

Original Research

Protective Role of Taraxasterol against Cardiovascular Aging and Aging-Induced Desensitization of Insulin Signaling

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Abstract

Background: Cardiovascular disease (CVD) has become one of the leading causes of death and disability worldwide, and its incidence continues to increase because of an aging population. Studies have shown that the function of cardiomyocytes decreases during aging, leading to changes in the functional and structural integrity of the heart, ultimately resulting in CVD. The decrease in the number of functional cardiomyocytes has a negative impact on cardiac function; thus, myocardial aging is one of the main factors that causes heart-related diseases (such as CVD). Therefore, alleviating cardiac aging is one of the main ways of treating aging-related cardiac diseases. In this study, we evaluated the potential effect of taraxasterol on myocardial aging. **Methods:** The effect of taraxasterol on the aging of cardiomyocytes was analyzed *in vivo* and *in vitro* using a D-galactose treatment mouse model of cardiomyocyte senescence. Furthermore, the effect of taraxasterol on aging-induced desensitization of insulin signaling was also evaluated. **Results:** The experimental results indicated that taraxasterol could reduce cardiomyocyte senescence, which was evaluated using Sa- β -gal staining and senescence-related marker molecules (e.g., p16 and p21). We found that taraxasterol could significantly alleviate cardiomyocyte senescence in the *in vitro* cell model. Furthermore, we found that taraxasterol had the potential to alleviate cardiomyocyte senescence via the regulation of oxidative stress and inflammatory processes. Additionally, taraxasterol could relieve the desensitization of insulin signaling caused by aging. Finally, we showed that cardiovascular aging and fibrosis were alleviated by taraxasterol treatment *in vivo*. **Conclusions:** Taken together, this work illustrated that taraxasterol could reduce cardiac aging and fibrosis and enhance insulin signaling sensitivity, indicating that taraxasterol may be an effective drug or health food additive for treating cardiac aging and fibrosis.

Keywords: taraxasterol; cardiovascular aging; p15; p16

1. Introduction

Aging is an inevitable life process that is accompanied by physiological and pathological changes in many tissues and organs [1]. However, with an increasing aging population, society will face the pressure of having to deal with issues specific to this aging population in the future [2]. Aging is accompanied by changes in body shape and physiological functions of tissues and organs [3]. Microscopically, it is manifested as increased cell damage and slowed metabolism; macroscopically, it is manifested as the appearance of organs and tissues aging and the weakening of organ functions [4]. Many studies have shown that age-related cardiovascular disease (CVD) has become the most important risk factor affecting the health of the elderly [5]. Cardiovascular damage is a common pathological state in heart disease [6], and it is the basis for the development of heart disease. Heart function gradually declines with aging. Therefore, anti-cardiovascular aging treatment can not only improve the quality of life of the elderly, but also help reduce the morbidity and mortality associated with CVD. Dandelion is a perennial herb. There has been a great deal of research on the chemical constituents of dandelions, and

researchers have extracted and separated many chemical constituents, including flavonoids, carotene, pigments, and volatile oils [7]. One of the many bioactive molecules of dandelions is taraxasterol, which has the molecular formula $C_{30}H_{50}O$ and a molecular weight of 426.72 [8]. The bio-pharmacological effects of taraxasterol have been studied extensively. Yang *et al.* [9] reported that taraxasterol could inhibit NLRP3 inflammasome activation and pyroptosis in macrophages. Furthermore, taraxasterol exhibits therapeutic effects against rheumatoid arthritis [10]. Additionally, taraxasterol exhibited a series of other biological activities, such as antitumor and anti-inflammatory effects, as well as others [11].

However, up to now, the effect of taraxasterol on cardiovascular aging has not been explored. For this, a mouse model of cardiomyocyte senescence induced by D-galactose was constructed and served as an aging model to investigate the effect of taraxasterol on D-gal-induced aging of cardiomyocytes. The experimental findings illustrated that taraxasterol could significantly alleviate cardiomyocyte senescence in the *in vitro* cell model. Furthermore, we found that taraxasterol had the potential to



alleviate cardiomyocyte senescence via the regulation of the SIRT1/p53/p21 signaling pathway. We also found that taraxasterol treatment alleviated cardiovascular aging and fibrosis *in vivo*. Taken together, we showed that taraxasterol could reduce cardiac aging and fibrosis, indicating that taraxasterol may be an effective drug or health food additive for treating/attenuating cardiac aging and fibrosis.

2. Materials and Methods

2.1 Antibodies and Reagents

The BCA protein concentration assay kit was from Pierce (Rockford, IL, USA). The cell counting kit-8 (CCK-8) and the β -galactosidase assay kit were obtained from Solarbio Biological Technology Co., Ltd (Beijing, China). Reactive oxygen species detection kits were purchased from Shanghai Beyotime Institute of Biotechnology, Inc. (Shanghai, China). Superoxide Dismutase (SOD) detection kits (No. S0033S) and MDA detection kits (S0131S) were purchased from Beyotime Institute of Biotechnology, Inc (Shanghai, China). Paraformaldehyde (4%) (No, P0099-100 mL), BSA (No, ST025-5g), phosphate buffered saline (PBS) (No, C0221A), RIPA lysate (No, P0013C), PMSF protease inhibitor (No, P1046), and PVDF membrane (No, FFP24) were purchased from Beyotime Institute of Biotechnology, Inc. D-galactose was from Thermo Fisher Scientific (Waltham, MA, USA). The SDS-PAGE gel preparation kit (No, P0670-250 mL) and BeyoECL Moon (P0018FS) were purchased from Beyotime Institute of Biotechnology, Inc. Anti- α -SMA antibodies (Cat No. A17910, 1:1000 dilution), p-53 antibodies (Cat no. A19585, 1:1000 dilution), and anti-p15 antibodies (Cat no. A11651, 1:1000 dilution) were purchased from Abclonal (Hangzhou, China). Anti-Col-1 antibodies (Cat no. A21059, 1:500 dilution) were purchased from Abcam (Cambridge, UK). Dulbecco's Modified Eagle Medium (DMEM) was purchased from Thermo Fisher Scientific.

2.2 Cell Culture

H9c2 cells were purchased from the American Type Culture Collection (ATCC) (Cat no. Crl-1446). The cells were cultured in DMEM (containing penicillin and streptomycin) with 10% fetal bovine serum (FBS). When H9c2 cells grew to a certain density, they were passaged at a ratio of 1:3. The primary human coronary artery endothelial cell line (HCAEC, PCS-100-020) was purchased from ATCC and cultured in DMEM.

2.3 Masson Staining

Paraffin sections of heart tissue were baked in a 55 °C incubator for 30 min. The sections were dewaxed using xylene. Distilled water was used to rinse the sections, and they were stained with hematoxylin (5 min). After rinsing the sections with distilled water, they were stained with scarlet solution for 5 min and then rinsed with distilled water. The sections were incubated in phosphoaluminate phospho-

tungstic acid aqueous solution for 15 min. After washing, the sections were stained with aniline blue for 5 min. After the sections were washed with 1% glacial acetic acid solution and distilled water, they were dehydrated and soaked in 70% ethanol for 1 s, 95% ethanol for 1 s, and 100% ethanol for 30 s. The above process was repeated three times. The sections were placed in xylene for 3 min (and repeated 3 times). Neutral resin was used to seal the sections. The sections were observed and imaged under a microscope.

