldehyde for more than 24 h, dehydrated, placed in wax for embedding, cut into 3- μ m sections, and stained. Histopathological changes in the sections were observed with a section scanner, and images were acquired for analysis.

2.6 Immunofluorescence staining

Paraffin sections of the mouse brain tissue samples were deparaffinized in water, antigenically repaired, blocked from endogenous peroxidase, and closed. Primary antibodies to p53, Sirt1, and β -gal were added for staining. Secondary antibodies were then added and incubated away from light. Finally, DAPI was used to restain the cell nuclei. The sections were observed under a fluorescence microscope (SOPTOP, CX40, Chongqing, China), and images were captured using Panoramic SCAN II (3DHISTECH Kft, Shanghai, China).

2.7 Measurement of β -gal content

Following the manufacturer's instructions, a β -gal assay kit was used to measure the amount of β -gal present in the mouse brain tissue samples.

2.8 Measurement of ATP content

In accordance with the instructions, the ATP levels in the mouse brain tissue samples were measured using an ATP assay kit.

2.9 Measurement of NAD⁺ activity

In accordance with the manufacturer's instructions, NAD⁺ levels in mouse brain tissue were measured using a NAD-dependent malate dehydrogenase activity assay kit.

2.10 Mitochondrial membrane potential assay (JC-1)

Mitochondrial membrane potential was measured using the mitochondria-specific dual probe JC-1. Fresh mouse brain tissue samples were made into a suspension and incubated with the JC-1 working solution at 37°C for 20 min. The cells were washed once with precooled $\times$ JC-1 assay buffer and analyzed with flow cytometry (Beckman Coulter, CytoFLEX, Guangzhou, China), using a 488-nm laser, fluorescein isothiocyanate, and phycoerythrin channel reception).

2.11 Western blot analysis

Total protein extraction was performed on the brain tissue samples, and then the protein concentration was measured using the bicinchoninic acid method. Protein samples were prepared, loaded on SDS-PAGE gels for electrophoresis, and transferred to polyvinylidene fluoride membranes. The isolated proteins were incubated with 5% skimmed milk (TBST dispensed) at room temperature and closed for 1 h before the primary antibodies were added. On the next day, the membrane was washed three times with TBST at room temperature and then incubated by adding the secondary antibody. Finally, the images were captured under chemiluminescent imaging (Shanghai Qinxian Scientific Instrument Co., ChemiScope 6100, China). The system uses an ECL chemiluminescent substrate (Biosharp, BL523B, China) and analyzed on a grayscale with chemical analysis software.

2.12 Quantitative PCR

Messenger RNA expression levels of the P53, AMPK, and SIRT1 genes were determined using qPCR. Total RNA was extracted from the mouse brain tissue samples, and RNA concentration and purity were detected using a nucleic acid analyzer (KAIAO, K2800, China). The extracted RNA was reverse transcribed into cDNA accordingly, and the RNA expression was detected on a fluorescence qPCR instrument (BIORAD, CFX Connect, China). The relative RNA expression level was calculated using the $2^{-\Delta\Delta CT}$ method, and the house-keeping β -actin gene was used as an internal control for the assay. By using the

Oligo 7 primer analysis software for primer design, all primers were synthesized by Shanghai Sangong Bioengineering Co. The primer sequences are described in **Table 1**.

Table 1. Primer Sequences

Gene	For sequence	Rev sequence
β -Actin	GTGCTATGTTGCTCTAGACTTCG	ATGCCACAGGATTCCATACC
P53	CGCCGACCTATCCTTACCATCATC	CTCCCAGGGCAGGCACAAAC
AMPK	CGAGTGTTGCGAGGAGGAGGTC	GTGGGCTGGTTGCTAGGTAGAAATC
SIRT1	GTGGCAGTAACAGTGACAGTGG	TCCAGATCCTCCAGCACATTCTG

2.13 Statistical analysis

All data were expressed as three or more replications. The SPSS 25.0 medical statistical software was used to analyze the data. The experimental data were expressed as mean $\pm$ standard deviation (SD), and one-way analysis of variance was used to compare the groups. The groups with variances in agreement were tested using the LSD method, and those with variances that were not in agreement were detected using the Tamhane T2 method. The t test was used to test two independent samples, with p values < 0.05 indicating statistically significant differences. The Origin 2022 software was used to draw the graphs. All experiments were performed in triplicate.

