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Antioxidant and inhibition of senescence effects of embelin

Jin Jung Ahn¹ and Hee Jung Yong^{2*}

Abstract

Background: Embelin is a major active ingredient of *Embelia ribes* Burm. belonging to Myrsinaceae, an important traditional medicinal plant of Indian origin. Embelin has a benzoquinone derivative structure and has been studied for anticancer, anti-inflammatory, and antioxidative effects. However, there have been no studies on the use of cosmetic raw materials for embelin. In this study, antioxidant and inhibition of senescence effects of embelin were examined using hydrogen peroxide (H₂O₂)-induced cellular aging model of human dermal fibroblast.

Methods: Cell viability was measured using the principle of water-soluble tetrazolium salt (WST-1) assay. qRT-PCR was used to quantitatively analyze gene expression patterns in human dermal fibroblasts (HDFs) by embelin. Dichlorofluorescein diacetate (DCF-DA) was used to measure changes in intracellular reactive oxygen species (ROS) concentration. Cell senescence was measured using the senescence-associated β -galactosidase (SA- β -galactosidase) assay, a method of staining β -galactosidase. A wound healing assay was used to observe cell mobility.

Results: The WST-1 assay demonstrated that decreased cell viability by H₂O₂ was restored by embelin in a dose-dependent manner. In addition, the expression of *SOD1*, *GPx1*, and *CAT* genes were upregulated by embelin suggesting that embelin-induced antioxidant capability may be enhanced through the upregulation of intracellular antioxidant-related genes. To determine whether embelin inhibits cell senescence in H₂O₂-induced senescence model of HDFs, the SA- β -galactosidase assay was performed. Embelin decreased the SA- β -galactosidase activity in a dose-dependent manner in H₂O₂-treated HDFs. Moreover, the expression of *p21* and *MMP1* genes were reduced by embelin in H₂O₂-treated HDFs in a dose-dependent manner. However, *COL1A1* genes were increased by embelin in H₂O₂-treated HDFs in a dose-dependent manner.

Conclusions: These results suggest that embelin has a potential as an antiaging cosmetic ingredient with antioxidant and anti-senescence properties.

Keywords: Embelin, Antioxidative, Anti-senescence, Fibroblast, Hydrogen peroxide

Background

The skin consists of the epidermis, dermis, and subcutaneous tissue. The dermis, which occupies the majority of the skin, consists of cells and extracellular matrix (ECM). The ECM comprises fibrous proteins and substrates, such as collagenous fibers and elastic fibers (Braverman and Fonferko 1982). In the dermis, fibroblasts are the most representative constituent cells, which produce collagen, elastin, and substrate that controls wrinkles, elasticity, and water retention.

Dysfunction of fibroblasts in the dermis promotes the expression of various proteases, thereby promoting collagen and elastin degradation. As a result, the dermis contracts to form wrinkles, and elasticity and moisture decrease, resulting in skin aging (El-Domyati et al. 2002; Matsumura and Ananthaswamy 2004; Kang et al. 2005).

Such skin aging is divided into endogenous aging and extrinsic aging due to changes in skin structure and function caused by the passing of time or environmental factors (Uitto 1986; Campisi 1998). Endogenous aging is caused by the depletion of skin cells gradually with aging, which is mainly caused by reactive oxygen species (ROS) generated during cellular metabolism in skin cells;

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exogenous aging is caused by external environmental factors (Yoon et al. 2013).

ROS, which is the main cause of endogenous aging, has a higher reactivity than O_2 in the general molecular state and includes O_2^- (superoxide anion), H_2O_2 (hydrogen peroxide), and OH^- (hydroxyl radical) (Thannickal and Fanburg 2000). These ROS are constantly produced by normal intracellular activity, and most of them are removed by antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Urso and Clarkson 2003). The body's antioxidant enzyme, SOD, converts O_2^- to H_2O_2 and O_2 , and the H_2O_2 produced in this process is eliminated by GPx and CAT (Heck et al. 2003).

