

ORIGINAL ARTICLE

The effect of *Dendrobium officinale* extract in inhibiting the senescence of H₂O₂-induced fibroblasts based on the senescence-associated secretory phenotype

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Ya Zhao, Shanghai Jahwa United Co., Ltd, Shanghai, China.

Email: zhaoya@jahwa.com.cn**Abstract**

Background: *Dendrobium officinale* is widely used for a long time in China, with effect of antioxidation, antitumor, enhancing immunity and so on. In recent years, *Dendrobium officinale* has been gradually used in cosmetics due to its powerful beauty effects.

Aims: Based on senescence-associated secretory phenotype (SASP), we studied the antiaging effect of *Dendrobium officinale* extract (DOE) on skin.

Methods: The senescent model of human skin fibroblasts was established by the induction of H₂O₂, and the content of SASP factors was tested after the treatment of DOE, such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and matrix metalloproteinase-1 (MMP-1).

Results: It was found that after the treatment with different concentrations of DOE, the contents of IL-6, MCP-1 and MMP-1 all decreased in different degrees.

Conclusions: It indicated that DOE could inhibit the secretion of SASP factors and was a promising natural antiaging agent.

KEYWORDSantiaging, *Dendrobium officinale* extract, human skin fibroblasts, SASP

1 | INTRODUCTION

Cell senescence was first proposed by Hayflick and Moorhead in 1961. It was originally used to describe the senescent fibroblasts that lose the reproductive ability under suitable growth conditions and enter a state of permanent growth cycle arrest.¹ Although senescent cells have permanently lost their ability to proliferate, they still have high metabolic activity and show great changes in chromatin tissue and gene expression. The secretion of SASP is one of

the important manifestations. The SASP is the general term of cytokines secreted by senescent cells, including cytokines, chemokines, growth factors, proteases, etc.²⁻⁷

Dendrobium officinale is the stem of *Dendrobium officinale* Kimura et Migo. At present, the reported skin beauty effects of *Dendrobium officinale* mainly include antioxidation, moisturizing, antiaging and so on. However, most of antiaging studies of *Dendrobium officinale* on skin are concluded through its antioxidation or moisturizing. There are few studies on the mechanism of antiaging of *Dendrobium*

officinale on skin. In this study, H_2O_2 was used to establish the senescent model of human skin fibroblasts, and the antiaging effect of *Dendrobium officinale* was evaluated by SASP factors.

2 | MATERIALS AND METHODS

2.1 | Materials

The fresh *Dendrobium officinale* was purchased from Anhui Homi Biotechnology Co., Ltd. (China). Human skin fibroblasts (Fb19052002), Dulbecco's modified Eagle's medium (DMEM) were provided by Guangdong BioCell Biotechnology Co., Ltd. (China). Phosphate-buffered saline (PBS) was purchased from BOSTER (America). H_2O_2 , dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dexamethasone (DXMS) were purchased from Sigma (America). Enzyme linked immunosorbent assay (ELISA) kits such as IL-6 and MMP-1 were purchased from Abcam (England). MCP-1 ELISA kit, transforming growth factor- β 1 (TGF- β 1) were purchased from Proteintech (America). RNAiso Plus, Reverse transcription kit were purchased from Takara (Japan). Senescence-associated beta-galactosidase (SA- β -gal) staining kit was purchased from Shanghai Biyuntian Biotechnology Co., Ltd. (China). Activated carbon powder was purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2 | Preparation of *Dendrobium officinale* extract (DOE)

Fresh *Dendrobium officinale* was extracted with deionized water at 95°C–105°C. The filtrate was obtained by separation of solid and liquid. 0.5% (w/w) active carbon powder (≥ 200 mesh) was added to the filtrate for over 30 min and was removed by separation of solid and liquid. The filtrate was lyophilized in a lyophilizer (Songyuan Freeze Dryer, China). Then DOE was obtained.