3. Results and discussion

3.1. Validation of the D-gal-induced aging mouse model

To evaluate the protective effects of DPC1 against aging in mice, the D-gal mouse model of aging was created. The β -gal content of mouse brain tissue samples was detected with the kit and stained using immunofluorescence staining for β -gal in mouse brain tissue. The experimental results showed that the β -gal content in the brain tissues of the mice in the model group was significantly higher than that in the normal control group ($p < 0.01$) (**Figure 3a**). In addition, the DPC1-treated groups (0.15, 0.30, and 0.60 g/kg) showed significantly suppressed D-gal-induced increase in β -gal content compared with the model group. These results indicate the successful establishment of an aging mouse model and suggest that DPC1 effectively attenuates the senescent effects of D-gal (**Figure 3b,c**).

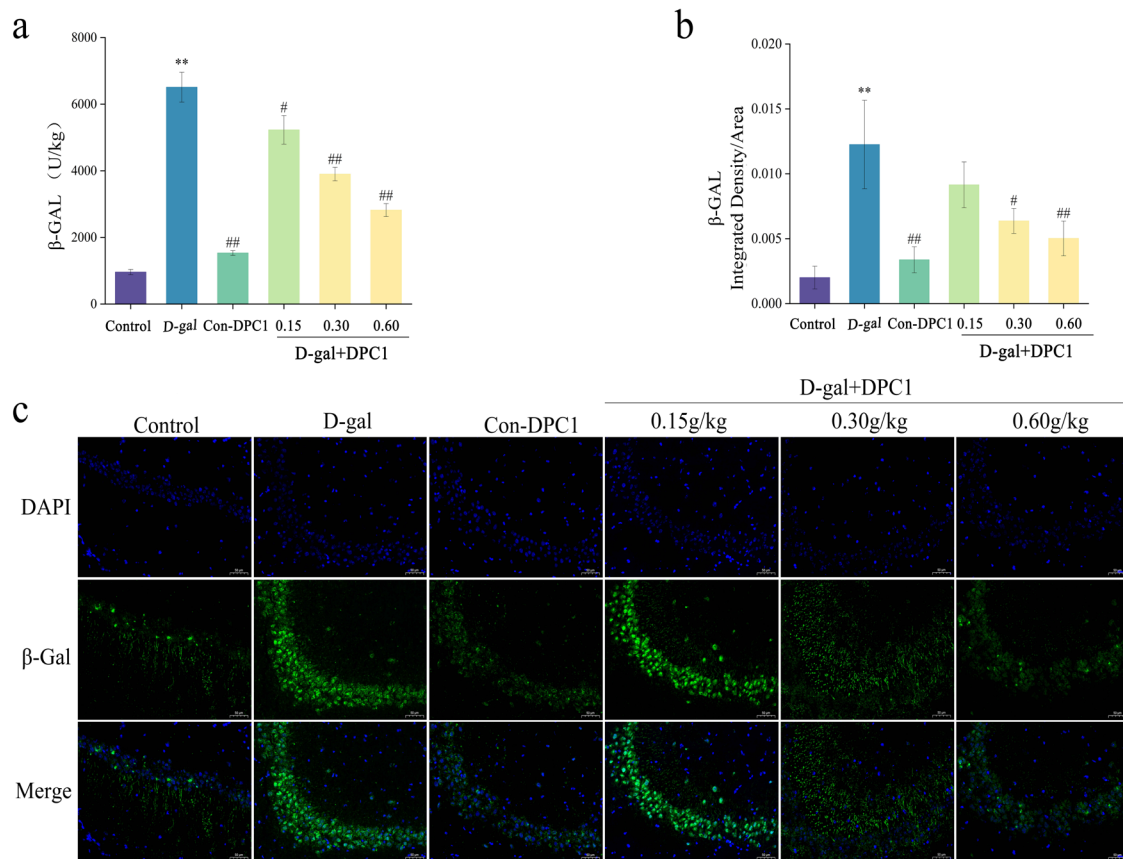


Figure 3. Effect of DPC1 on oxidative stress in the aging mouse model: (a) β -Gal level in a mouse brain tissue sample; (b) Quantitative analysis of β -gal staining ($n = 3$); (c) Immunofluorescence staining of β -Gal in a mouse brain tissue sample (original magnification, $\times 400$; scale bar, $50 \mu\text{m}$). * $p < 0.05$, ** $p < 0.01$, compared with the normal control group. # $p < 0.05$, ## $p < 0.01$, compared with the D-gal model group.