DNA damage caused by oxidative stress activates p53, a transcription factor that determines cell repair or apoptosis (Miliani de Marval and Zhang 2011), and expresses the senescence-promoting gene p21 (Smits and Medema 2001). p21 inhibits cyclin D/cyclin-dependent kinase 4 (CDK4) and cyclin E/cyclin-dependent kinase 2 (CDK2), thereby inhibiting cell growth (Stein and Dulic 1995).

A total of 90% of the dermis is occupied by collagen, contributing to the firmness and elasticity of the skin (Philips et al. 2011). ROS stimulate collagen degradation in the dermis by activating nuclear factor κ B (NF- κ B) and increasing the expression of matrix metalloproteinase 1 (MMP1) (Yoon et al. 2013) and collagen type I alpha 1 chain (COL1A1). Inhibition of this expression leads to skin elasticity and wrinkles, resulting in skin aging (Varani et al. 2002; Lee et al. 2012).

Embelin is a major active ingredient in *Embelia ribes* Burm. (Myrsinaceae), an important traditional medicinal plant in India. It has a benzoquinone derivative structure and a wide range of biological activities, such as antioxidant, anticancer, anti-inflammatory, analgesic, insect repellent, and antimicrobial effects (Radhakrishana and Gnanamani 2014). Research on embelin has focused on its effects against various cancers, such as pancreatic cancer and breast cancer (Mori et al. 2007; Li et al. 2013), its anti-inflammatory effects in arthritis (Dharmapatni et al. 2015), and its antioxidant effects in liver damage and diabetes (Singh et al. 2009; Naik et al. 2013). Other studies on its neuroprotective effects in brain cells have also been reported (Dhadde et al. 2016).

Research on the development of cosmetic materials for antiaging has been increasing worldwide, and anticancer, anti-inflammation, and antioxidant studies on embelin in the field of medicine have been reported, but to the best of our knowledge, research on embelin as a cosmetic raw material has not been conducted. Therefore, we investigated the antioxidative and senescence-inhibiting effects of embelin in H_2O_2 -induced human dermal fibroblasts, as well as the applicability of embelin as an antiaging component of cosmetics.

Methods

Cell culture

In this study, human dermal fibroblasts (HDFs) (ATCC, USA) were used. Cell culture was performed with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (penicillin 100 IU/mL, streptomycin 100 μ g/mL; Invitrogen, USA) in DMEM (Dulbecco's modified Eagle's medium), and the cells were cultured under conditions of 5% CO_2 and 37 °C.

Sample treatment

Embelin (Sigma-Aldrich, USA) was purchased in pure purified (>98%) powder form and dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at the appropriate concentration for use in the experiment. HDFs were cultured in a cell culture dish for 24 h. Embelin was added to the culture medium for 24 h, after which H_2O_2 was applied at a constant concentration for 3 h, followed by analysis.

Cell viability estimation

Cell viability was measured using the water-soluble tetrazolium salt (WST-1) assay. A total of 100 μ L of HDFs (3×10^3 cells/well) were inoculated on a 96-well plate and incubated for 24 h. Then, embelin was applied at concentrations of 2.5, 5, 7.5, and 10 μ M for 24 h. After incubation for 1 h, the absorbance was measured at 490 nm using a microplate reader (Bio-Rad, USA). This was repeated three times to derive the mean and standard deviation of cell viability.

qRT-PCR analysis

Quantitative real-time PCR (qRT-PCR) was performed for quantitative analysis of gene expression patterns in HDFs upon exposure to embelin. I mixed 0.2 μ M primers, 50 mM KCl, 20 mM Tris/HCl pH 8.4, 0.8 mM dNTP, 0.5 U Extaq DNA polymerase, 3 mM $MgCl_2$, and 1 \times SYBR Green (Invitrogen) in PCR tube to manufacture reaction solution and used Linegene K (BioER, China). After denaturation with temperature at 94 °C for 30 min, I performed denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and polymerization at 72 °C for 30 s for 40 cycles and proceeded PCR. I verified the effectiveness of PCR with melting curve, and by standardizing the expression of β -actin, I performed the comparative analysis for each gene expression. The primer used in experiment is shown in Table 1.