2.4 β -Galactosidase (SA- β -Gal) Staining

An SA- β -Gal kit was used to stain H9c2 cardiomyocytes according to the manufacturer's instructions. The specific procedure was as follows: H9c2 cells were fixed in 4% formaldehyde at room temperature for 15 min. The cells were washed 3 times with PBS at room temperature, and then the cells or slides were immersed in freshly prepared SA- β -gal staining solution and incubated overnight in a CO₂-free incubator at 37 °C. After the stained cells were washed twice with PBS, they were visually examined using an inverted microscope.

2.5 Establishment of a Cell Senescence Model

D-gal was used to induce H9c2 cardiomyocytes to establish a cell aging model. H9c2 cells were seeded into 6-well plates. After the cells grew to 90% confluence, they were subcultured into a 12-well plate. When the cells grew to 30%–40% confluence, they were starved in DMEM containing 0.5% FBS for 12 h and then replaced with DMEM without FBS. The various concentrations of D-gal were added to induce cell aging, after which the cells were washed three times. Fresh DMEM (plus 5% FBS) was added, and cells were cultured for three more days. SA- β -Gal staining was performed to evaluate cell senescence and apoptosis.

2.6 Anti-Aging Effect of Taraxasterol *in Vivo*

Mice were divided into two groups. The control group received drinking water only for 6 weeks, while the experimental group received taraxasterol (5.0 mg/kg body weight) given in the drinking water for 6 weeks [10].

2.7 Indirect Immunofluorescence Assay

The heart tissue or cell sample was fixed with 4% paraformaldehyde. The heart tissue was embedded in paraffin, and then 5 μ m thick tissue sections were cut. The paraffin heart tissue sections were baked at 55 °C for 30 min. Tissue was dewaxed and treated with xylene I for 5 min and then subjected to a series of treatments. They were incubated in xylene II for 5 min followed by xylene III for 5 min. The sections were then placed into ethanol for 30 min. The sections were blocked using BSA at room temperature for 30 min. After the sections were washed, the primary antibody was added, and the sections were incubated in a refrigerator at 4 °C for 12 h. After the tissue was washed,

the diluted secondary antibody was added, and the tissue was incubated at room temperature for 1 h. The sections were washed 3 times (5 min each time). DAPI was used to counterstain the nuclei and the sections were incubated in a wet box at room temperature for 15 min. The tissue was washed 3 times. The tissue or cell samples were observed using confocal laser scanning microscopy.

2.8 Hematoxylin Staining

The heart tissues from mice were taken out and then treated in 4% formaldehyde solution for 48 h. The fixed tissue was rinsed with running water to remove any residual fixative. The tissues were dehydrated in successively increasing percentages of ethanol: 70%, 80%, 90%, 95%, and 100%. The tissue was embedded in wax and then sectioned. The slides were incubated in hematoxylin dye for 3 min. After distilled water was used to wash tissue sections approximately 2–3 times, the slides were put into eosin staining solution for 3 min. The slides were put into 95% alcohol I for 30 s, 95% alcohol II for 30 s, absolute ethanol I for 3 min, and absolute ethanol II for 3 min. After the slides were sealed with neutral gum, the histopathological changes in the tissues were observed using a microscope.

2.9 RT-PCR Analysis

For tissue samples, the appropriate amount of myocardial tissue was added to a microtube, and then 1 mL Trizol was added to each microtube. The microtubes were put in a grinder (30 Hz, 60 s) and then placed on ice. For cell samples, the cells were washed three times with PBS. After adding 1 mL Trizol to each tube, 200 μ L of chloroform was added and mixed, and then the microtubes were placed on ice for 5 min. The microtubes were then centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was discarded, and absolute ethanol was added to wash the RNA once. The microtubes were centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was discarded and placed on ice and allowed to stand for 5 min. An appropriate amount of 0.1% DEPC water was added to dissolve the RNA precipitate. RNA concentrations were determined by measuring absorbance at 260 nm and 280 nm using an ultraviolet spectrophotometry (Thermo-Fisher company, Beijing, China). When the purity of the nucleic acid sample was determined as having an OD₂₆₀ nm/OD₂₈₀ nm ratio between 1.8 and 2.0, the RNA sample was then used in subsequent experiments. RT-PCR was then performed.

2.10 Animal Administration in Vivo

The animals used in the current experimental group (C57 mice) were purchased from Beijing Huafukang Company (Beijing, China); 3-month-old mice (young group) and 18-month-old mice (aged group) were chosen. The mice were placed in a clean animal room and the temperature was maintained at 22 \pm 2 °C. Mice were given taraxasterol, which was added to their drinking water (5.0 mg/kg

body weight).

2.11 Western Blot Analysis

After the tissue/cell protein was extracted, the protein concentration was determined using a BCA kit. The protein sample was subjected to SDS-PAGE and then transferred to a PVDF membrane. The membranes were put into TBST solution containing 5% skim milk powder and incubated at room temperature for 3 h. After washing with TBST solution 2–3 times, the corresponding primary antibody was added and incubated at 4 °C overnight (the primary antibody could be recycled and reused). After washing the PVDF membrane with TBST solution for 6 min, the corresponding horseradish peroxidase coupled-IgG antibody was added and incubated at room temperature for 2–3 h. The secondary antibody solution was discarded, and the PVDF membranes were washed with TBST solution for 10 min/time (6 times). ECL chemiluminescence reagent was used as the detection agent, and the exposure time was selected according to the staining intensity of the PVDF membrane.

2.12 Analysis of Cell Apoptosis

Cells (3 \times 10⁴ cells/well) were seeded into 24-well cell culture plates. Trypsin (0.25%) was added to the cells. After collecting the cells by centrifugation, Annexin V-FITC and propidium iodide were added to the cells. After incubation for 0.5 h at RT, the apoptosis rate was determined using flow cytometry.

2.13 Determination of MDA and SOD

Cells were collected using centrifugation (1000 rpm). The levels of MDA and SOD were determined using an MDA/SOD kit according to the instructions provided with the kit.

2.14 Detection of Mitochondrial Membrane Potential

Mitochondrial membrane potential was analyzed using a mitochondrial membrane potential kit according to the instructions provided with the kit.

2.15 Statistical Analysis

The results of all experimental data are expressed as the mean \pm S.D. The differences between groups were analyzed using ANOVA. A $p < 0.05$ indicated a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results and Discussion

3.1 Establishment of a Model of Cardiomyocyte Aging Induced Using D-gal

To induce senescence in H9c2 cells, H9c2 cells were pre-treated with various concentrations of D-gal for 2 h (10 g/L, 20 g/L, 40 g/L, 60 g/L, and 80 g/L). After fresh medium was added to the H9c2 cells and they were cultured for an additional 48 h, senescence and apoptosis were evaluated.

First, a CCK8 assay was performed to evaluate cell viability. The experimental results showed that the cell viability gradually decreased with increasing D-gal concentrations (Fig. 1A). Sa- β -gal staining was used to detect the aging of H9c2 cells. The results showed that the aging of cells in the D-gal treatment group was significantly greater than those in the control group (Fig. 1B). The results of flow cytometry showed that when the concentration of D-gal exceeded 80 g/L, the cell apoptosis rate increased significantly (Fig. 1C). Therefore, we chose 60 g/L D-gal to establish the aging cell model. In addition, we found that p16 and p21 in H9c2 cardiomyocytes were significantly upregulated in the D-gal treatment group (Fig. 1D).

In the pre-experiment, we used the CCK8 cell viability assay to determine the concentration of taraxasterol (0–100 μ M) to use. We found that taraxasterol could significantly promote cell proliferation when the concentration used was ≤ 30 μ M. But when the concentration of taraxasterol exceeded 50 μ M, there was no significant change in cell viability (Fig. 1E). Therefore, we chose 30 μ M taraxasterol in the current experiments.

