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Inhibitory effect of phytol on cellular senescence

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Abstract

Background: Phytol is a component of chlorophyll with demonstrated anticancer and immune-enhancing effects. In particular, it has been reported that phytol enhances the activity of natural killer cells that detect and remove cancer cells and promotes macrophage functions in immunity. In this study, based on the recently published precedent articles, we examined whether phytol can also inhibit cellular senescence to provide evidence for its possible use as a base material for cosmetics.

Methods: HaCaT keratinocytes were pretreated with phytol for 24 h and then treated with oxidative stress-inducing hydrogen peroxide (H_2O_2) for 6 h before the analysis; anti-inflammation analysis, cell cycle analysis, cytoprotection analysis, caspase 3 (CASP3) activity analysis, and senescence-associated β-galactosidase (SA-β-gal) assay were performed to examine the cell potency of phytol.

Results: In this study, HaCaT keratinocytes, treated with 750 μM H_2O_2, were tested with a phytol concentration below 10 μM to obtain the following results. First, phytol pretreatment suppressed H_2O_2-induced inflammation in a concentration-dependent manner, as indicated by the reduced expression of the mRNA levels of *TNF-α* (tumor necrosis factor-alpha), *IL6* (interleukin 6), *IL8* (interleukin 8), and *COX2* (cyclooxygenase 2). Second, the quantitative analysis of cell cycle, *NRF2* (nuclear factor erythroid 2-related factor 2), *HO-1* (heme oxygenase 1), *14-3-3σ* (Stratifin), *cyclin B* mRNA, and CASP3 was performed to examine the cytoprotective effects of phytol, as evidenced by increased mRNA expression of the cytoprotective genes *NRF2* and *HO-1* and decreased mRNA expression of the G2 cell cycle arrest-inducing genes *14-3-3σ* and *Cyclin B*. CASP3, a protein that induces apoptosis, was reduced in a concentration-dependent manner, confirming that phytol protected cells from apoptosis. Third, the SA-β-gal assay revealed that phytol reduced H_2O_2-induced senescence in a concentration-dependent manner, and the cellular senescence was delayed on treatment using phytol.

Conclusions: This study verifies that phytol can inhibit oxidative stress-induced senescence of HaCaT keratinocytes. The results suggest the use of phytol as a base material for cosmetics to inhibit cellular senescence.

Keywords: Phytol, HaCaT keratinocytes, Antioxidant, Anti-inflammation

Background

Oxidative stress induces both proinflammatory and cytoprotective genes. The NRF2-KEAP1 (kelch-like ECH-associated protein 1) system is one of the major intracellular mechanisms for maintaining reductive homeostasis in cells and for protecting cells from oxidative stress (Motohashi and Yamamoto 2004) (Fig. 1). Under normal physiological conditions, nuclear factor erythroid 2-related factor 2 (NRF2) resides in the cytoplasm bound to the actin-binding protein KEAP1. Under oxidative stress, however, NRF2 separates from KEAP1, becomes activated, and translocates to the nucleus where it binds to the antioxidant response element (ARE) promoter and increases the expression of antioxidant enzymes such as HO-1 (Paine et al. 2010; Kang et al. 2005). HO-1 plays an important role in regulating cellular heme levels by oxidation and degradation, which releases biliverdin, carbon monoxide, and free Fe^{2+}. Biliverdin is then converted to the antioxidant bilirubin by cytoplasmic reductase. In addition, HO-1 is a heat shock protein that protects cells and regulates inflammation when cells are exposed to various oxidative stressors such as hypoxia–reperfusion, heavy metals, ultraviolet (UV) light, H_2O_2,

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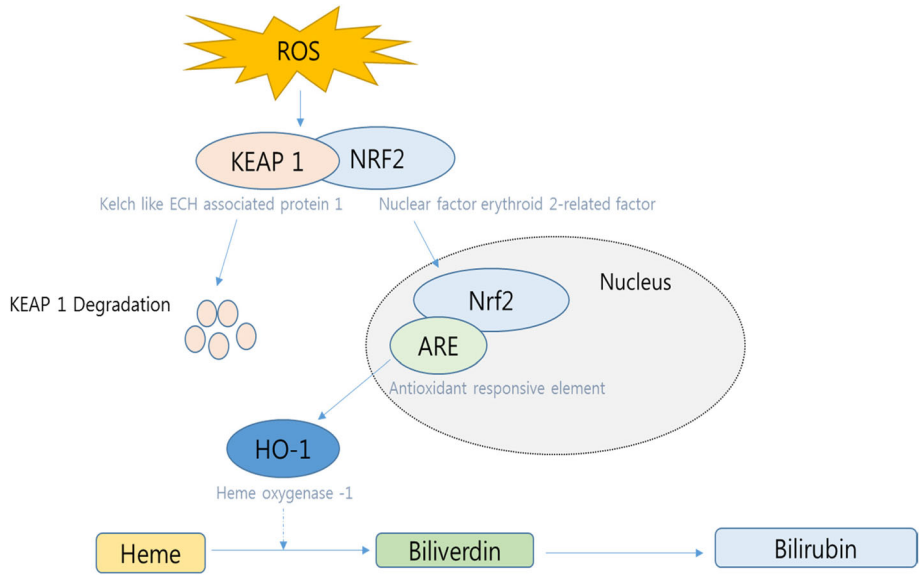