Cell senescence measurement

Cell senescence was measured by the senescence-associated β -galactosidase (SA- β -galactosidase) assay. The senescence detection kit (Biovision, USA) was used, which produced blue staining by degrading X-gal by β -galactosidase activation only in aged cells. Cells

Table 1 List of primers used in this study

Gene	Forward primer	Reverse primer
<i>β-actin</i>	GGATTCCTATGTGGGCGACGA	CGCTCGGTGAGGATCTTCATG
<i>SOD1</i>	GGGAGATGGCCCAACTACTG	CCAGTTGACATGCAACCGTT
<i>GPx1</i>	TTCCCGTGCAACCAGTTTG	GGACGCTACTTGAGGGAATTCAGA
<i>CAT</i>	ATGGTCCATGCTCTCAAACC	CAGGTCATCCAATAGGAAGG
<i>p21</i>	GTCCAGCGACCTTCTCATCCA	CCATAGCCTCTACTGCCACCATC
<i>MMP1</i>	TCTGACGTTGATCCAGAGAGCAG	CAGGGTGACACCACTGACTGCAC
<i>COL1A1</i>	AGGGCCAAGACGAAGACATC	AGATCACGTCATCGACAACA

were inoculated on a 60-mm culture dish at 2×10^5 cells/well and cultured for 24 h. Then, the cells were treated with the sample and the stimulus appropriately and further cultured. The medium was removed from the cultured cells and washed with 1 mL of PBS, and 0.5 mL of fixing solution was added thereto, followed by immobilization at room temperature for 15 min. A total of 0.5 mL of a staining solution (470 μ L of staining solution, 5 μ L of staining supplement, 20 μ L of 20 mg/mL X-gal in dimethylformamide) was added to the immobilized cells, and the cells were stained by incubation at 37 °C for 24 h. The stained cells were then washed with PBS, the number of stained cells was measured using an optical microscope (Olympus, Japan), and the ratio of cells was analyzed.

ROS measurement

Dichlorofluorescein diacetate (DCF-DA) was used to measure changes in intracellular ROS concentration. DCF-DA is oxidized by ROS in the presence of peroxides associated with hydrogen peroxide, resulting in green fluorescence (Karlsson et al. 2010). Cells were inoculated in a 60-mm culture dish at 2×10^5 cells/well, cultured for 24 h, treated with embelin, and cultured further. For harvesting of cells, 10 μ M DCF-DA, a staining reagent for measuring ROS, was added and incubated for 30 min. After the addition of PBS, the cells were released and analyzed by flow cytometry (BD Biosciences) to measure ROS change. *N*-Acetyl-L-cysteine (NAC; Calbiochem, USA), which has ROS scavenger function as a positive control, was also measured in the same manner to compare the efficacy of ROS removal.

Wound-healing assay

A wound-healing assay was used to observe the change of cell migration distance. This technique allows the movement of cells and their filling of a damaged area to be visualized. Cells were seeded on a six-well plate and cultured until reaching 90% confluence. After 24 h, the medium was changed to low-serum medium. A marker line was introduced on the bottom of the cell culture dish, and three scratched lines were created using the tip of a 200- μ L pipette. After 24 h, phase contrast $\times 10$

photographs were taken using an optical microscope (Olympus, Japan), and images were analyzed.

Statistical processing

All experiments in this study were performed in triplicate independently under the same conditions, and the results are expressed as mean \pm standard deviation. The test results were analyzed by Student's *t* test, and statistical significance ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$) was considered when the *p* value was 0.05, 0.01, or 0.001 or less.