3.2. DPC1 protects against oxidative stress senescence in the D-gal-induced aging mouse model

We then investigated the alleviating effect of DPC1 on oxidative stress in the aging mouse model. The results (Figure 4) showed that MDA content and MAO activity levels in the brain tissue and serum samples from the D-gal-treated mice in the D-gal model group were significantly higher than those in the normal control group ($p < 0.01$). However, the DPC1-treated groups (0.15, 0.30 and 0.60 g/kg) significantly inhibited the D-gal-induced increase in MDA content and MAO activity compared to the D-gal model group ($p < 0.01$). Moreover, the SOD, CAT, and GSH-PX activity levels in the brain tissue and serum samples from the D-gal-treated mice in the model group were significantly lower than those in the normal control group ($p < 0.01$). The DPC1-treated groups (0.15, 0.30 and 0.60 g/kg) showed a significant ($p < 0.01$) and gradient-dependent increase in SOD, CAT and GSH-PX activities compared to the D-gal model group ($p < 0.01$) in a gradient-dependent manner. These results indicate that DPC1 administration inhibited D-gal-induced oxidative stress in the mice, as evidenced by the

decrease in MDA content and MAO activity levels, and the increase in the SOD, CAT, and GSH-PX activity levels.

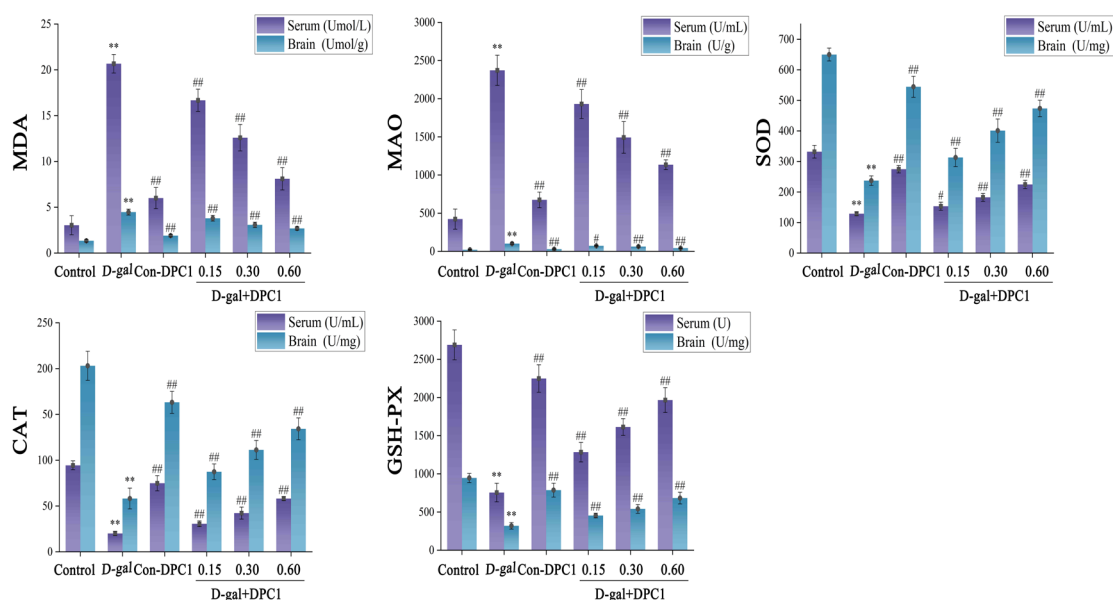


Figure 4. Effects of DPC1 on the MDA, MAO, SOD, CAT, and GSH-PX levels in the brain and serum samples from D-gal-induced aging mouse model. Results are expressed as mean $\pm$ SE (n = 5). *p < 0.05, **p < 0.01, compared with the normal control group. #p < 0.05, ##p < 0.01, compared with the D-gal model group.

3.3. Effect of DPC1 on the histopathology of the aging brains of D-gal-induced aging mouse model

We performed hematoxylin and eosin (H&E) staining to observe whether DPC1 could attenuate the pathological changes in aging mouse brain tissues. The H&E staining results showed that the hippocampus of the mice in the normal control group had an intact tissue structure, neatly arranged neuronal cells, clear cell differentiation, and no apparent pathological damage. By contrast, the neuronal cells in the brain of the mice in the D-gal model group were degenerated, wrinkled, and deeply stained, and the pericellular gap was widened. Meanwhile, no significant change was observed in the DPC1 low-dose group. However, compared with the model group, the DPC1 medium-dose group showed a complete tissue structure, neatly arranged neuronal cells, and small vacuole formation in the brain of the mice. The brain tissues of the mice in the high-dose DPC1 group were structurally intact, with neatly arranged neuronal cells and clear cell demarcation, with no obvious pathological damage. These results suggest that DPC1 prevents D-gal damage to brain tissue (**Figure 5**).