Fig. 1 Nrf2-antioxidant response element signaling pathway

and nitric oxide (NO) (Marcinkiewicz et al. 2006; Black et al. 2008).

The P53 (tumor protein P53) acts as an "emergency brake" for the cell cycle and determines whether cells damaged by reactive oxygen species (ROS) are repaired or proceed to apoptosis. If the P53 gene does not function properly, cells will replicate uncontrollably to form tumors. The P53 protein regulates the expression levels of various cell cycle-related genes such as cyclin-dependent kinase inhibitor 1A (*P21*), *14-3-3σ*, and *CASP3* (Kortlever et al. 2006). In particular, P21 promotes G1 cell cycle arrest, *14-3-3σ* and cyclin B promote G2 cell cycle arrest, and CASP3 triggers cellular apoptosis (Brown et al. 1997; El-Deiry 1998) (Fig. 2).

Phytol ($C_{20}H_{40}O$, molecular weight 296.5) is a component of chlorophyll that is known to be a strong cytoprotectant against oxidative stress. It is found at high levels in perilla leaves and shepherd's purse and is produced by chlorophyll hydrolysis upon destruction of plant tissue (Gross 1991). Phytol is known to have strong anticancer and immune-enhancing properties. Phytol not only enhances the activity of natural killer cells that remove cancer cells but also strengthens immunity by regulating macrophage function (Park 1978). Indeed, most recent research on phytol has focused on these anticancer and anti-inflammatory properties (Komiya et al. 1999; Silva et al. 2014). In contrast, few studies have evaluated the potential anti-senescence efficacy of phytol, a property desirable for antiaging products. Therefore, we examined the cytoprotective and anti-senescence effects of phytol to evaluate it suitability as a base material for cosmetics.

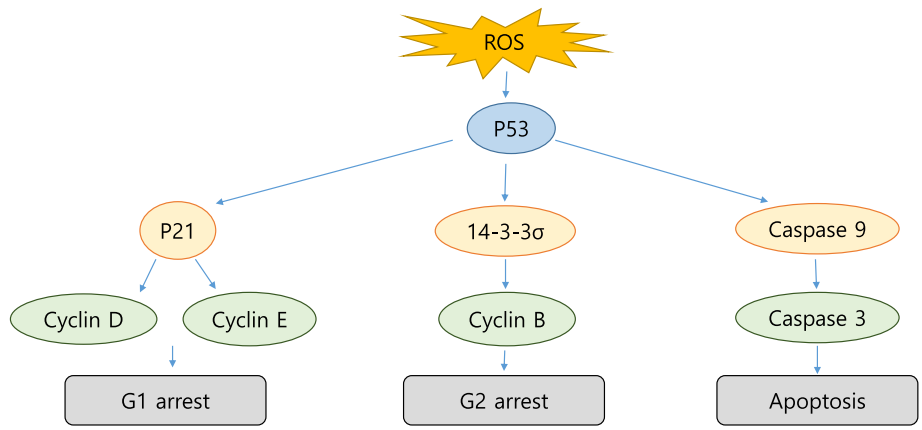

Fig. 2 Modeling of p53 pathway to cell cycle arrest and apoptosis

Methods

Cell culture

The human keratinocyte cell line HaCaT was purchased from the American Type Culture Collection (Manassas, VA, USA) for this study. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone™, GE Healthcare Life Sciences, Little Chalfont, Bucks, UK) supplemented with 10% fetal bovine serum (FBS; Hyclone™) and 1% penicillin/streptomycin (penicillin 100 IU/mL, streptomycin 100 μg/mL; Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C under a 5% CO_2 atmosphere.