3.2 Taraxasterol Inhibited the Senescence of H9c2 Cells Induced by D-gal

To study the effect of taraxasterol on cell aging, H9c2 cells were incubated in D-gal at a concentration of 60 g/L to establish the cell aging model. Sa- β -Gal staining was performed to detect the senescence of H9c2 cells. The results showed that there was no significant difference in the number of aging cells between the taraxasterol group and the control group ($p > 0.05$). The number of senescent cells in the D-gal group was significantly higher than that in the control group ($p < 0.05$). The number of senescent cells in the taraxasterol + D-gal group was significantly lower than that in the D-gal group ($p < 0.05$) (Fig. 2A). Furthermore, we used western blotting to detect the expression of aging marker molecules p16 and p21. The results showed that the expression levels of p16, p21, and p53 in H9c2 cells in the D-gal group were significantly higher than those in the control group (Fig. 2B), which was statistically significant ($p < 0.05$). The expression levels of p16 and p21 in H9c2 cells in the taraxasterol + D-gal group were significantly lower than those in the D-gal treatment group. Cell cycle analysis showed that in the D-gal group, S phase decreased and G0-G1 phase cells increased, while in the taraxasterol treatment group, the proportion of S phase cells was partially restored (Fig. 2C). The mitochondrial membrane potential also increased significantly in the taraxasterol treatment group (Fig. 2D).

3.3 Taraxasterol Inhibited D-gal-Induced Cardiomyocyte Senescence-Associated Secretory Phenotype (SASP)

We examined the effect of taraxasterol on the cardiomyocyte SASP. In the D-gal group, the mRNA expression levels of IL-1 β , IL-6, and TNF α increased. At the pro-

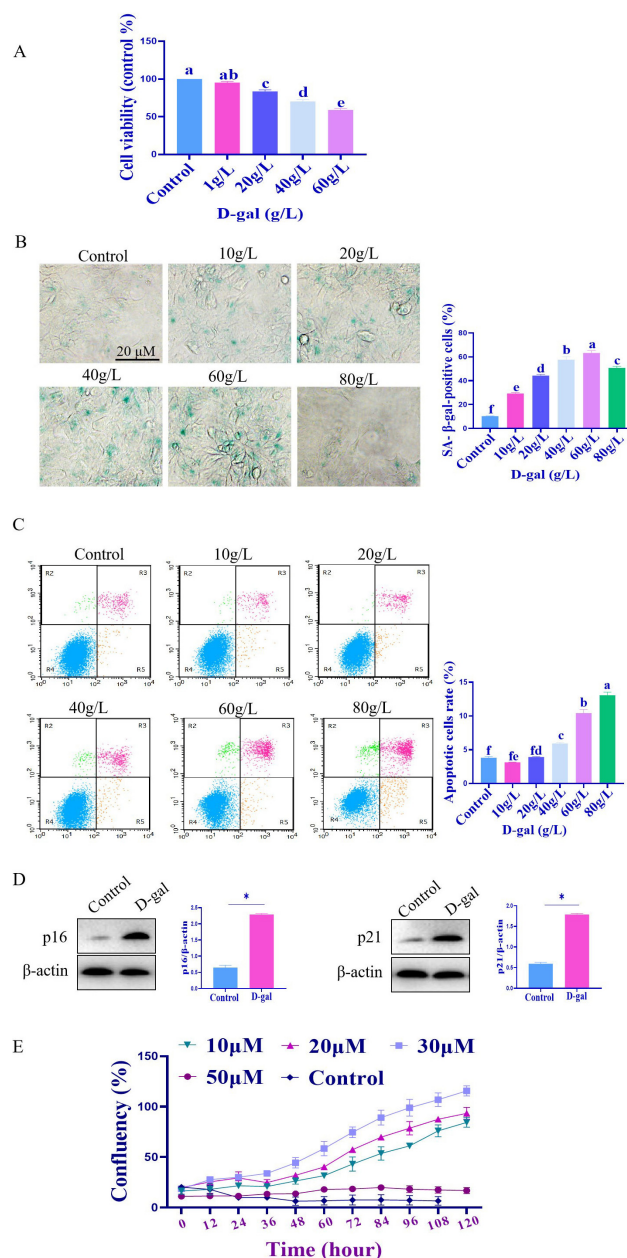


Fig. 1. Establishment of the senescent cell model. (A) D-gal treatment leads to the decline of cell viability of H9c2 cells. The cells were treated with different concentrations of D-gal. Cell viability was detected using the CCK8 assay kit. (B) Sa- β -Gal staining showed that cardiomyocytes were aging under D-gal treatment. (C) Cell apoptosis was evaluated using flow cytometry with D-gal treatment. (D) p16 and p21 in H9c2 cardiomyocytes were significantly upregulated. (E) Determination of the taraxasterol concentration used in the current work using the CCK8 assay kit. $p < 0.05$ indicates a significant difference. Different low case letters above columns indicate statistical differences at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

tein level, the above-mentioned three proinflammatory factors were also significantly increased. In contrast, taraxasterol inhibited the D-gal-induced cardiac SASP (Fig. 3A).