Results

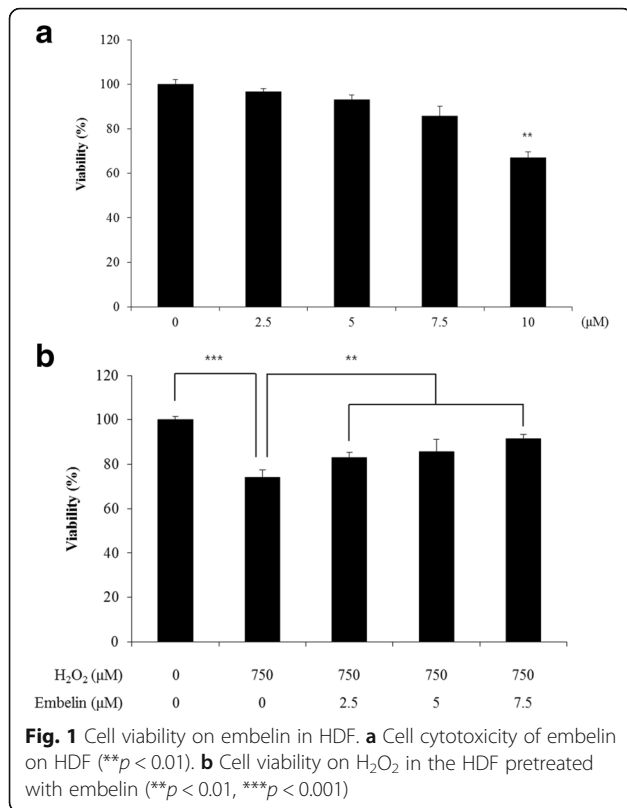
Cell viability analysis

The viability of HDFs was measured after human dermal fibroblast cell treatment with 2.5, 5, 7.5, and 10 μ M embelin for 24 h. The results showed that cell viability was 97% at 2.5 μ M, 93% at 5 μ M, and 86% at 7.5 μ M, depending on the concentration of embelin. However, cell viability was reduced to 67% at 10 μ M and was considered to be cytotoxic. Therefore, 7.5 μ M was used at the maximum concentration (Fig. 1a).

To investigate the cytoprotective effect of embelin against damage to human dermal fibroblast cells by H_2O_2 , embelin was pretreated for 24 h at 2.5, 5, and 7.5 μ M, followed by treatment with 750 μ M H_2O_2 for 3 h. Experimentally, at higher concentrations of hydrogen peroxide, more than half of the cell deaths were observed, making it difficult to confirm the effect on embelin. Cell viability was reduced to 74% with 750 μ M H_2O_2 without embelin treatment in HDFs. Cell viability was also reduced by embelin to 83, 86, and 91% upon pretreatment with 2.5, 5, and 7.5 μ M embelin (Fig. 1b).

Antioxidant effect of embelin

The effect of embelin on *SOD1*, a representative antioxidant enzyme, was confirmed by qRT-PCR. The results showed that *SOD1* gene expression decreased from 1 to 0.53 by 750 μ M H_2O_2 , but *SOD1* gene expression increased to 0.74, 0.88, and 0.97 after treatment with 750 μ M H_2O_2 after 2.5, 5, and 7.5 μ M embelin pretreatment (Fig. 2a).



The effect of embelin on the expression of the antioxidant enzyme encoded by the *GPx1* gene was confirmed by qRT-PCR. The results showed that *GPx1* gene expression was decreased from 1 to 0.73 by 750 μM H_2O_2 , but *GPx1* gene expression was increased to 1.04, 1.16, and 1.31 upon treatment with 750 μM H_2O_2 after 2.5, 5, and 7.5 μM embelin pretreatment, respectively (Fig. 2b).

The effect of embelin on the expression of the antioxidant enzyme encoded by the *CAT* gene was confirmed by qRT-PCR. The results showed that *CAT* gene expression was decreased from 1 to 0.72 by 750 μM H_2O_2 , but it increased to 0.88, 1.15, and 1.43 upon treatment with 750 μM H_2O_2 after pretreatment with embelin at 2.5, 5, and 7.5 μM , respectively (Fig. 2c).

DCF-DA was used to confirm that ROS in HDFs produced by H_2O_2 were removed by embelin. DCF-DA is deacetylated in cells and reacts with ROS to convert to DCF, a fluorescent substance. The fluorescence values of the DCF thus formed were measured using flow cytometry (BD Biosciences, USA). NAC, also known as a ROS scavenger, was compared with a positive control. The results showed that ROS production was increased from 1 to 4.05 upon treatment with 750 μM H_2O_2 but decreased to 2.81 and 1.19 upon treatment with 750 μM H_2O_2 after pretreatment with embelin at 2.5 and 7.5 μM . In the case of the positive control group NAC, ROS production was reduced to 1.12 upon treatment

with 750 μM H_2O_2 after 10 mM pretreatment (Fig. 2d). In particular, at low concentrations of embelin, the levels of NAC were similar to those of the positive control, suggesting that the ROS removal effect was superior.