Cell viability analysis

The water-soluble tetrazolium salt (WST-1) assay was performed to evaluate the cytotoxicity of phytol and H_2O_2 in HaCaT keratinocytes. Phytol was purchased in pure powder form (> 97%, Sigma-Aldrich, St. Louis, Mo, USA) and dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) before use. HaCaT cells (3×10^3 cells/well) were cultured on 96-well plates for 24 h and treated with phytol at 1, 2, 5, 10, and 20 μM for 24 h. Cells were then treated with 10 μL EZ-Cytox cell viability assay kit reagent (ItsBio, Seoul, Korea) and incubated for 6 h. Absorbance was measured at 490 nm on a microplate reader (Bio-Rad, Hercules, CA, USA). Each trial was repeated three times to obtain the mean and standard deviation of cell viability.

qRT-PCR analysis

After the indicated treatments, cells were dissolved in Trizol reagent (Invitrogen™) and cellular RNA was isolated by addition of 0.2 mL chloroform (Biopure, Tulln, Austria). The mRNA was dissolved in diethylpyrocarbonate (DEPC; Biopure) water for use in quantitative real-time polymerase chain reaction (qRT-PCR) assays. Only highly pure mRNA samples were used for qRT-PCR, as indicated by an 260/280 nm ratio ≥ 1.8 measured in a Nanodrop cuvette (Maestrogen, Hsinchu, Taiwan). PCR was validated via melting curve analysis, and the expression of each gene of interest was compared after standardization with respect to the expression of *β-actin*. All primers used are listed in Table 1.

To examine the cytoprotective effects of phytol, the expression levels of genes related to the NRF2-ARE and P53 pathways were measured by qRT-PCR. HaCaT cells (2×10^5 cells/well) were cultured for 24 h in 60-mm culture dishes and then pretreated with culture medium containing vehicle or 1, 5, or 10 μM phytol for 24 h. After washing with PBS, cells were treated with 750 μM H_2O_2 for 6 h, and the expression levels of *NRF2*, *HO-1*, *14-3-3σ*, and *Cyclin B* mRNAs were measured.

Cell cycle analysis

Cell cycle was analyzed by measuring the cell numbers in G0/G1, S, and G2/M stages using a BD FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA). Cells were seeded on 60-mm culture dishes at 2×10^5 cells/well, incubated for 24 h, and then pretreated with vehicle or phytol (1, 5, or 10 μM) for 24 h. After washing with PBS, 750 μM H_2O_2 was added for 6 h. Cells were then harvested and centrifuged at 5000 rpm for 5 min at 4 °C. The supernatant was removed, and 300 μL phosphate buffered saline (PBS) was added to resuspend the cell pellet. Then, 700 μL absolute ethanol was gradually added while vortexing. The cells were then frozen and stored at 4 °C for at least 3 h for immobilization, after which 1 mL PBS was added, and the supernatant was removed by centrifugation at 5000 rpm for 5 min at 4 °C. Cells were then resuspended in 200 μL propidium iodide (PI) staining buffer (Sigma-Aldrich) and allowed to stand at 37 °C for 1 h. HaCaT cells were centrifuged at 5000 rpm for 5 min at 4 °C to remove excess staining buffer, washed with PBS, and resuspended in 1 mL PBS. The number of cells in each cell cycle stage was measured using a BD FACSCalibur™ flow cytometer.

Quantitative analysis of CASP3 activity

The ApoAlert™ caspase-3 colorimetric assay kit (Takara Bio, Shiga, Japan) was used to examine the expression of CASP3, which induces apoptosis. HaCaT cells were seeded

Table 1 List of primers

Gene (symbol)	Forward primer	Reverse primer
β-actin	GGATTCCTATGTGGGCGACGA	CGCTCGGTGAGGATCTTCATG
14-3-3σ	GTGTGTCCCCAGAGCATGG	ACCTTCTCCCGGTACTCACG
TNF-α	CCCAGGGACCTCTCTCTAATC	GGTTTGCTACAACATGGGCTACA
IL6	TAACAGTTCCTGCATGGGCGGC	AGGACAGGCACAAACACGCACC
IL8	CTCTCTTGGCAGCCTTCC	CTC AATCACTCTCAGTTCTTTG
NRF2	TACTCCCAGGTTGCCCACA	CATCTACAAACGGGAATGTCTGC
HO-1	GCCTGCTAGCCTGGTTCAAG	AGCGGTGTCTGGGATGAACTA
COX2	CGCGGATCCGCGGTGAGAACCGTTTAC	GCGAGGAAGCGGAAGAGTCTAGAGTCGACC
Cyclin B	GGCCAAAATGCCTATGAAGA	AGATGTTTCCATTGGGCTTG

in 60-mm culture dishes at 2×10^5 cells/well for 24 h and then pretreated with vehicle or 1, 5, or 10 μM phytol for an additional 24 h. After PBS washing, cells were treated with 750 μM H_2O_2 for 6 h. The cell pellet was isolated as described above and resuspended in chilled cell lysis buffer, left on ice for 10 min, and centrifuged at 15,000 rpm for 3 min at 4 °C. The supernatant was added to 96-well plates containing ApoAlert™ reaction buffer and incubated at 37 °C for 30 min. Caspase-3 substrate was then added, the mixture was incubated for 1 h at 37 °C, and the absorbance was measured at 405 nm on a microplate reader (Bio-Rad).