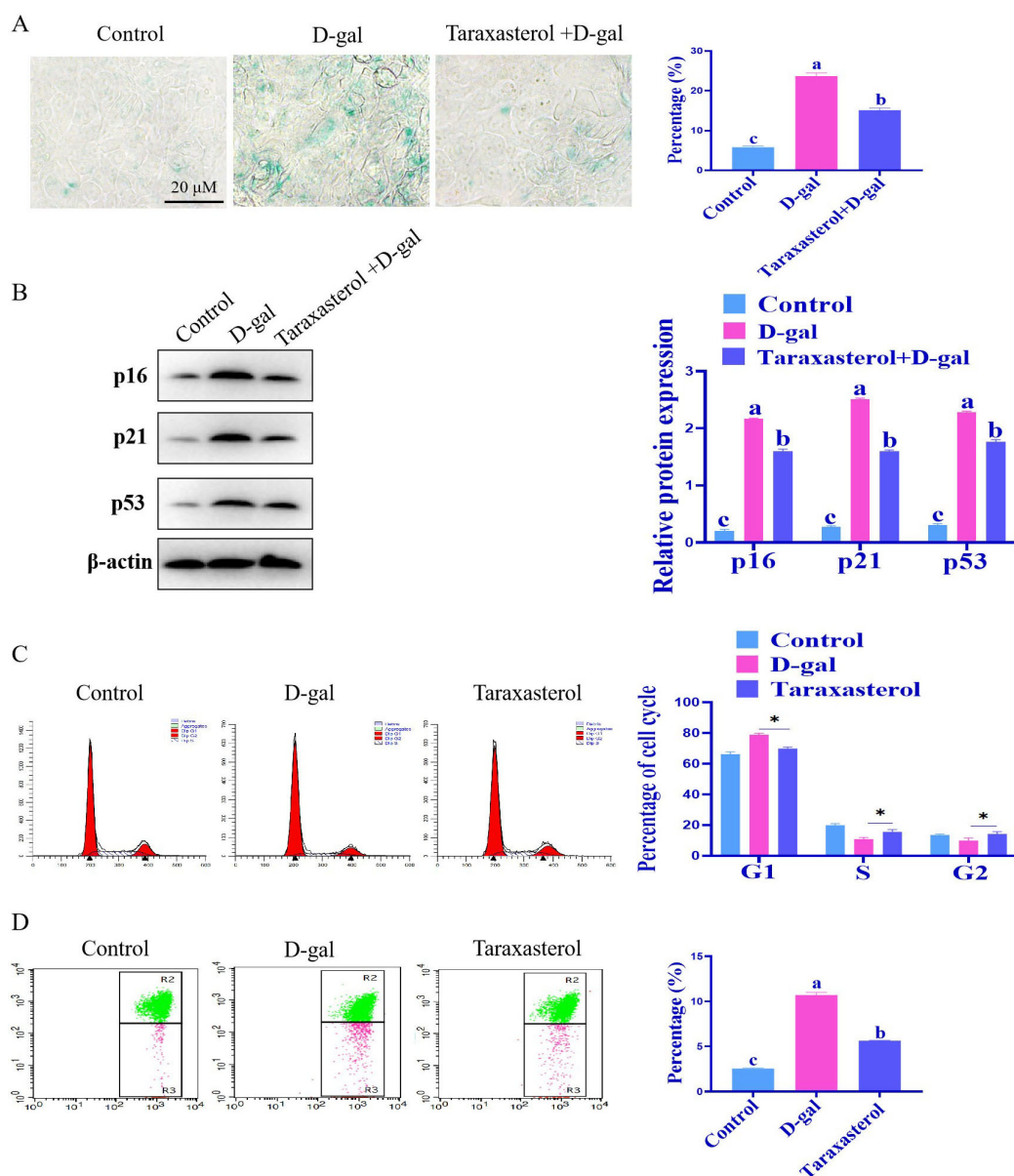


Fig. 2. Effects of Taraxasterol on cell senescence. (A) Taraxasterol reduced the number of senescent cells induced by D-gal. (B) The expression of p16, p21, and p53 was downregulated under taraxasterol treatment. (C) The proportion of S phase cells was increased under taraxasterol treatment. (D) The mitochondrial membrane potential also increased significantly in the taraxasterol treatment group. Different low case letters above columns indicate statistical differences at $p < 0.05$.

Further, we analyzed the effect of taraxasterol on NF- κ B signaling. In the aging cell model, the phosphorylation levels of NF- κ B, p65, and I κ B were significantly higher than those in the control group. However, taraxasterol treatment could significantly reduce the phosphorylation levels of NF- κ B, p65, and I κ B ($p < 0.05$), indicating that taraxasterol inhibited activation of the NF- κ B signal transduction pathway (Fig. 3B) in a dose-dependent manner. Further analysis showed that the expression levels of p-p38, p-ERK1/2, and p-JNK (shown using flow cytometry) were also significantly downregulated in the taraxasterol treatment group (Fig. 3C).

3.4 The Effect of Taraxasterol on Oxidative Damage

In the current work, D-gal significantly increased the levels of ROS and MDA, but the level of SOD decreased significantly. However, in the taraxasterol treatment group, the levels of ROS and MDA decreased significantly, while SOD increased significantly (Fig. 4). These experimental results show that taraxasterol can alleviate oxidative stress.

3.5 The Potential Anti-Aging Mechanism of Taraxasterol

To determine the potential anti-aging molecular mechanism of taraxasterol, we assessed the expression of p53 as well as its upstream regulatory factor SIRT1 and down-

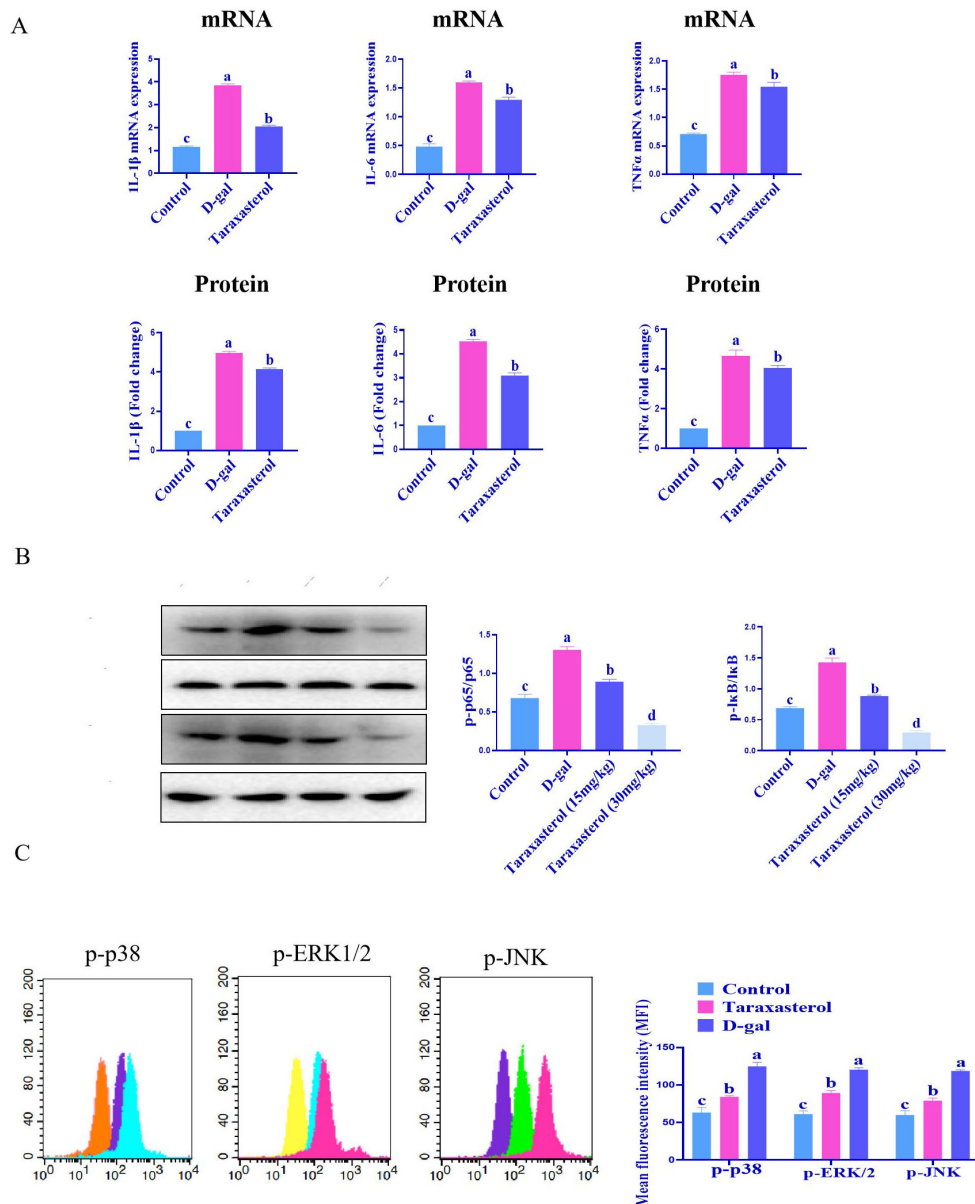


Fig. 3. Effects of Taraxasterol on SASP. (A) Taraxasterol could inhibit the D-gal-induced cardiac SASP. (B) Taraxasterol treatment significantly reduced the phosphorylation level of NF- κ B, p65, and I κ B. (C) The expression levels of p-p38, p-ERK1/2, and p-JNK (shown using Flow cytometry) were also significantly downregulated in the taraxasterol treatment group. Different low case letters above columns indicate statistical differences at $p < 0.05$.

stream factor p21 in cardiomyocytes to confirm whether taraxasterol's anti-aging effect may be related to the SIRT1/p53/p21 pathway. The results showed that compared with the normal group, in the aging cardiomyocyte model group, the expression levels of SIRT1 and cyclin D1 proteins decreased significantly, while the expression levels of p16, p21, and p53 proteins increased (Fig. 5). Taraxasterol upregulated the expression levels of SIRT1 and cyclin D1 proteins, and downregulated the expression levels of p16, p21, and p53 proteins ($p < 0.05$). The above results suggest that taraxasterol may delay the aging of cardiomyocytes through the SIRT1/p53/p21 signaling pathway

(Fig. 5A). We performed rescue experiments to determine whether the anti-aging effects of taraxasterol were related to the SIRT1 molecule and found that inhibiting the function of SIRT1 neutralized the anti-aging effects of taraxasterol (Fig. 5B).