2.3 | Establishment of the senescent model of H_2O_2 -induced fibroblasts

The control group and H_2O_2 -induced group were set up in the experiment. Fibroblasts in logarithmic phase were selected and seeded into 6-well plates, and were treated according to groups when the plates were spread about 40%–60%. Fibroblasts in H_2O_2 -induced group were incubated with serum-free low glucose DMEM containing 400 μ M H_2O_2 , and the control group were incubated with serum-free low glucose DMEM. After 2 h, the fibroblasts in each group were washed by PBS for three times. Then low glucose DMEM medium containing 10% serum was added, and the fibroblasts were incubated for 24 h. Repeat the above operation for three times starting

TABLE 1 The primer sequence of p16.

Forward primer sequence(5'-3')	Reverse primer sequence(5'-3')
CTCTGAGAAACCTCGGGAAC	ATGAAACTACGAAAGCGGG

from H_2O_2 processing step. Then the obtained fibroblasts were cultured for five passages and were collected for SA- β -gal staining and testing of p16 mRNA expression.

Fibroblasts cultured for five passages were stained with SA- β -gal staining kit. According to the rate of plate laying is 80%–90%, they were seeded into 6-well plates and incubated for 24 h under 5% CO_2 at 37°C. Then fibroblasts were washed once with PBS. 1 mL SA- β -gal staining fixative solution was added to each well. After 15 min the fibroblasts were washed by PBS for three times. 1 mL SA- β -gal staining working solution was added to each well. Then the fibroblasts were incubated overnight at 37°C, and the 6-well plates were sealed with sealing film to prevent evaporation. They were observed, photographed and counted under an ordinary light microscope (Olympus, Japan). The positive fibroblasts of SA- β -gal staining is blue. The number of positive fibroblasts and total fibroblasts in five different areas in each film was counted to calculate the proportion of the positive fibroblasts of SA- β -gal.

According to the rate of plate laying is 80%–90%, fibroblasts cultured for five passages were seeded into 6-well plates and incubated for 24 h under 5% CO_2 at 37°C. Then fibroblasts were washed twice with PBS. 1 mL RNAiso Plus was added to each well, the fibroblasts were lysed by blowing. RNA was extracted, reverse transcribed to cDNA, and then tested by CFX96 Touch Real-time PCR Detection System (BioRad, America). The primer sequence of p16 is showed in Table 1. $2^{-\Delta\Delta CT}$ was used to calculate and relative gene expression was presented as fold change in relation to the control group (Control).

2.4 | Cell viability assays

The cell viability was determined by MTT assay. According to the rate of plate laying is 80%–90%, the fibroblasts induced by H_2O_2 according to point 2.3 were seeded into 96-well plates and incubated under 5% CO_2 at 37°C. Then DOE was diluted two concentrations (2.5%, 5%, m/V) by normal medium and treated by 200 μ L per well. The solvent control group (SC) was treated by 200 μ L normal medium. The positive control group (PC) was treated by 200 μ L 10% V/V DMSO in normal medium. The wells without cells but only 200 μ L normal medium were used as the blank control group (BC). Three wells were used for each concentration of the aforementioned drugs. After 24 h, the medium was removed and 200 μ L MTT solution (0.5 mg/mL) was added to each well and incubated at 37°C in dark Place for 4 h. The MTT solution was then removed, and 150 μ L DMSO was added to each well. Cell viability was calculated using the following formula: cell viability (%) = $\frac{([OD]_{test} - [OD]_{BC})}{[OD]_{PC}} \times 100$

$[(OD)_{SC} - (OD)_{BC}] \times 100\%$. Absorbance was read by Enzyme-labeled Instrument (BioTek, America) at 490 nm.

2.5 | SASP factors assays

According to the rate of plate laying is 80%–90%, fibroblasts induced by H_2O_2 according to point 2.3 were seeded into 6-well plates and incubated for 24 h. Then fibroblasts were treated by DOE and incubated for 24 h. The supernatants were collected and used to test the content of IL-6, MCP-1 and MMP-1 by ELISA kits. The PC of IL-6 content assay was 0.1 mg/g DXMS. The PC of MCP-1 content assay and MMP-1 content assay was 100 ng/mL TGF- β 1.