Cell senescence analysis

The effect of phytol on cell senescence was examined using SA-β-gal detection kit (BioVision, Milpitas, CA, USA). HaCaT cells were seeded on 60-mm dishes at 2×10^5 cells/well, cultured for 24 h, pretreated with vehicle or phytol at 1, 5, or 10 μM for 24 h, and then with 750 μM H_2O_2. Keratinocytes were then removed from the medium, washed with 1 mL PBS, and fixed with 0.5 mL fixing solution at room temperature for 15 min. The fixed cells were incubated in 0.5 mL staining mixture containing 470 μL staining solution, 5 μL staining supplement, and 25 μL X-gal in dimethylformamide (DMF; 20 mg/mL) at 37 °C for 24 h. Cells were washed with PBS and the number of stained cells to the total was measured using an optical microscope (Olympus, Tokyo, Japan).

Statistics

All experiments in this study were conducted at least three times on separate cultures. Group means were compared by paired *t* tests. A *p* value of ≤ 0.05 was considered statistically significant.

Results

Cytotoxicities of phytol and H_2O_2

The WST-1 assay was performed to determine the cytotoxicities of phytol and H_2O_2 on HaCaT keratinocytes. Cell viability was 101% at 1, 106% at 2, 114% at 5, 109% at 10, and 103% at 20 μM phytol compared with the vehicle-treated control group, indicating that phytol alone is non-toxic to HaCaT cells at the concentrations used. Concentrations of 1, 5, and 10 μM were chosen for subsequent experiments (Fig. 3a). In contrast, cell viability was 98% at 100, 92% at 250, 85% at 500, 76% at 750, and 59% at 1000 μM H_2O_2 compared with the untreated control group. Due to the robust but not excessive cell death at this concentration, 750 μM H_2O_2 was chosen for subsequent experiments (Fig. 3b).

Anti-inflammatory effects of phytol

To examine the potential anti-inflammatory effects of phytol on HaCaT keratinocytes, the expression levels of proinflammatory genes were compared among control, H_2O_2-treated, and phytol-pretreated/H_2O_2-treated cultures. Treatment with H_2O_2 alone increased *TNF-α* mRNA expression by approximately 4.3-fold compared with untreated controls, whereas pretreatment with phytol markedly reduced this H_2O_2-induced response to 4.0-fold at 1, 2.5-fold at 5, and to 1.1-fold at 10 μM. This concentration-dependent decrease in *TNF-α* mRNA expression indicates that phytol has anti-inflammatory effects against H_2O_2 (Fig. 4a). Furthermore, H_2O_2 alone enhanced *IL6* mRNA expression by 6.7-fold and *IL8* mRNA expression by 8.7-fold compared with the untreated controls, whereas phytol pretreatment reduced the H_2O_2-induced *IL6* expression to 5.2-fold at 1, 3.8-fold at 5, and to 1.9-fold at 10 μM and *IL8* mRNA expression to 6.0-fold at 1, 4.0-fold at 5, and 3.2-fold at 10 μM (Fig. 4b, c). Finally, phytol reduced *COX2* mRNA upregulation induced by H_2O_2 alone to 0.91-fold at 1, 0.74-fold at 5, and 0.66-fold at 10 μM. Collectively, these results indicate that phytol is a potent suppressor of the H_2O_2-induced inflammatory responses (Fig. 4d).

Cell cycle recovery by phytol

Analysis of cell cycle progression in HaCaT keratinocytes using fluorescence-activated cell sorting indicated

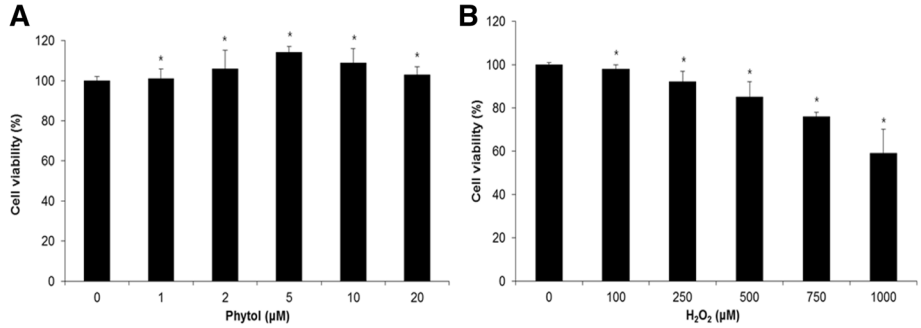

Fig. 3 Cytotoxicity of phytol and H_2O_2 in HaCaT keratinocytes. Cell viability of phytol (**a**) and H_2O_2 (**b**) on HaCaT cells was tested by performing WST-1 assay. The HaCaT (3×10^3 cells/well) were seeded on 96-well plates. Phytol and H_2O_2 were treated with each of indicated concentrations for 24 and 6 h. The data are expressed as means ± standard deviation from three independent experiments (*$p < 0.05$)