3.6 Taraxasterol Enhanced Insulin/Insulin Receptor (IR)-Mediated Intracellular Signaling

To clarify the effect of taraxasterol on insulin resistance in aging cardiomyocytes, western blot assays were conducted. A previous study had demonstrated that the insulin receptor (IR)-mediated signaling pathway (signaling proteins p-IRS-1, p-AKT, and p-GSK-3 β) was significantly

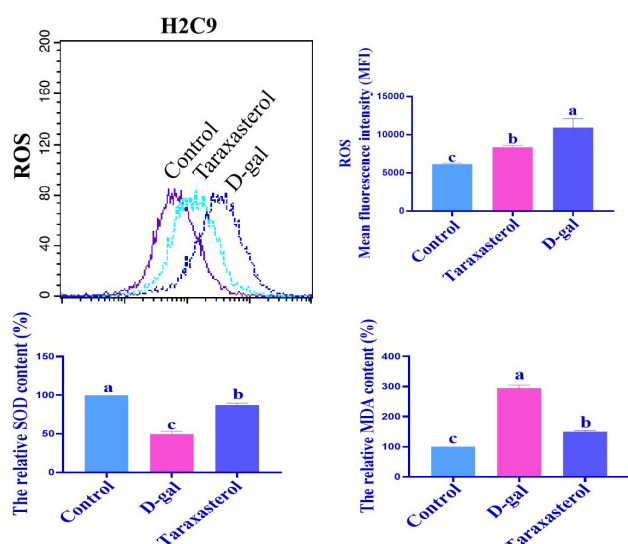


Fig. 4. Taraxasterol can alleviate oxidative stress. The levels of ROS, MDA, and SOD were determined using the corresponding assay kit as described in the Materials and Methods section. Different low case letters above columns indicate statistical differences at $p < 0.05$.

downregulated in aging cell models and that these signaling molecules caused a series of cascade effects to reduce blood glucose. Compared with the aging cell model, IR-mediated downstream signaling molecules were increased, indicating that taraxasterol may improve insulin sensitivity (Fig. 6) ($p < 0.05$).

3.7 Evaluation of the Effect of Taraxasterol on Aging in Vivo

We used the aged mice model in which mice were treated with taraxasterol provided in their drinking water to study the effect of taraxasterol on aging *in vivo*. We evaluated the aging of the heart in the aged mice and found that the SA- β -Gal staining area increased in the aged heart. Additionally, there were extensive p16 and p21 stained cells in the aged mice compared with the young mice. However, in the taraxasterol group, SA- β -Gal staining showed that in the taraxasterol treatment group, the aging of the heart was significantly reduced (Fig. 7A). Furthermore, the number of p16 and p21 stained cells decreased significantly (Fig. 7B). We further analyzed the effect of taraxasterol on cardiac inflammation *in vivo*. To this end, we detected the expression of related inflammatory molecules in the heart tissue, including IL-1 β , IL-6, and TNF α . The results showed that taraxasterol treatment inhibited the expression of these proinflammatory-related molecules (Fig. 7C). These data suggest that taraxasterol can alleviate SASP caused by cardiac aging.

The effects of taraxasterol on SOD, MDA, and GSH in serum of aging mice were also analyzed. The results illustrated that in aging mice, the contents of SOD and GSH in serum were lower than those in the control group ($p <$

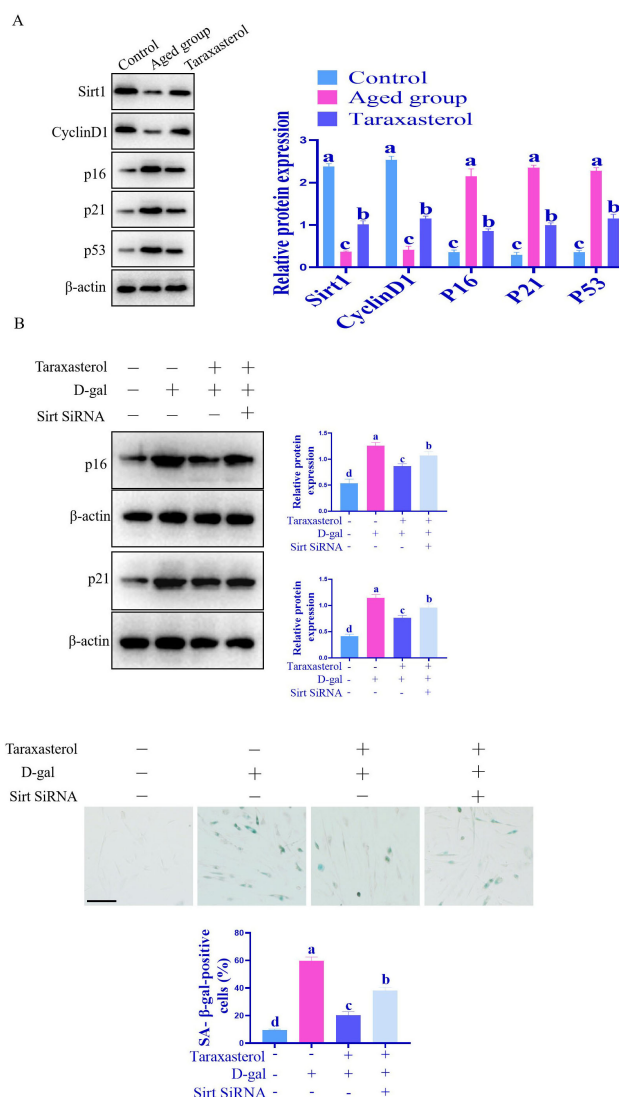


Fig. 5. Taraxasterol might delay the aging of cardiomyocytes through the SIRT1/p53/p21 signaling pathway. $p < 0.05$ indicates a significant difference.

0.05), while in the taraxasterol group, the contents of SOD and GSH were significantly higher. In addition, the contents of ROS and MDA decreased in the control group. In contrast, in the taraxasterol group, the expression of ROS and MDA decreased (Fig. 7D).

3.8 Taraxasterol Inhibited Myocardial Fibrosis in Aging Mice in Vivo

In the process of aging, the heart experiences obvious pathological changes, with one of the most important being myocardial fibrosis. Therefore, we studied the effect of taraxasterol on myocardial fibrosis in aged mice. Masson staining showed that the fibrotic staining area in the taraxasterol treatment group was significantly reduced (Fig. 8A). In addition, in the taraxasterol group, the α -SMA immunostaining area was also significantly decreased compared with the aging model group (Fig. 8B). These results suggest that