Inhibition of senescence effect of embelin

The senescence-associated β -galactosidase (SA- β -galactosidase) assay was used to determine the effect of embelin on H_2O_2 -induced HDFs. The aging of cells increased the activity of SA- β -galactosidase at pH 6.0. The results showed that cell senescence was increased from 14 to 84% by 750 μM H_2O_2 , but after pretreatment with embelin at 2.5, 5, and 7.5 μM , treatment with 750 μM H_2O_2 was associated with reductions of cell viability by 60, 39, and 29%, respectively (Fig. 3a).

The expression of *p21* by embelin was confirmed by qRT-PCR. Specifically, the expression of the *p21* gene was increased from 1 to 9.86 by 750 μM H_2O_2 but was decreased to 7.50, 3.24, and 1.88 upon treatment with 750 μM H_2O_2 after pretreatment with 2.5, 5, and 7.5 μM embelin (Fig. 3b).

The expression of the *MMP1* gene, which degrades most of the dermis, type I collagen, was determined by qRT-PCR. The results showed that expression of the *MMP1* gene was increased from 1 to 4.48 by 750 μM H_2O_2 but was decreased to 3.39, 1.78, and 1.22 upon treatment with 750 μM H_2O_2 after pretreatment with embelin at 2.5, 5, and 7.5 μM , respectively (Fig. 3c).

To confirm the effect of embelin on skin aging, expression of the *COL1A1* gene, which encodes collagen type I, was determined by qRT-PCR. The results showed that the expression of the *COL1A1* gene was decreased from 1 to 0.60 by 750 μM H_2O_2 but was increased to 0.75, 0.81, and 0.84 upon treatment with 750 μM H_2O_2 after 2.5, 5, and 7.5 μM embelin pretreatment, respectively (Fig. 3d).

A wound-healing assay was also used to determine the effect of embelin on cell mobility. The results showed that cell mobility was decreased from 1 to 0.32 by 750 μM H_2O_2 , but cell mobility increased to 0.59, 0.69, and 0.98 upon treatment with 750 μM H_2O_2 after pretreatment with embelin at 2.5, 5, and 7.5 μM , respectively (Fig. 3e).

Discussion

Antioxidant effect of embelin

SOD, a typical antioxidant enzyme that reduces oxidative stress in humans, converts superoxide anion into hydrogen peroxide to lower the active oxygen concentration in the body (McCord and Fridovich 1969; Fridovich 1995). These SODs are classified into three types in humans: SOD1 is present in the cytoplasm, SOD2 is present in the mitochondria, and SOD3 is present outside the cell (Huang et al. 1999). Among these, SOD1 is the most important antioxidant enzyme and decreases ROS in all tissues in which oxygen, including cytoplasm, is present (Crapo et al. 1992).