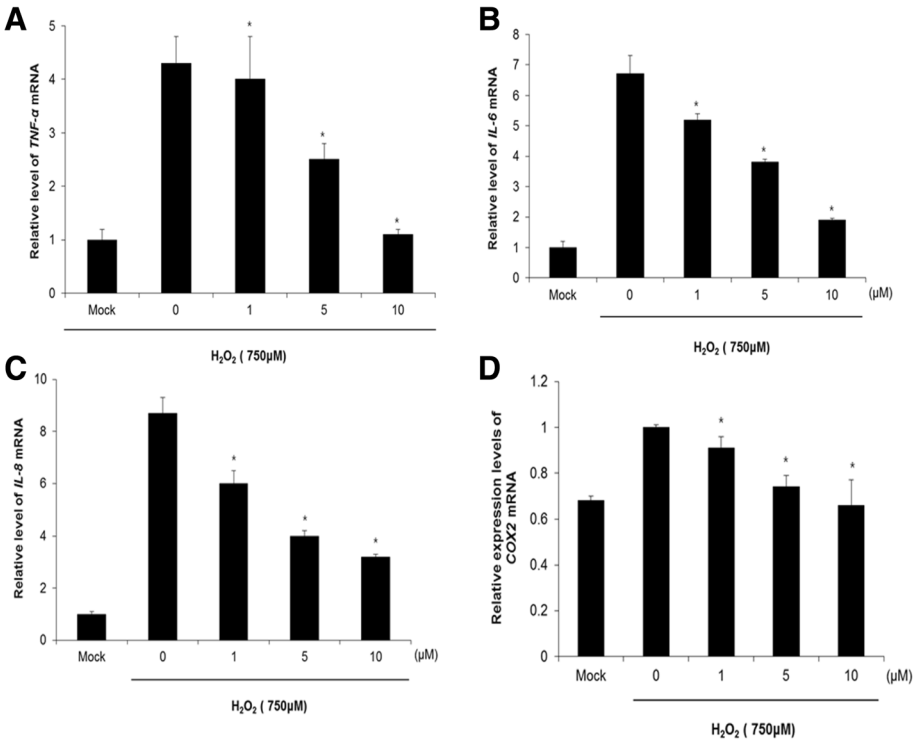

Fig. 4 Anti-inflammatory effect of phytol in HaCaT keratinocytes. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. Expression of *TNF-α* (**a**), *IL6* (**b**), *IL8* (**c**), and *COX2* (**d**) mRNA was determined using the qRT-PCR. The data are expressed as means ± standard deviation from three independent experiments (*$p < 0.05$)

that H_2O_2-treated cells aged more rapidly than the untreated control cells, as evidenced by a decreased proportion of HaCaT cells in the G0/G1 phase. Pretreatment with phytol (1, 5, and 10 μM) dose-dependently increased the proportion of H_2O_2-treated cells in the G0/G1 phase (Fig. 5). Therefore, phytol reversed the suppressive effect of H_2O_2 on cell cycle progression.

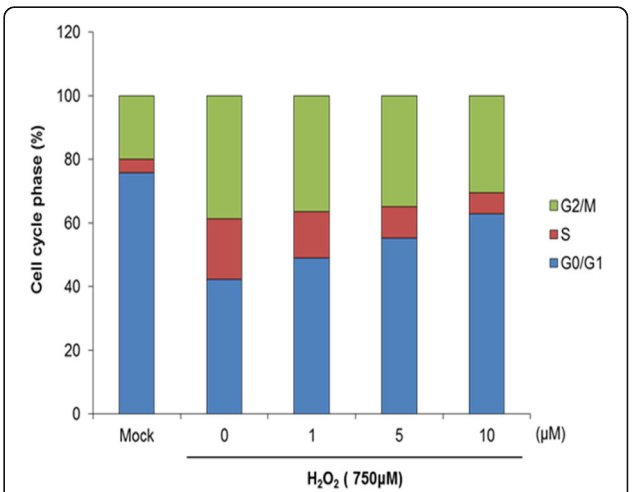

Fig. 5 Effect of phytol on cell cycle distribution by flow cytometry in HaCaT keratinocytes. Restoration of cell cycle arrest by phytol in H_2O_2-treated HaCaT keratinocytes was tested. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. After further incubation for 24 h, cell cycle was determined using the PI staining and BD FACSCalibur™ flow cytometer (*$p < 0.05$)