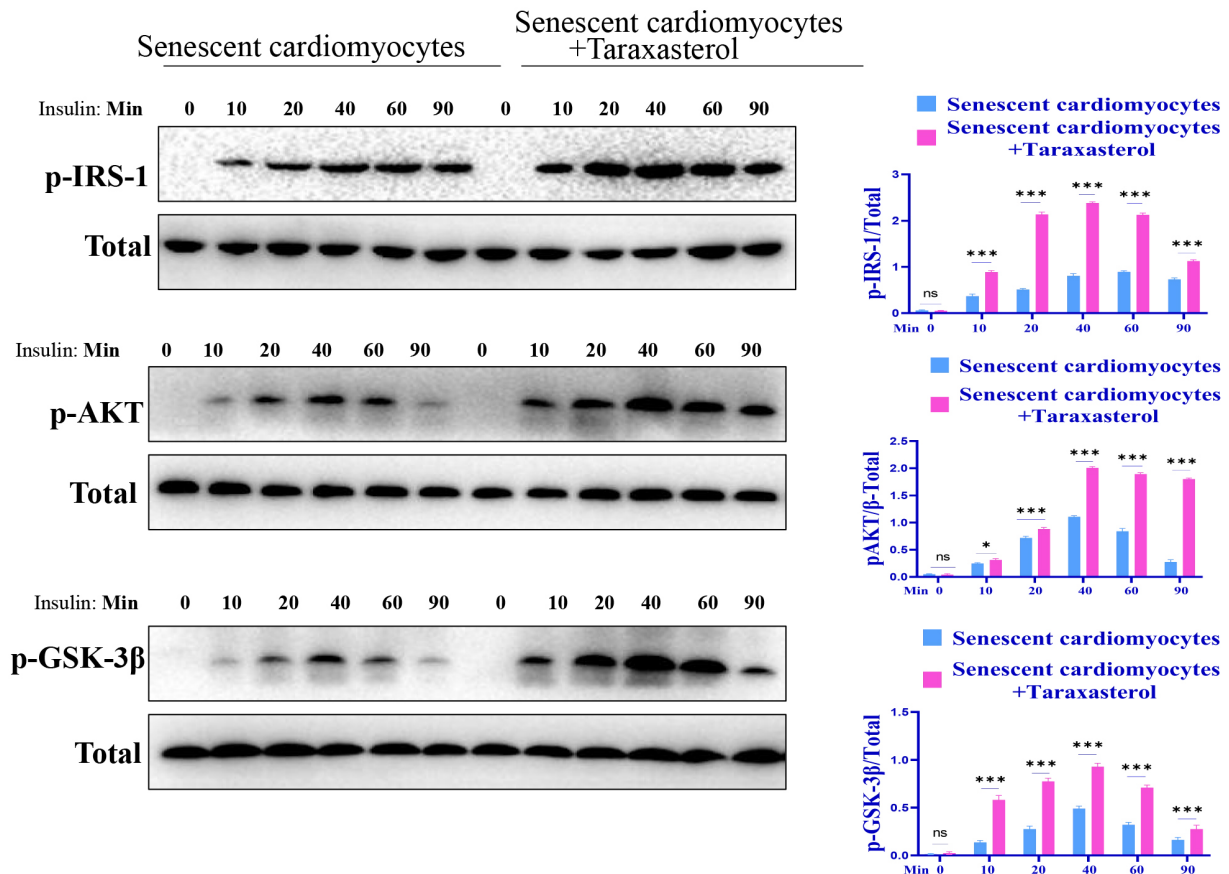


Fig. 6. Taraxasterol may improve insulin sensitivity. Different low case letters above columns indicate statistical differences at $p < 0.05$. *** $p < 0.01$.

taraxasterol could inhibit cardiac fibrosis. In addition, the hematoxylin and eosin (HE) staining showed that taraxasterol significantly improved the pathological changes of the myocardial tissue (Fig. 8C).

3.9 Taraxasterol Inhibited Oxidative Stress-Induced Cellular Senescence of HCAEC

In the above-mentioned study, we analyzed the effect of taraxasterol on cardiomyocyte aging. Here, the effect of taraxasterol on the aging of HCAEC was studied. The HCAEC were stimulated with 60 g/L D-gal. Sa- β -Gal staining was done to detect the aging of HCAEC. The results showed that there was no significant difference in the number of aging cells between the taraxasterol treatment group and the control group ($p > 0.05$) (Fig. 9A). The number of senescent cells in the D-gal group was significantly higher than that in the control group ($p < 0.05$). The number of senescent cells in the taraxasterol + D-gal group was significantly lower than that in the D-gal group ($p < 0.05$). Furthermore, western blotting was performed to detect the expression of aging marker molecules, such as p16, p21, and p53. The results showed that the expression levels of p16, p21, and p53 in HCAEC in the D-gal group were significantly higher than those in the control group. Compared with the D-gal group, the expression levels of p16, p21, and

p53 in H9c2 cells decreased significantly in the taraxasterol + D-gal group (Fig. 9B). The mitochondrial membrane potential also increased significantly in the taraxasterol treatment group (Fig. 9C).

The incidence of CVD continues to increase because of population aging, and CVD has become one of the leading causes of death and disability worldwide [12]. During myocardial aging, cardiomyocytes gradually lose their functional and structural integrity, resulting in a reduction in the number of cardiomyocytes capable of functioning normally, and ultimately leading to CVD. Cardiomyocyte aging is a key factor in the decline of cardiac function and the development of CVD such as cardiac hypertrophy and heart failure [13]. With an increasing aging population, the study of cardiomyocyte aging and its corresponding protection strategies has important clinical significance for preventing the occurrence and development of CVD. To this end, in the current study, we evaluated taraxasterol myocardial aging *in vivo* and *in vitro* and found that taraxasterol exhibited an anti-aging effect on myocardial cells, which provided a theoretical basis for intervening with or even reversing cardiomyocyte aging.

Cellular senescence is the irreversible arrest of cell growth [14]. Cellular senescence can be divided into two categories: replicative senescence and premature senes-