2.6 | Statistical analysis

All data were represented by mean $\pm$ SD ($n=3$) and were analyzed by one-way analysis of variance (ANOVA) using "Graph Pad Prism 9 Software (USA)". A p -value of <0.01 and <0.05 was considered as a statistically significant difference.

3 | RESULTS

3.1 | Establishment of the senescent model of H_2O_2 -induced fibroblasts

H_2O_2 is a commonly used inducer of cell senescence. It can affect cellular DNA directly, then lead to specific DNA damage response (DDR), thereby activate p53/p21 or p16/pRb pathways, induce cell senescence finally.^{8–11} One of the marks of cell senescence is that the activity of SA- β -gal is Enhanced. SA- β -gal can catalyze 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) to galactose, then form blue precipitates after dimerization, and the senescent cells are stained blue finally.^{12–15} In a variety of normal tissues, the expression of p16 gene is inhibited, and it can only be activated when the tissue is damaged or the cell has stress reaction. Therefore, in most senescent tissues, its expression level increases exponentially.^{16–23}

After fibroblasts were induced by H_2O_2 , the senescent cells are stained blue (Figure 1). The positive rate of SA- β -gal staining (Figure 2) and p16 mRNA expression (Figure 3) were significantly

higher than control group. It indicated that 400 μ M H_2O_2 could induce the senescence of fibroblasts successfully.

3.2 | Cell viability

The MTT assay was used to evaluate cytotoxicity of DOE on H_2O_2 -induced fibroblasts. Both the two concentrations of DOE had no significant effect on the viability of H_2O_2 -induced fibroblasts (Table 2). It indicated that the evaluated concentrations of DOE had no toxicity to H_2O_2 -induced fibroblasts.

3.3 | SASP factors assays

SASP is a typical characteristic of cell senescence, including cytokines, chemokines, growth factors, proteases, etc. IL-6 is one of the main cytokines in SASP signaling and plays a direct mediating role in inflammation. MCP-1, a member of chemokine CC subfamily, has a mediated effect on inflammatory response and can also induce the generation of inflammatory mediators.²⁴ MMP-1 is a proteolytic enzyme that promotes cell invasion and metastasis.²⁵

The content of IL-6, MCP-1 and MMP-1 were tested by ELISA kit. Based on the cytotoxicity results, two concentrations of DOE were selected for SASP factors assays. As shown in, Figure 4A–4C the content of IL-6, MCP-1 and MMP-1 decreased significantly after the treatment with DXMS or TGF- β 1. Also The content of IL-6, MCP-1 and MMP-1 decreased significantly with the treatment with different concentrations of DOE. It shows that DOE can inhibit the secretion of SASP factors in senescent fibroblasts.

4 | DISCUSSION

Fibroblasts are an important part of the dermis, which can synthesize and secrete collagen, elastin and other extracellular matrix (ECM) to maintain skin elasticity and prevent the senescence of skin. Traditionally, it is believed that the senescence of skin fibroblasts leads to the decrease of the number of fibroblasts, the change of their morphology, the weakening or decline of the function of secreting and synthesizing ECM. Eventually, skin aging appears. It is speculated that the senescence of fibroblasts may be the main

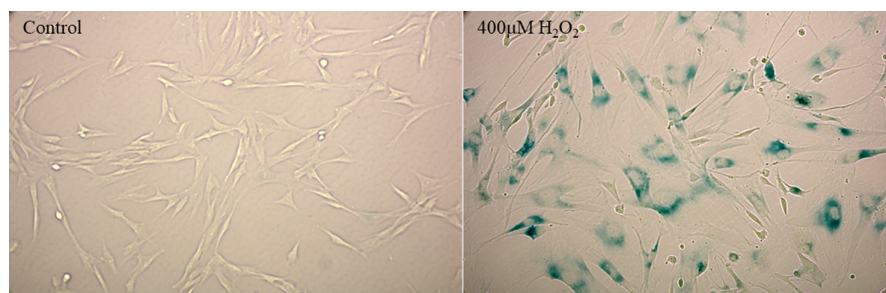


FIGURE 1 H_2O_2 -induced fibroblasts.