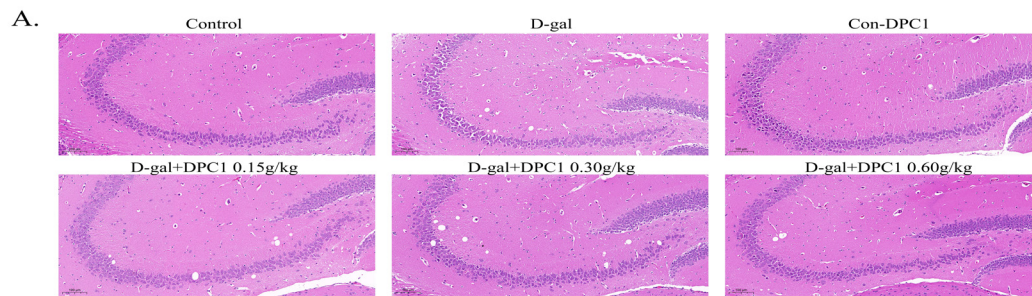


Figure 5. Hematoxylin and eosin (H&E) staining of the hippocampal region of the mouse brain tissue, showing round and intact normal cells, with clearly visible nuclei. By contrast, the degenerated cells are more heavily stained (pyknosis) and have blurred nucleocytoplasmic borders ($n = 3$; original magnification, $\times 200$; scale bar, $100 \mu\text{m}$).

3.4. Effect of DPC1 on mitochondrial function in the D-gal-induced aging mouse model

Meanwhile, we tested the effect of DPC1 on mitochondrial function. JC-1 levels were detected using a mitochondrial potential assay kit. The results showed that the mitochondrial membrane potential was reduced in the D-gal model group compared with the normal control group, and the difference was significant ($p < 0.01$). Compared with the D-gal model group, the DPC1-treated groups (0.30 and 0.60 g/kg) showed increased membrane potential, and the difference was significant ($p < 0.01$). ATP level and mitochondrial membrane potential were measured. The results (Figure 6a) showed that the ATP content was significantly reduced in the D-gal model group compared with the normal control group ($p < 0.01$). Compared with the normal group, the D-gal model group showed abnormal energy metabolism. However, the DPC1-treated groups (0.15, 0.30, and 0.60 g/kg) showed significantly increased D-gal-induced reduction in ATP content ($p < 0.01$) in a gradient-dependent manner. These results indicate that DPC1 can increase ATP production, stabilize mitochondrial membrane potential, reduce mitochondrial damage, and protect the mitochondrial function in mouse brain tissue (Figure 6b, c).

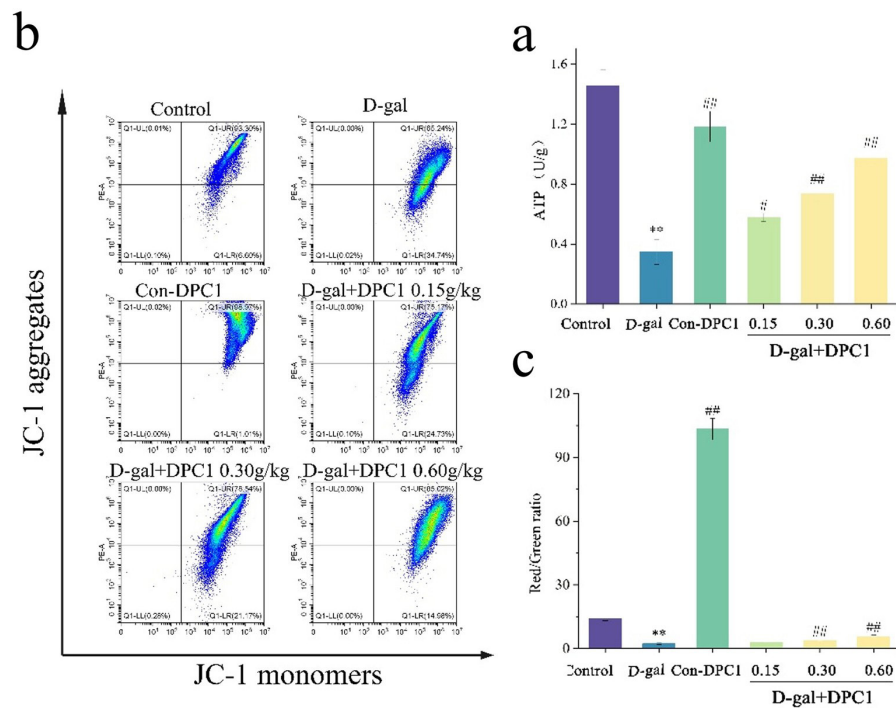


Figure 6. Effect of DPC1 on the mitochondrial function of the aging mouse model: (a) Measurement of mitochondrial ATP levels in mouse brain tissue samples (n = 5); (b) Effect of DPC1 on mitochondrial membrane potential in mouse brain tissue; (c) The ratio of JC-1 red to green fluorescence (n = 4). *p < 0.05, **p < 0.01, compared with the normal control group. #p < 0.05, ##p < 0.01, compared with the D-gal model group.