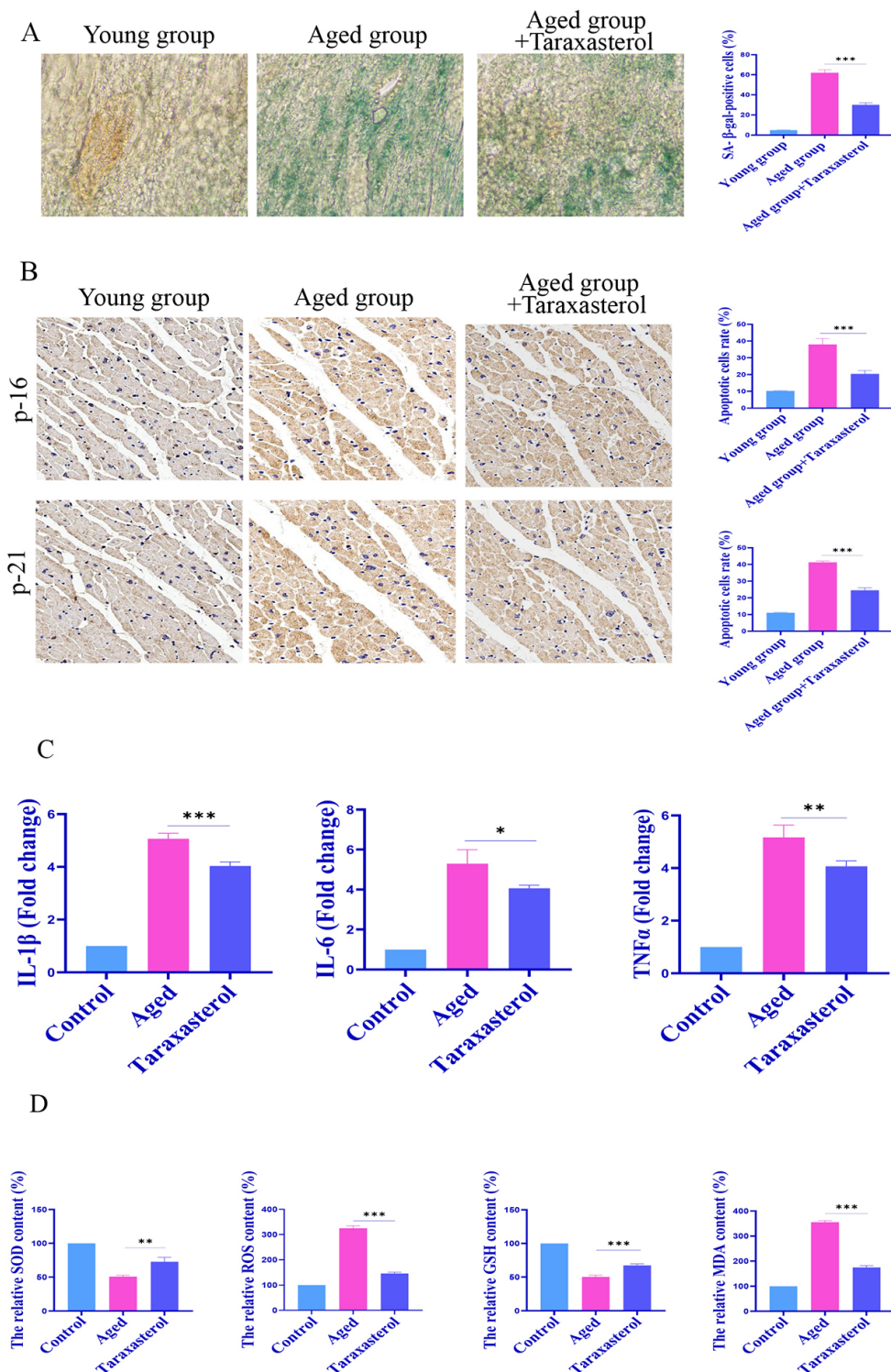


Fig. 7. Evaluation of the anti-aging effect of taraxasterol *in vivo*. (A) The taraxasterol treatment group showed a significant reduction in the aging of the heart. (B) p16 and p21 stained cells decreased significantly under taraxasterol treatment. (C) Taraxasterol treatment inhibited the expression of proinflammatory molecules. (D) The effect of taraxasterol on SOD, GSH, MDA, and ROS content. $p < 0.05$ indicates a significant difference. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

cence [15]. Cellular senescence is the basic unit of biological aging and the common basis for the pathogenesis of human geriatric diseases [16]. During physiological aging, the total number of cardiomyocytes decreases. It has been

suggested that the aging of cardiomyocytes is involved in many cardiovascular events, and aging cardiomyocytes exhibit many functional and structural changes. In *in vitro* experiments, the effect of taraxasterol on the aging of car-

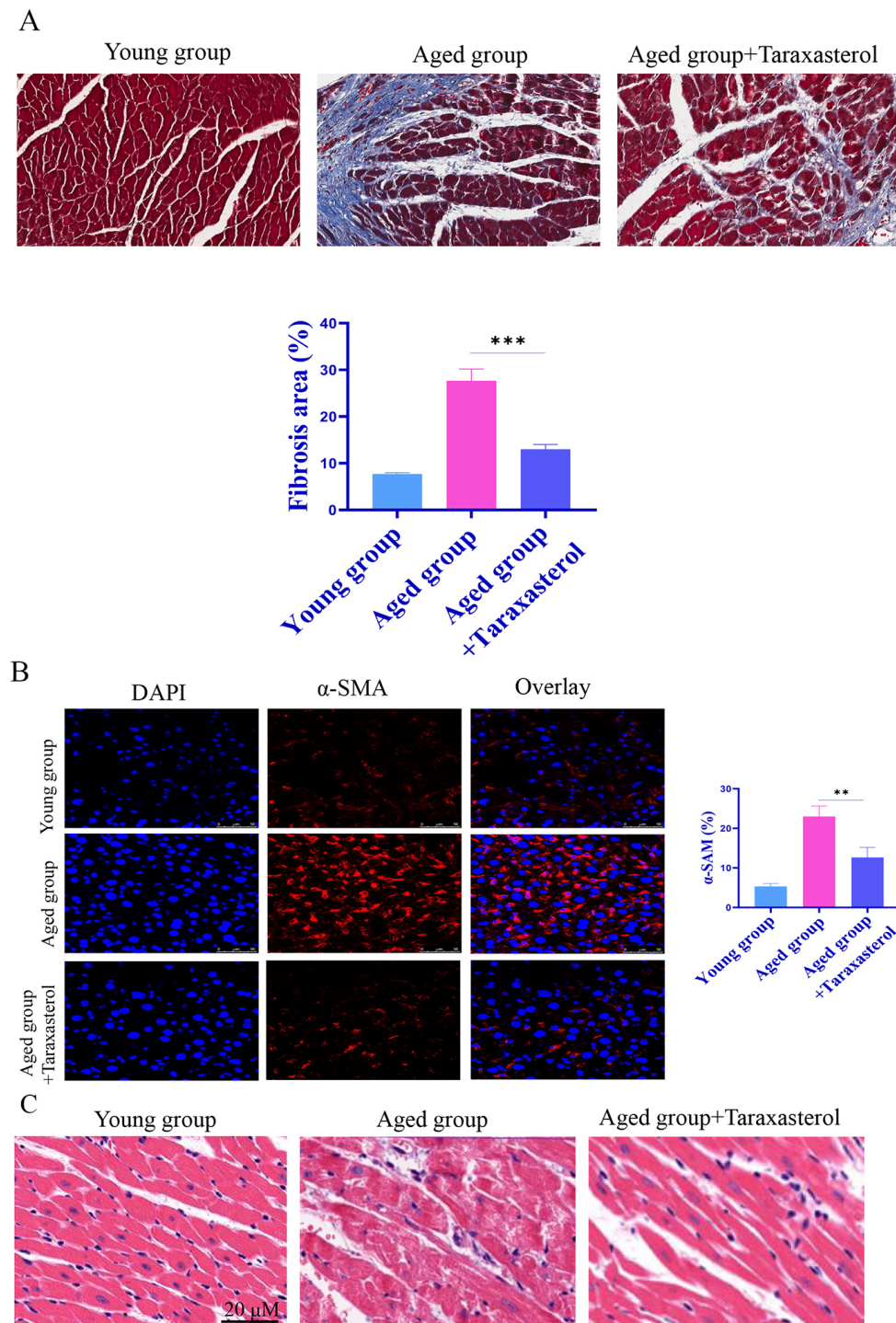


Fig. 8. Effects of Taraxasterol on myocardial fibrosis. (A) Masson staining showed that the fibrotic staining area in the taraxasterol treatment group was significantly reduced. (B) The α -SMA immunostaining area was also significantly decreased under taraxasterol treatment. (C) Analysis of the morphology of myocardial tissue using HE staining. $p < 0.05$ indicates a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

diomyocytes was first evaluated. Taraxasterol is a pentacyclic three-shielded compound, an active ingredient extracted and isolated from dandelions. We evaluated a series of senescence-related markers and found that taraxasterol significantly alleviated cardiomyocyte senescence.