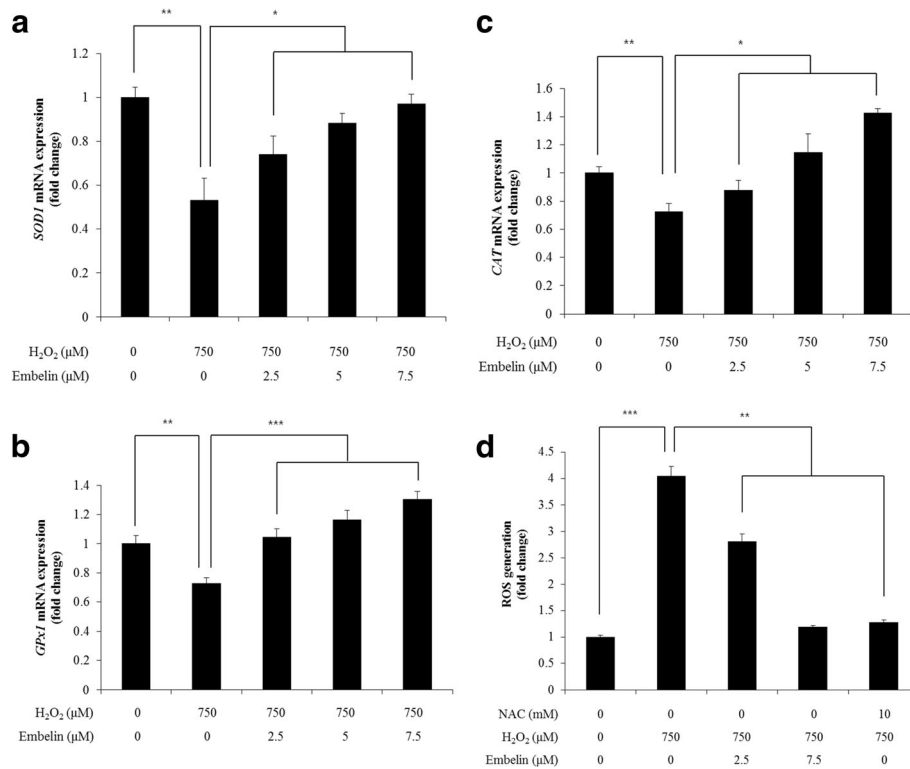


Fig. 2 Antioxidant effect of embelin. **a** Elevation of *SOD1* gene expression by embelin treatment in H₂O₂-treated HDFs (**p* < 0.05, ***p* < 0.01). **b** Elevation of *GPx1* gene expression by embelin treatment in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001). **c** Elevation of *CAT* gene expression by embelin in H₂O₂-treated HDFs (**p* < 0.05, ***p* < 0.01). **d** The ROS scavenging effect of embelin in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001)

Therefore, it is suggested that embelin induces the antioxidative effect of superoxide anion by converting it into hydrogen peroxide by increasing the expression of the *SOD1* gene, a cytotoxic antioxidant enzyme, in a concentration-dependent manner.

Among the antioxidant enzymes, SOD catalyzes the conversion of superoxide into O₂ and H₂O₂, and the H₂O₂ produced is decomposed into H₂O and O₂ by glutathione peroxidase 1 (GPx1). GPx1 is present in the cytoplasm and mitochondria and protects cells from oxidative damage (Masella et al. 2005; Orhan et al. 2005). Therefore, it is considered that embelin increases antioxidative enzyme *GPx1* gene expression in a dose-dependent manner, which is more effective in decomposing H₂O₂ into H₂O and O₂, which are in turn safer for the body than antioxidants.

The antioxidant enzyme CAT acts as a catalyst to convert H₂O₂ produced by SOD to H₂O and O₂ (Brioukhanov and Netrusov 2004). Therefore, it is considered that embelin increases antioxidative enzyme *CAT* gene expression in a dose-dependent manner and acts as a catalyst to convert H₂O₂ into noxious H₂O and O₂.

ROS produced by H₂O₂ damage the cellular function of normal skin and disrupt the balance of antioxidant defenses there, resulting in the loss of gloss, formation of wrinkles, and loss of elasticity (Yaar and Gilchrist 2007).

Embelin may decrease ROS that are increased by H₂O₂ in a dose-dependent manner and inhibit cellular damage of normal skin.

Inhibition of senescence effect of embelin

Aging of cells involves the in vivo loss of their regenerative ability, with no further cell division, and occurs when the DNA damage is slightly weaker than the level required to cause cell death (Campisi and d'Adda di Fagagna 2007). Senescence-associated β-galactosidase (SA-β-galactosidase) activity is a characteristic of aged cells and is widely used as an aging cell indicator (Dimri et al. 1995). In view of the obtained findings, H₂O₂ seems to induce cell senescence, and it is considered that embelin inhibits cell senescence in a concentration-dependent manner.

When DNA damage is caused by H₂O₂, p53 is activated and induces p21 gene expression. These results suggest that p21 inhibits the function of cyclin-dependent kinase (CDK), arrests the cell cycle, and inhibits cell growth (Smits and Medema 2001; Gewirtz et al. 2008). Therefore, it is considered that embelin inhibits cell senescence by activating CDK function by decreasing the expression of *p21*, a gene that stops the cell cycle and promotes senescence in a concentration-dependent manner.