3.5. Determination of NAD⁺ content

We determined whether DPC1 could affect the content of NAD⁺, the only coenzyme of SIRT protein. The NAD⁺ content of the D-gal model group was significantly lower than that of the normal control group (p < 0.01), according to the results, and it was significantly higher than that of the DPC1-treated groups (0.15, 0.30, and 0.60 g/kg) than it was of the D-gal model group (p < 0.01). The results indicated that DPC1 had a promoting effect on the recovery of NAD⁺ levels (Figure 7).

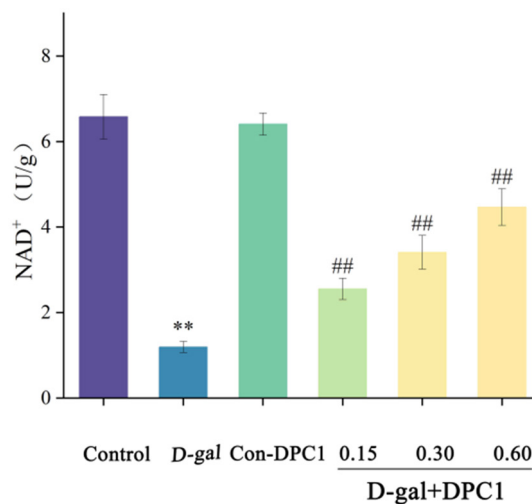


Figure 7. Determination of NAD⁺ content in the brain tissue samples of senescent mice (n = 5). *p < 0.05, **p < 0.01, compared with the normal control group. #p < 0.05, ##p < 0.01, compared with the D-gal model group.

3.6 Effect of DPC1 on the expression of proteins related to AMPK/SIRT 1 signaling pathway in aging mice

We focused on the AMPK/SIRT1 signaling pathway, which is closely related to aging and the mitochondria. The following experiments were performed to demonstrate that DPC1 can activate the AMPK/SIRT1 signaling pathway (**Figure 8** and **Figure 9**).

Through immunofluorescence staining, we observed that the P53 expression level in the brain tissue of the mice in the D-gal model group was significantly higher than that in the normal control group and that the DPC1 intervention alleviated these changes. We then demonstrated in Western Blot experiments that the p-P53 and P21 expressions levels were significantly higher in the brain tissues of the mice in the D-gal model group than in those in the normal control group, and were significantly reduced in the DPC1-treated groups (0.15, 0.30, and 0.60 g/kg) compared with the D-gal model group. These data demonstrate that DPC1 ameliorates D-gal-induced senescence in mice. Next, we analyzed the effect of DPC1 on AMPK/SIRT1 activation in mice. In the immunofluorescence staining, we observed that the SIRT1 expressions levels in the brain tissue samples of the mice in the D-gal model group were significantly lower than those in the normal control group, and the DPC1 intervention significantly increased the D-gal-induced reduction in SIRT1 expressions levels in a gradient-dependent manner. We also demonstrated by western blot analysis experiments that the expression levels of the p-AMPK, SIRT1, and NAMPT proteins were significantly reduced in the model group compared with the normal control group (p < 0.01). The DPC1 high-dose group showed significantly increased p-AMPK, SIRT1, and NAMPT protein levels (p < 0.05) and significantly reduced Ac-FoxO1/FoxO1 protein lev-

els ($p < 0.05$). These results suggest that DPC1 may activate the AMPK/SIRT1 signaling pathway and regulate oxidative stress, thereby delaying aging.