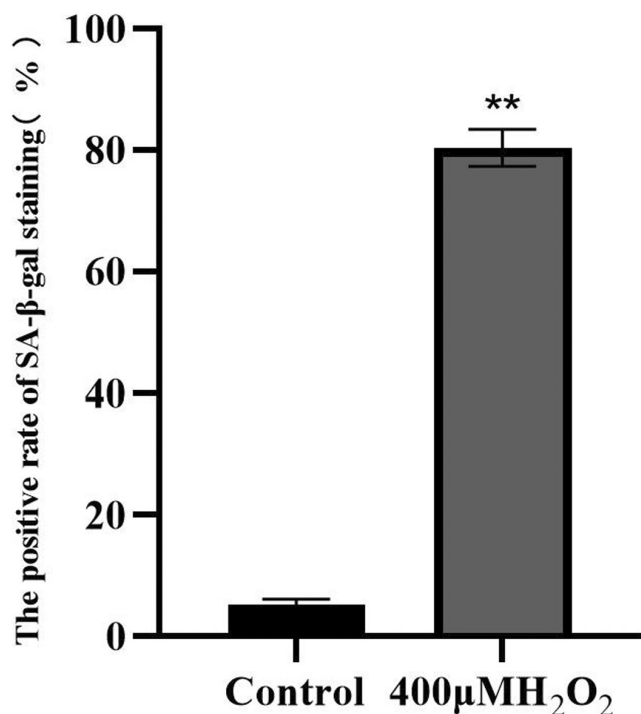


FIGURE 2 SA-β-gal staining of H₂O₂-induced fibroblasts. The results represent mean ± SD from three parallel experiments. $p < 0.01$ (**) means significantly different from the control value.

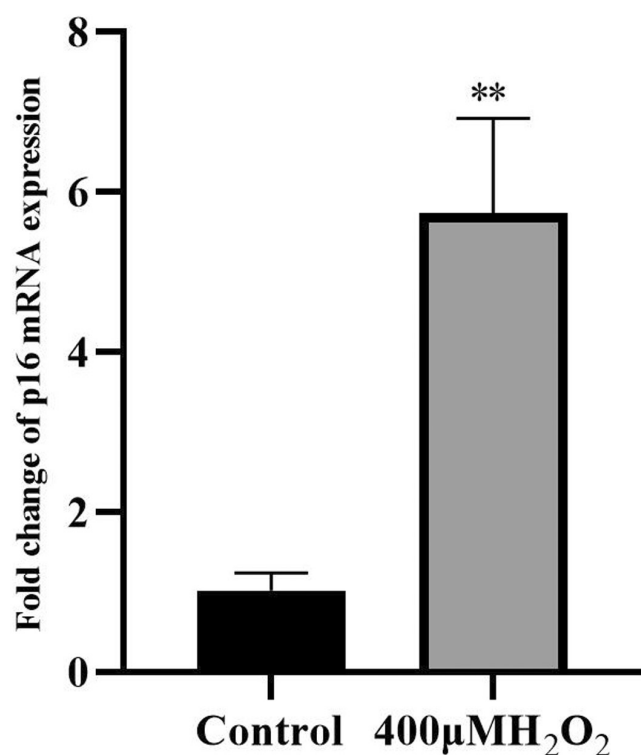


FIGURE 3 p16 mRNA expression of H₂O₂-induced fibroblasts. The results represent mean ± SD from three parallel experiments. $p < 0.01$ (**) means significantly different from the control value.

TABLE 2 Cell viability.

Test sample	Viability
SC	100.0% ± 2.92%
0.25% DOE	104.88% ± 0.3%
0.5% DOE	101.56% ± 1.18%

Note: The results represent mean ± SD from three parallel experiments. SC represents solvent control.

factor of skin aging. Therefore, slowing down or preventing the senescence of skin fibroblasts is one of the effective means to prevent skin aging. Fibroblasts are also a common cell type for studying the mechanism of cellular senescence in vitro. Recent SASP studies suggest that senescent fibroblasts can also enhance senescent phenotype and induce senescence of adjacent fibroblasts by secreting SASP factors. Therefore, further study to obtain SASP inhibitors of senescent fibroblasts can provide a new idea for the development of antiaging agents for skin.