Cytoprotection by phytol through the NRF2-ARE signaling pathway

To examine if phytol can initiate a cytoprotective response against oxidative stress, the expression of *NRF2* mRNA was measured by qRT-PCR. The expression of *NRF2* mRNA was reduced 0.7-fold by H_2O_2 compared with the control group, whereas pretreatment with phytol increased the expression to 0.9-fold at 1, 1.4-fold at 5, and 1.6-fold at 10 μM, verifying its cytoprotective efficacy (Fig. 6a). The expression of *HO-1* mRNA was increased 7.9-fold by H_2O_2 alone compared with the control group, and phytol pretreatment enhanced this protective response, increasing the expression to 9.2-fold at 1, 11.8-fold at 5, and 16.6-fold at 10 μM (Fig. 6b).

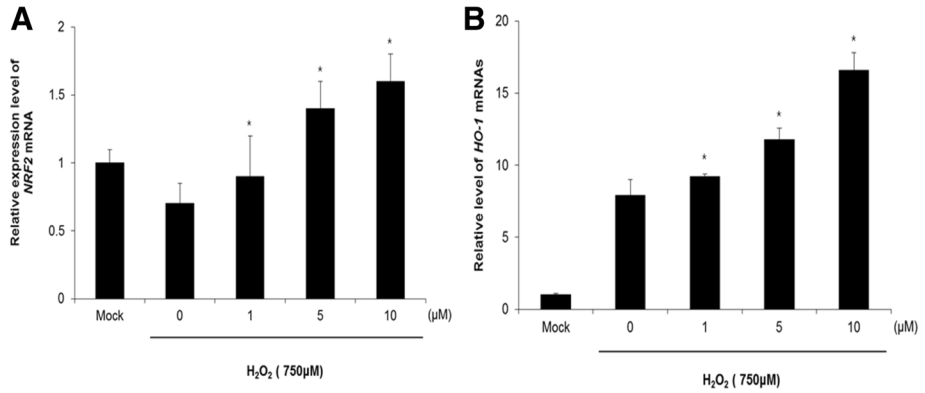

Fig. 6 Protective effect of phytol via NRF2-ARE pathway in HaCaT keratinocytes. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. Expression of *NRF2* (**a**) and *HO-1* (**b**) mRNA was determined using the qRT-PCR. The data are expressed as means ± standard deviation from three independent experiments (*$p < 0.05$)

Cytoprotective effect of phytol through suppression of P53 target genes

Cyclin B and 14-3-3σ are downstream targets of P53 known to arrest the cell cycle at G2. To investigate the cytoprotective efficacy of phytol via the P53 pathway, the mRNA expression levels of *14-3-3σ* and *Cyclin B* were examined in HaCaT keratinocytes. The expression level of *14-3-3σ* mRNA increased 3.1-fold and *Cyclin B* mRNA by 4.6-fold in H_2O_2-treated cells compared with untreated controls, whereas phytol pretreatment reduced *14-3-3σ* mRNA expression to 2.8-fold at 1, 1.5-fold at 5, and 0.9-fold (below baseline) at 10 μM and *Cyclin B* mRNA expression to 3.2-fold at 1, 2.0-fold at 5, and 1.2-fold at 10 μM, indicating substantial cytoprotective efficacy via P53 signaling (Fig. 7a, b).

Cytoprotective effect of phytol through inhibition of CASP3 activity

Caspases are involved in mediating cellular apoptosis signaling and directly degrading intracellular proteins (Riedl and Shi, 2004). CASP3 activity was measured to examine if phytol protects H_2O_2-treated HaCaT keratinocytes by suppressing apoptosis. Treatment with H_2O_2 alone increased CASP3 expression by 12.7-fold compared with the phytol and H_2O_2 untreated groups, whereas phytol pretreatment reduced this increase to 9.6-fold at 1, 5.8-fold at 5, and 4.1-fold at 10 μM, suggesting that cytoprotection by phytol is mediated in part by the suppression of apoptosis (Fig. 8).

Inhibitory effect of phytol on cellular senescence

In HaCaT keratinocytes, the expression ratio of SA-β-gal was 8% in phytol and H_2O_2 untreated control groups but was markedly increased to 87% by H_2O_2. However, the ratio of SA-β-gal decreased in a concentration-dependent manner to 49% at 1, 38% at 5, and 21% at 10 μM phytol, indicating that phytol can suppress cellular senescence induced by oxidative stress (Fig. 9).