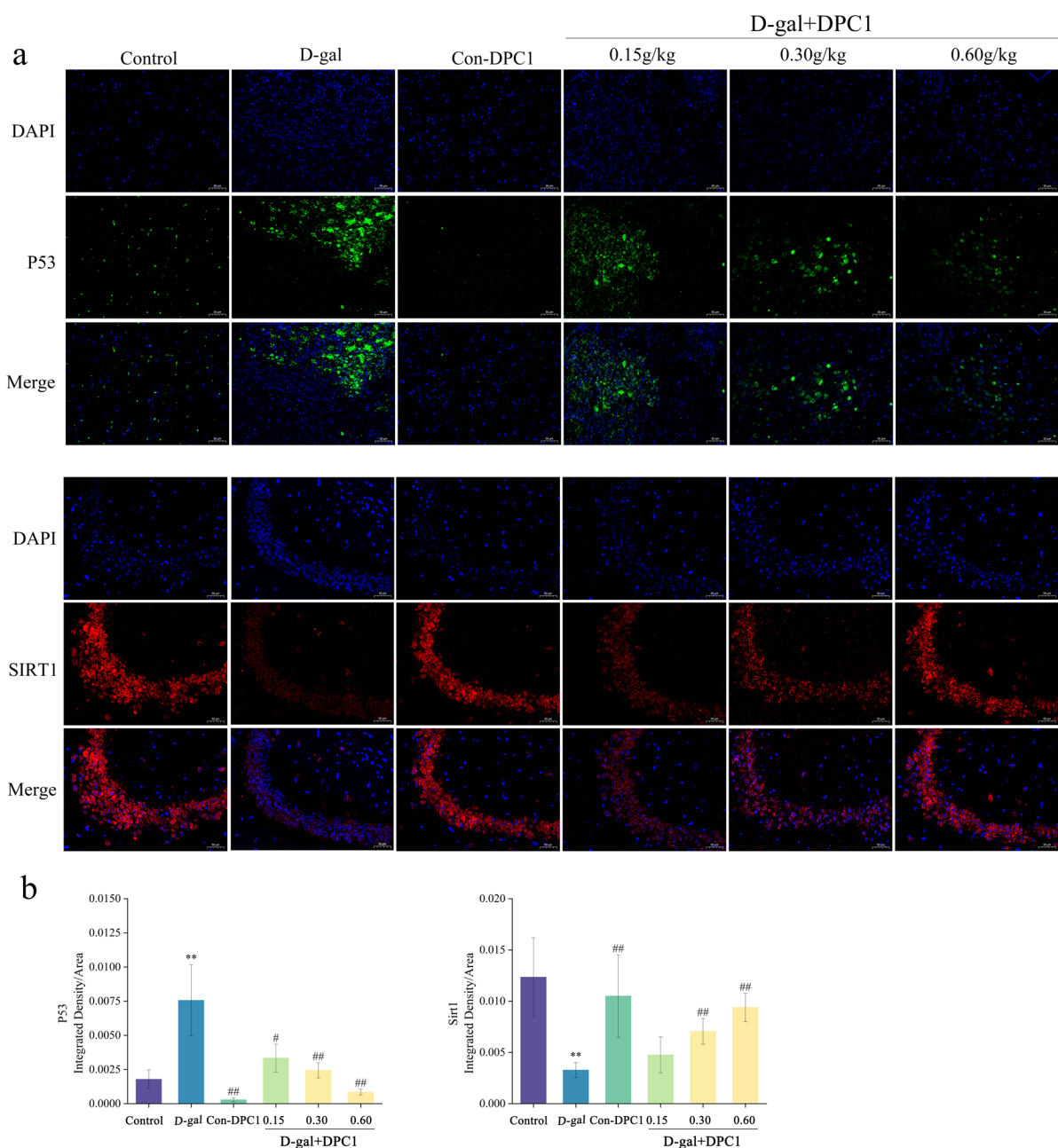


Figure 8. Fluorescent staining in mouse brain tissue: (a) Immunofluorescence staining of P53 and SIRT1 in mouse brain tissue samples (original magnification, $\times 400$; scale bar, 50 μm); (b) Quantitative analyses of the P53 and SIRT1 staining results ($n = 3$). * $p < 0.05$, ** $p < 0.01$, compared with the normal control group. # $p < 0.05$, ## $p < 0.01$, compared with the D-gal model group.