In this study, fibroblasts were induced by H₂O₂, and the rate of the positive cells of SA-β-gal staining and p16 mRNA expression were used as indicators to evaluate the establishment of senescent model of fibroblasts. Three representative SASP factors, IL-6, MCP-1 and MMP-1, were selected to evaluate the effects of DOE on the secretion of SASP from H₂O₂-induced fibroblasts. The results showed that DOE could inhibit secretion of SASP from senescent fibroblasts. The effect of 0.25% DOE was better than 0.5% DOE. However, with only two concentrations, the relationship between concentration and efficacy cannot be determined. It was speculated that the two concentrations were not the best for DOE to inhibit the secretion of SASP by senescent fibroblasts, and further studies on the dose-effect relationship were needed. However, this did not affect our discovery that DOE can inhibit the secretion of SASP from senescent fibroblasts, which clarified the new antiaging mechanism of DOE, and provided more sufficient basis and more modern scientific support for its application in skin beauty.

5 | CONCLUSIONS

Traditional Chinese medicine (TCM) is a great treasure of China. *Dendrobium officinale* ranks first among the "nine immortal herbs" and has high value of beauty research. In this study, it was found that *Dendrobium officinale* could effectively inhibit the secretion of SASP. It shows that *Dendrobium officinale* has good antiaging ability and is a natural skin antiaging agent for skin. In the 21st century, green and natural are still important guidelines for global sustainable development. We deeply study TCM, especially valuable *Dendrobium officinale*, and use it in cosmetics. It will certainly be pursued and loved by people all over the world, and will also contribute to the sustainable development of the world.

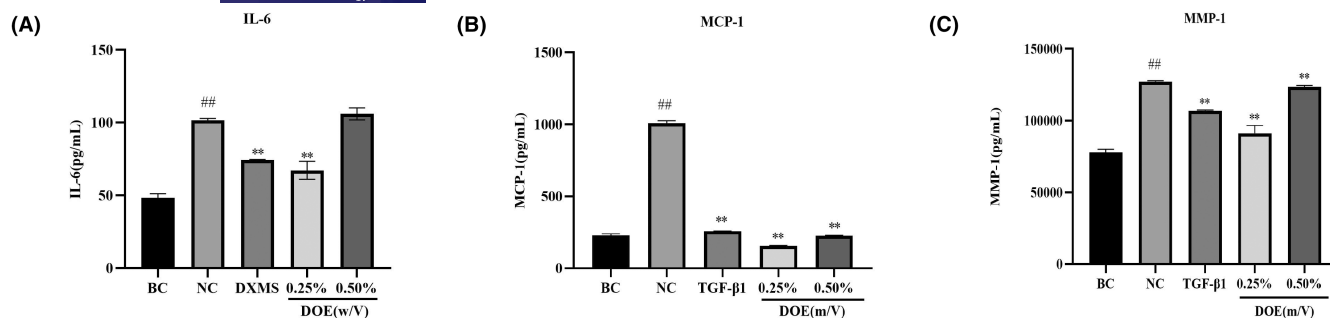


FIGURE 4 (A–C) The content of SASP factors. The results represent mean $\pm$ SD from three parallel experiments. BC represents normal fibroblasts. NC represents fibroblasts induced by H_2O_2 . $p < 0.01$ (##) means significantly different from BC. $p < 0.01$ (**) means significantly different from NC.

AUTHOR CONTRIBUTIONS

Lingyu Li: Conceptualization, investigation, methodology, visualization, writing—original draft. **Le Zhu:** Methodology, visualization, writing—review & editing. **Mo Chen:** Methodology. **Ya Zhao:** Project administration, writing—review & editing. **Haidong Jia:** Supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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