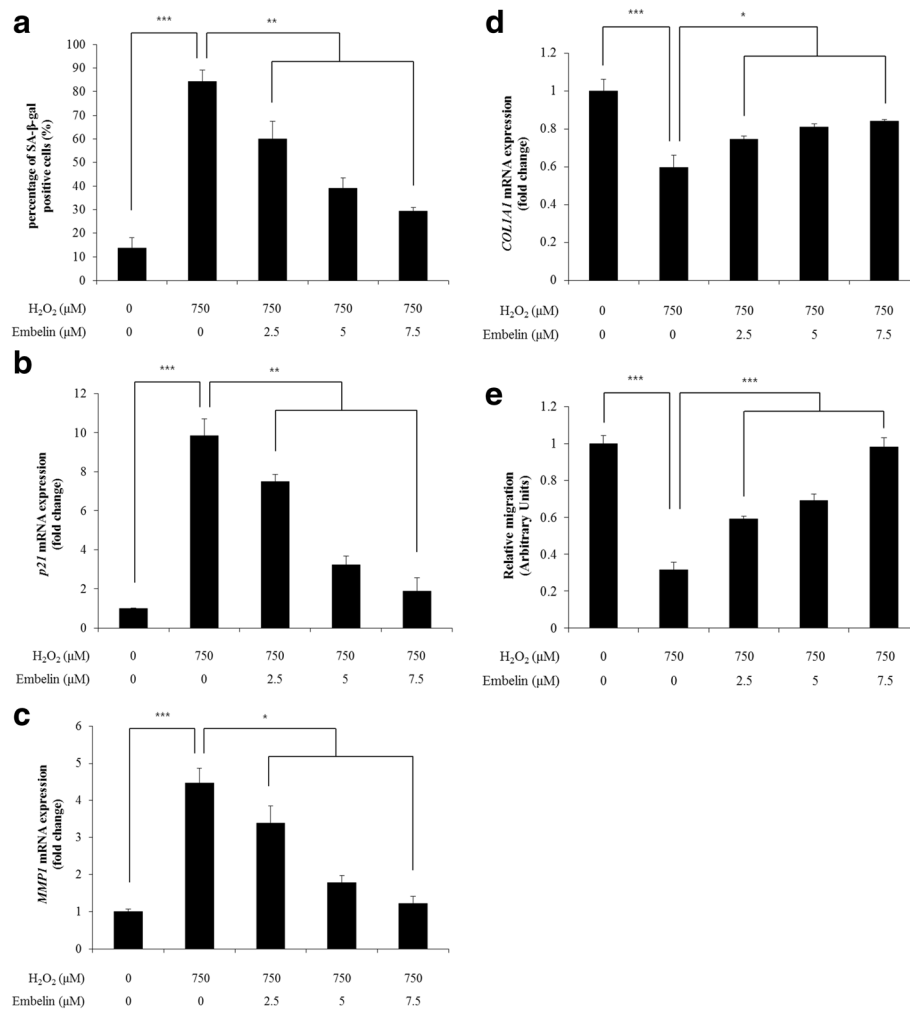


Fig. 3 Inhibition senescence effect of embelin. **a** Inhibiting of cellular senescence by embelin in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001). **b** Inhibition of *p21* gene expression by embelin in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001). **c** Inhibition of *MMP1* gene expression by embelin in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001). **d** Elevation of *COL1A1* gene expression by embelin treatment in H₂O₂-treated HDFs (**p* < 0.05, ***p* < 0.01). **e** Elevation of cell migration by embelin treatment in H₂O₂-treated HDFs (***p* < 0.01, ****p* < 0.001)

MMP1 is mainly produced or secreted by fibroblasts and is a representative enzyme that reduces the elasticity of the dermis and breaks down the collagen fibers; it is used as an index of skin aging with collagen fibers (Steinhoff et al. 2004; Lee et al. 2006). Therefore, it is concluded that embelin inhibits type I collagen degradation by inhibiting *MMP1* expression, which promotes collagen fiber degradation in a dose-dependent manner.

HDFs play a role in producing collagen, which accounts for 90% of the skin dermis. Collagen is involved in denseness of dermis, elasticity, binding force of tissue, adhesion of cells, cell proliferation, and differentiation (Perlish et al. 1988). Collagen has various types, of which 84% are type I. Type I is present in the skin, muscles, and bones (Cutroneo 2003). It is suggested that embelin increases *COL1A1* expression, which leads to collagen synthesis in a dose-dependent manner,

thereby activating collagen synthesis, which occupies most of the dermis.