In addition, previous studies have found that taraxasterol has a range of biological activities. It has been reported that taraxasterol exhibited a protective effect on acute lung injury in mice [17]. Furthermore, taraxasterol has anti-inflammatory and anti-arthritis effects [18]. In addition,

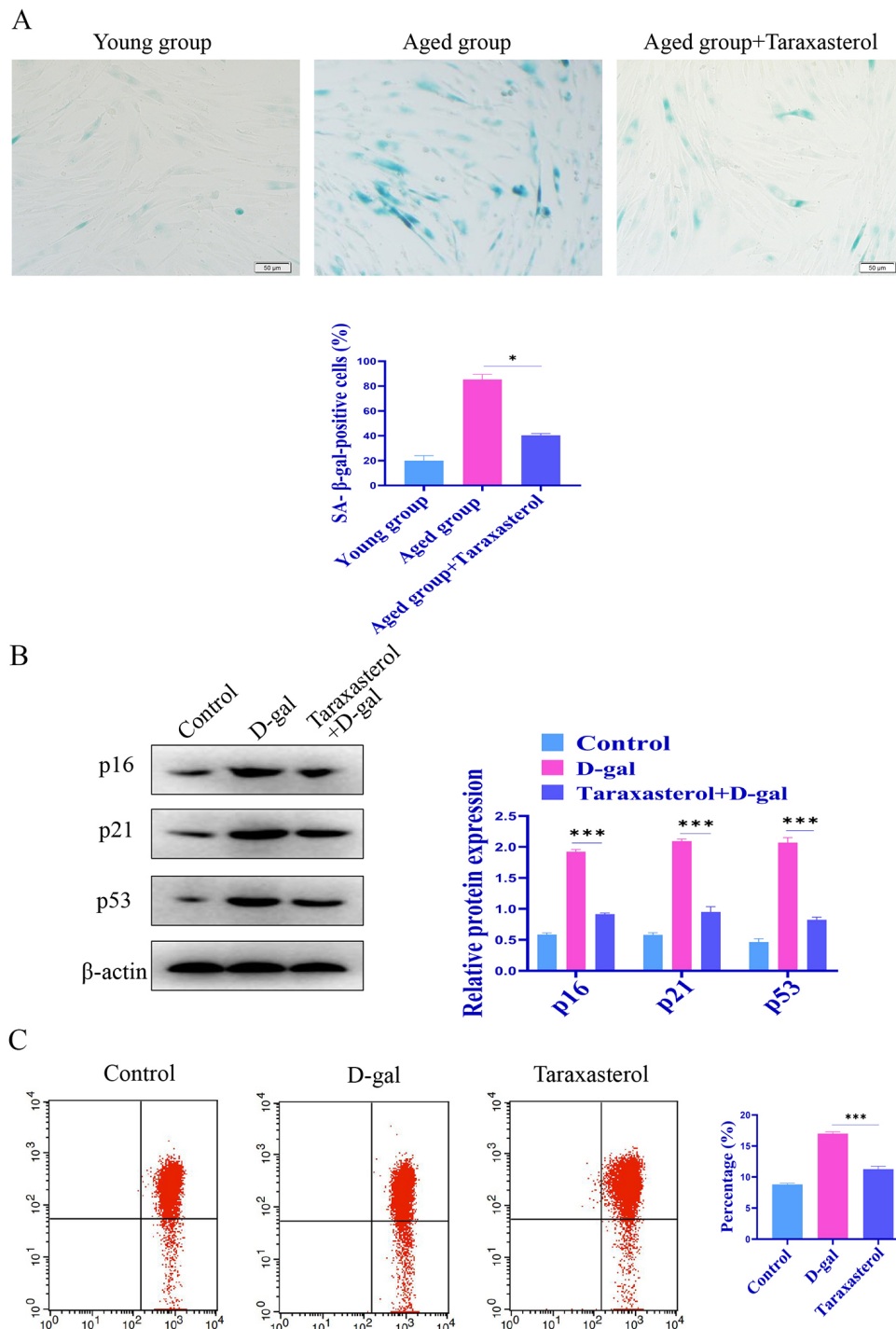


Fig. 9. Effect of taraxasterol on cardiomyocyte aging. (A) Sa-β-Gal staining showed that the number of senescent cells decreased significantly under taraxasterol treatment. (B) The expression levels of p16, p21, and p53 in H9c2 cells were decreased in the taraxasterol treatment group. (C) The mitochondrial membrane potential also increased significantly in the taraxasterol treatment group. $p < 0.05$ indicates a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

taraxasterol exhibited protective effects on ethanol-induced liver injury [19].

With aging, there is a progressive increase in the proinflammatory response in the body. Franceschi *et al.* [20] were the first to name this phenomenon inflammatory aging. Inflammatory aging is closely related to a variety of

geriatric diseases. The proinflammatory factors and anti-inflammatory factors in the elderly change, and the final manifestation is the excessive proinflammatory response and the imbalance of inflammatory homeostasis, which leads to inflammatory aging. In the current study, we found that taraxasterol significantly reduced proinflammatory fac-

tor expression (such as IL-6 and IL-1 β), suggesting that taraxasterol has the potential to inhibit inflammatory aging.

Oxidative stress is closely related to aging [21]. The theory of free radical aging was first proposed by Harmana, who believed that free radicals attack and destroy biological macromolecules, causing damage to tissue cells and finally leading to aging [22]. Oxidative stress refers to the imbalance between the antioxidant system and the oxidative system whereby the body's antioxidant system is insufficient to resist and repair foreign oxidants (active oxygen free radicals and reactive nitrogen free radicals) when the body is subjected to various harmful stimuli. This results in tissue and cellular damage. In the current study, we found that taraxasterol has significant anti-oxidative stress effects *in vitro* and *in vivo*.

We further analyzed the molecular mechanism by which taraxasterol exhibited the anti-aging effect. The results showed that taraxasterol upregulated the expressions of SIRT1 and cyclin D1 and downregulated the levels of p53, p21, and p16. At the same time, taraxasterol upregulated the mRNA levels of SIRT1 and cyclin D1 and decreased the mRNA levels of p53, p21, and p16. These findings suggest that taraxasterol exhibited the anti-aging effect possibly via p21 and the p16-Cyclin D1 signaling pathway. However, the in-depth molecular mechanism still needs to be elucidated in the future.

Aging can cause myocardial fibrosis [23], and this leads to a significant increase in the incidence of and mortality from heart failure in the elderly. Aging is an inevitable process and one of the recognized causative factors of CVD. Myocardial fibrosis refers to the transformation of fibroblasts into myofibroblasts caused by various pathological factors. In CVD, the synthesis and degradation of collagen become unbalanced, the proportion of collagen is unbalanced, the arrangement of cells is disordered, and the deposition of extracellular matrix is common. Elucidating the role and mechanism of aging in myocardial fibrosis may help prevent and treat age-related CVD and improve the quality of life in elderly people. In the current study, we found that taraxasterol could inhibit myocardial fibrosis *in vivo*.

Studies have confirmed that aging can lead to a decline in insulin sensitivity in the heart (insulin resistance) [24]. In the current study, we found that taraxasterol could alleviate cardiac aging, so it could correspondingly increase insulin sensitivity (insulin sensitivity will significantly decrease because of cardiac aging). The results showed that taraxasterol partially improved insulin sensitivity.

The incidence of CVD such as myocardial ischemia, heart failure, and myocardial fibrosis also increases with aging [25,26]. Therefore, scientists are looking for anti-cardiovascular aging solutions. In addition to cardiomyocytes, we also assessed the potential effect of taraxasterol on arterial aging and found that taraxasterol was able to significantly alleviate vascular endothelial cell aging.

4. Conclusions

Taken together, this work illustrates that taraxasterol could reduce cardiac aging and fibrosis, indicating that taraxasterol may be an effective drug or health food additive for treating cardiac aging and fibrosis. The current study provides a rationale for intervening in and treating cardiac aging.

Abbreviations

CVD, cardiovascular disease; PBS, phosphate buffered saline; MMP, Mitochondrial membrane potential; IR, insulin receptor.

Author Contributions

GL and DZ designed the research study; SW, NJ performed the research; YQ provided help and advice on the conclusions; GL and NJ analyzed the data; GL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The establishment of the experimental mouse model was approved by the Animal Ethics Committee of Jiangsu College of Nursing (2021-0301).

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Conflict of Interest

The authors declare no conflict of interest.

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