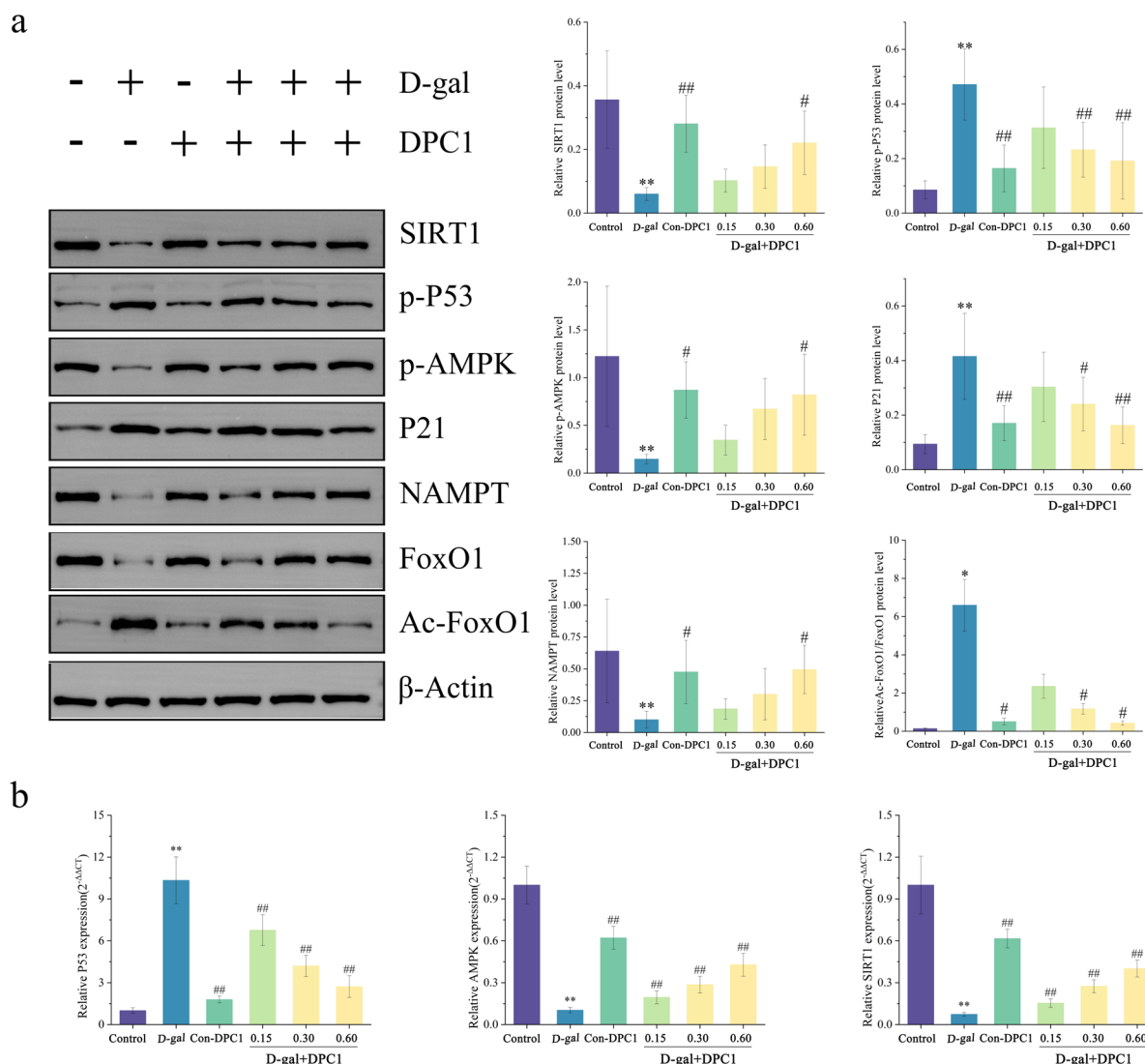


Figure 9. DPC1 slows down senescence by activating the AMPK/SIRT1 pathway: (a) Protein blotting showing the expressions of the senescence-associated proteins SIRT1, p-P53, p-AMPK, P21, NAMPT, FoxO1, and Ac-FoxO1 in the brain tissue samples of senescent mice. Quantification of SIRT1, p-P53, p-AMPK, P21, NAMPT, FoxO1, and Ac-FoxO1 protein expressions in mouse brain tissue samples by protein blotting. All data are expressed as mean $\pm$ SD ($n = 3$); (b) mRNA expression levels of P53, AMPK, and SIRT1 detected by qPCR in the brain tissue samples of senescent mice. * $p < 0.05$, ** $p < 0.01$, compared with the normal control group. # $p < 0.05$, ## $p < 0.01$, compared with the D-gal model group.

3.7 Effects of DPC1 on the AMPK, SIRT1, and P53 mRNA expression levels detected by qPCR in the D-gal-induced aging mouse model

We again used qPCR experiments for further verification. Fortunately, consistency with the previous results was maintained. Compared with the normal control group, the D-gal model group showed decreased mRNA expression levels of AMPK and SIRT1 ($p < 0.01$), and increased mRNA expression level of P53. Compared with the D-gal model group, the DPC1-treated groups (0.15, 0.30, and 0.60 g/kg) showed increased mRNA expression levels of AMPK and SIRT1 ($p < 0.01$). The mRNA expression level of P53 was decreased ($p < 0.01$) with gradient dependence. The results suggest that DPC1 can protect the mitochondrial function through the AMPK/SIRT1 signaling pathway (**Figure 9b**).

4. Discussion

We investigated the effect of DPC1 on the D-gal-induced senescence mouse model, and the results showed that DPC1 correlated with the AMPK pathway. Long-term repeated injections of D gal will enhance oxidative stress, reduce the activity of antioxidant enzymes in mice, cause accelerated aging in mice, and also cause degenerative changes in many tissues and organs, especially brain tissue^[20-22]. In addition, increased oxidative stress can damage mitochondria and lead to mitochondrial dysfunction in the long term^[23, 24]. The results of the study showed that D-gal can induce an increase in the content of β -gal in mouse brain tissue, an increase in the content of MDA and MAO activity in serum and brain tissue, which are related to oxidative stress, and a decrease in the activity levels of SOD, CAT and GSH-PH. The cells in the mouse brain tissue were shriveled and degenerated, the mitochondrial membrane potential of the brain tissue decreased, ATP production decreased, and the expression of aging-related proteins P53 and P21 increased. Administration of DPC1 reversed these phenomena. It can enhance the activity of antioxidant enzymes in mice, improve oxidative damage caused by D-gal in mice, and restore their physiological functions. It has a protective effect on the brain tissue of aging mice, significantly reducing the content of β -Gal and the expression of aging markers in brain tissue. In addition, DPC1 can restore mitochondrial membrane potential, upregulate ATP content, and improve mitochondrial function. Therefore, we further investigated the AMPK/SIRT1-FoxO1 signaling pathway, which is closely related to both aging and mitochondria.