Discussion

HaCaT keratinocytes were pretreated with phytol for 24 h and then treated with oxidative stress-inducing H_2O_2 for

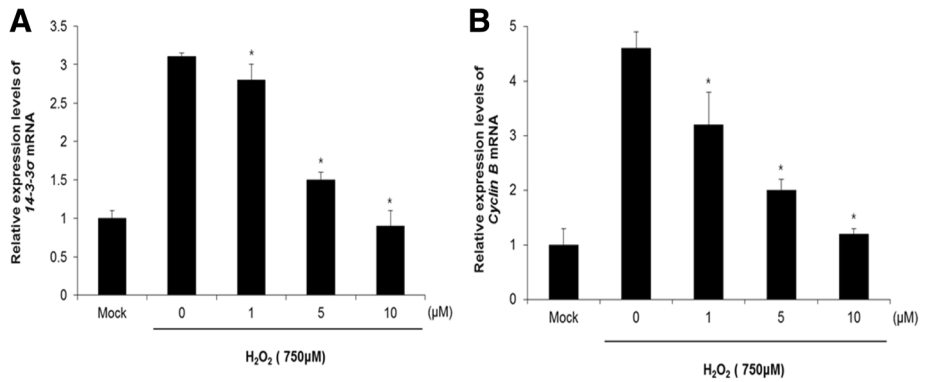

Fig. 7 Protective effect of phytol via P53 pathway in HaCaT keratinocytes. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. The expression of *14-3-3σ* (**a**) and *Cyclin B* (**b**) mRNA was determined using the qRT-PCR. The data are expressed as means ± standard deviation from three independent experiments (*$p < 0.05$)

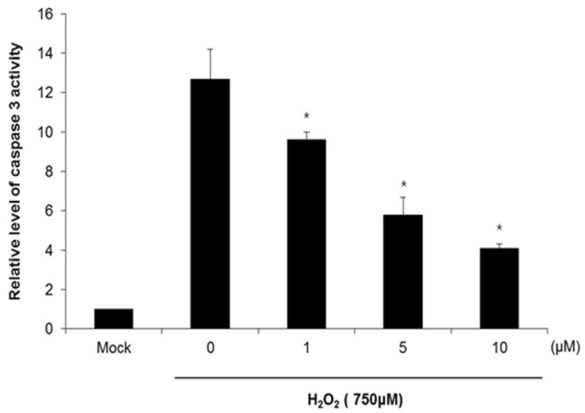

Fig. 8 Effect of phytol on levels of activated CASP3 in HaCaT keratinocytes. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. After further incubation for 6 h, level of activated CASP3 was determined using the CASP3 assay. The data are expressed as means ± standard deviation from three independent experiments (*$p < 0.05$)

6 h. Effects on inflammation, cell cycle, cytoprotective signals, CASP3, and senescence were assessed. The following results were obtained.

Firstly, phytol suppressed the H_2O_2-induced increases in mRNA expression levels of the inflammatory genes *TNF-α*, *IL6*, *IL8*, and *COX2* in a concentration-dependent manner. Environmental stressors such as ROS and foreign antigens induce the expression of pro-inflammatory cytokines and enzymes such as TNF-α, IL6, IL8, and COX2. TNF-α is produced mainly by

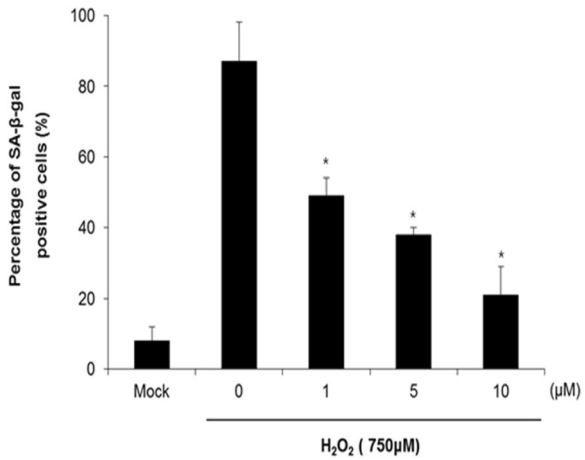

Fig. 9 Effect of phytol on senescence using SA-β-gal assay in HaCaT keratinocytes. HaCaT (2×10^5 cells/well) were seeded and then treated with the indicated concentrations of phytol for 24 h. Then, the cells were washed with PBS and exposed to H_2O_2 for 6 h. Cellular senescence by phytol was determined using the SA-β-gal assay. The data are expressed as mean ± standard deviation from three independent experiments (*$p < 0.05$)

macrophages and T lymphocytes in response to microbial infection, and it is involved in various inflammatory responses through activation of the stress-related transcription factor NF-κB (nuclear factor kappa B) (Wajant et al. 2003). The multi-functional cytokines IL6 and IL8 are known to mediate inflammatory and immune responses (Pang et al. 1994; Zhai et al. 2001). The amount of COX2 is increased in inflamed and cancerous tissues when stimulated by various growth factors, cytokines, tumor promoters, or TNF-α (Masferrer et al. 1994).