Damaged cells express the genes typically expressed in cells such as fibroblasts to regain a normal state, accompanied by cell migration and cell proliferation. Cell proliferation and cell mobility of HDFs are essential for the retention of skin tissue and inhibition of aging (Stevenson and Thornton 2007). This study suggests that embelin activates the regeneration of cells damaged by H₂O₂ in a concentration-dependent manner and promotes cell mobility.

Conclusions

Research on the development of antiaging cosmetics has been increasing worldwide; anticancer, anti-inflammation, and antioxidant research in the fields of medicine and pharmacology for embelin has been reported, but research

on the use of embelin as a cosmetic raw material has not been conducted, to the best of our knowledge. To confirm the possibility of using embelin as an antiaging component of cosmetics, HDF was pretreated with embelin concentration and oxidative stress was induced by H_2O_2 . I confirmed the antioxidant and inhibition of senescence effect of embelin through the changes.

Here, I examined the cytotoxicity of embelin and its cytoprotective effect on H_2O_2 in HDFs. The results showed that the survival rates with embelin at 2.5, 5, and 7.5 μM were 97, 93, and 86%, respectively, but cell viability was reduced to 67% at a concentration of 10 μM . In subsequent experiments, 7.5 μM was thus applied as the maximum concentration. In addition, it was confirmed that cell viability was recovered in a concentration-dependent manner upon pretreatment of human dermal fibroblasts with 750 μM H_2O_2 at embelin concentrations of 2.5, 5, and 7.5 μM .

I confirmed the antioxidant effect of embelin in HDFs. Specifically, quantitative analysis of ROS by DCE-DA confirmed that ROS generated by H_2O_2 in HDFs were removed by embelin, showing that the radical scavenging ability increased in a concentration-dependent manner after embelin pretreatment, as was also the case for the positive control NAC. In addition, the expression of the *SOD1*, *GPx1*, and *CAT* genes, which encode antioxidative enzymes, was also increased in a dose-dependent manner after embelin pretreatment.

The inhibitory effect of embelin on HDFs was also confirmed. The activity of β -galactosidase, an indicator of aging cells, was decreased by embelin in a concentration-dependent manner. In addition, it was confirmed that aging was inhibited by decreasing the expression of *p21*, which is a gene that stops the cell cycle and promotes senescence. As the concentration of embelin increased, the expression of *MMP1*, a typical enzyme for reducing the elasticity of collagen and of the dermis, was also decreased in a dose-dependent manner. On the other hand, the expression of *COL1A1*, a gene that produces collagen type I, was increased in a dose-dependent manner. As a result of measuring cell mobility using a wound-healing assay, embelin is thought to stimulate cell regeneration and cell migration in a concentration-dependent manner.

The results of this study confirm that embelin restores the survival rate of HDFs damaged by H_2O_2 in a concentration-dependent manner and has antioxidant and antiaging effects. Therefore, it is considered that embelin can be used as a cosmetic material having antiaging effects on HDFs. However, further studies involving clinical trials are needed to verify the results of this work.

Abbreviations

CAT: Catalase; CDK2: Cyclin-dependent kinase 2; CDK4: Cyclin-dependent kinase 4; COL1A1: Collagen type I alpha 1 chain; DCF-DA: Dichlorofluorescein diacetate; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dimethyl sulfoxide; ECM: Extracellular matrix; FBS: Fetal bovine serum; GPx1: Glutathione peroxidase 1;

HDF: Human dermal fibroblast; MMP1: Matrix metalloproteinase 1; NAC: N-Acetyl-L-cysteine; NF- κ B: Nuclear factor κ B; p21: Cyclin-dependent kinase inhibitor 1A; PBS: Phosphate-buffered saline; qRT-PCR: Quantitative real-time polymerase chain reaction; ROS: Reactive oxygen species; SA- β -galactosidase: Senescence-associated β -galactosidase; SOD1: Superoxide dismutase 1; WST: Water-soluble tetrazolium salt

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Authors' contributions

JJ did all of the research background such as experiments, data collecting, and statistical analysis and drafted the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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