The AMPK/SIRT1-FoxO1 signaling pathway is closely related to oxidative stress-induced senescence and plays a very important role in the aging and senescence process^[25-27]. AMPK is a major regulator of intracellular energy metabolic homeostasis and a key regulator of mitochondrial biosynthesis during energy deficiency^[28]. SIRT1 is a class of histone deacetylases with NAD⁺ as a cofactor, which regulates physiopathological processes such as inflammation, metabolism,

cell proliferation, apoptosis, and aging through substrate deacetylation^[29-32]. In addition, SIRT1 interacts with various senescence signaling molecules, such as FoxO1 and P53, to regulate oxidative stress, apoptosis, and autophagy^[33, 34]. NAMPT, the rate-limiting enzyme that redefines the synthesis of NAD⁺, catalyzes the conversion of nicotinamide to nicotinamide mononucleotide, which plays a role in regulating intracellular NAD⁺ levels. AMPK affects NAD⁺ levels by regulating NAMPT expression, which in turn regulates sirt1^[35]. Western blot results showed that the protein levels of p-AMPK, NAMPT and SIRT1 were significantly increased in the brain tissues of DPC1-administered mice compared with those of the model group. activation of AMPK is usually determined by p-AMPK, and DPC1 activates the AMPK/SIRT1 signaling pathway.

Another study showed that the AMPK/SIRT1 pathway has a significant regulatory effect on oxidative stress. FoxO1 is an important deacetylated substrate of AMPK/SIRT1, and translational modifications such as acetylation/deacetylation regulate its activity^[36, 37]. Studies have confirmed that the expression levels of antioxidant enzymes such as CAT and SOD are upregulated after the transcriptional activity of FoxO1 is enhanced by deacetylation, thereby resisting oxidative stress^[38, 39]. As the transcriptional activity of FoxO1 is regulated by its own acetylation/deacetylation status, we further detected the Ac-FoxO1 protein expression to observe the change in Ac-FoxO1/FoxO1 ratio. The results show that the model group significantly increased the Ac-FoxO1/FoxO1 ratio, whereas DPC1 inhibited this promotion. Taken together, these results indicate that DPC1 may inhibit FoxO1 acetylation through the AMPK/SIRT1 signaling pathway, thereby regulating oxidative enzymes, restoring pro-oxidant/antioxidant balance, restoring mitochondrial function, and resisting D-galactose-induced senescence results to achieve a delayed aging effect.

DPC1 is a fructan similar to agavin-type fructan. Agavin-type fructan is a fructan that regulates antioxidant responses and inhibits stress-related oxidative stress. It can be used to prevent and treat various diseases, including colorectal cancer, overweight, obesity, diabetes and osteoporosis, and has brain-protective properties^[40]. Agavin-type fructans, which include inulin or agave inulin, have been widely used in functional foods in recent years. They are also known as prebiotics and can regulate the intestinal flora. Previous studies have also found that DPC1, as a prebiotic, promotes the growth of probiotics. Many probiotics have been shown to activate AMPK activity . We speculate that DPC1, as a new type of prebiotic, can regulate aging through the intestinal flora. We will use other models in the future to further demonstrate this.

5. Conclusion

In summary, this study demonstrates that the novel Chinese medicine *P. cyrtonema*-derived fructan DPC1 has a significant anti-aging effect, which may inhibit FoxO1 acetylation through the AMPK/SIRT1 signaling pathway, thereby

regulating oxidases, restoring the pro-oxidative/antioxidative balance, alleviating D-gal-induced aging and oxidative stress, improving mitochondrial function and brain tissue damage, and achieving an anti-aging effect. This is the first study of DPC1 on aging and related pathways, which strengthens the scientific basis of *P. cyrtonema*'s anti-aging effects. The careful mining of downstream target genes and pathways that produce therapeutic effects has filled the gaps in mechanism research. These findings suggest that DPC1 may be a candidate for the prevention or treatment of aging-related diseases. This study provides a theoretical basis for the development of DPC1 as a functional food additive and pharmaceutical ingredient, and is of great significance for promoting the in-depth industrial development of DPC1.

Author contributions:

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Conflict of interest:

All authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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