Secondly, the quantitative analysis of cell cycle, *NRF2*, *HO-1*, *14-3-3σ*, *Cyclin B* mRNA, and CASP3 to confirm the cytoprotective effect of phytol revealed that the mRNA expression levels of cytoprotective genes, *NRF* and *HO-1*, increased, whereas those of the G2 cell cycle arrest-inducing genes, *14-3-3σ* and *Cyclin B*, decreased, confirming the recovery of the cytoprotective effect by phytol. CASP3, a protein inducing apoptosis, decreased in a concentration-dependent manner, confirming that phytol protected cells from apoptosis.

The transcription factor NRF2 coordinates cellular defenses against oxidative stress by inducing antioxidant genes. Under non-stressed conditions, NRF2 exists in an inactive cytoplasmic form bound to KEAP1, an intracellular sensor of oxidative stress. Upon induction of oxidative stress, NRF2 separates from KEAP1 and translocates into the nucleus through phosphorylation, where it binds to ARE elements in the promoters of antioxidant genes such as *HO-1* (Itoh et al. 1999; Niture et al. 2009). HO-1 plays a protective role in maintaining cell homeostasis under various stresses in the environment. The protein HO-1 is widely expressed throughout the body and is induced when cells are exposed to various types of oxidative stressors such as hypoxia–reperfusion, heavy metals, high temperature, UV, and ROS (Marcinkiewicz et al. 2006; Black et al. 2008). The aging process is characterized by progressive oxidation and damage of cellular components partly due to the reduced expression of antioxidant defense system components, particularly genes under the control of the NRF2-ARE signaling pathway.

Cell replication, which is one of the most important characteristics of cells, proceeds through a tightly regulated series of steps comprising the cell cycle. Tissues maintain proper function and systemic homeostasis through cell cycle control or apoptosis, which allows each tissue to control cell number (Martinsoon et al. 1994). This process can be mainly divided into replication and division. The cell cycle comprises the G1 phase to prepare for growth and chromosomal replication, the S phase to clone chromosomes, the G2 phase to prepare for division, and the M phase of division. In addition, there is a G0 phase, in which cells no longer divide or participate in the cell cycle, i.e., differentiated cells that do not divide forever eventually pass from the G1 phase to the G0 phase. This transition from

one stage to the next is characterized by checkpoints (Hartwell and Weinert 1989). The most important factors regulating the cell cycle are cyclin and cyclin-dependent kinase, which are responsible for the initiation, progression, and completion of the cell cycle (Nurse 1994). These cell cycle factors determine whether to repair cell damage or induce apoptosis. Analysis of cell cycle progression in HaCaT keratinocytes using fluorescence-activated cell sorting indicated that H_2O_2-treated cells aged more rapidly than the untreated control cells. Pretreatment with phytol dose-dependently increased the proportion of H_2O_2-treated cells in the G0/G1 phase. Therefore, phytol reversed the suppressive effect of H_2O_2 on cell cycle progression.

Thirdly, the SA-β-gal cell aging assay showed that the concentration of SA-β-gal decreased in a concentration-dependent manner, confirming that the cellular senescence was delayed on treatment using phytol. Cellular senescence stops the cell cycle and changes the cell state (Collado et al. 2007; Kuilman et al. 2010). Cellular senescence is thought to underlie aging because the body loses the capacity of tissue repair (Campisi 2007). The SA-β-gal assay, which is based on the observation that β-gal activity detected at pH 6.0 increases as the cell enters senescence, was used to examine if phytol could promote cellular senescence recovery in response to oxidative stress.

Conclusions

In this study, the effect of phytol on the inhibition of cellular senescence in HaCaT keratinocytes was studied. Phytol is a component of chlorophyll, a green pigment in plant tissues, and has strong anticancer and immune-enhancing effects. It is known that this substance not only enhances the activity of natural killer cells that detect and remove cancer cells but also enhances immunity through macrophage function. Based on recently published articles related to anticancer and anti-inflammation efficacies, we tested the anti-senescence efficacy of phytol to determine its suitability as a base material for cosmetics.

In this study, phytol was shown to inhibit cellular senescence from H_2O_2-induced oxidative stress. These results suggest the possibility of use of phytol as a base material for cosmetics base material to inhibit cellular senescence.

Abbreviations

14-3-3σ: Stratifin; ARE: Antioxidant response element; CASP3: Caspase 3; COX2: Cyclooxygenase 2; DEPC: Diethylpyrocarbonate; DMEM: Dulbecco's modified Eagle's medium; DMF: Dimethylformamide; DMSO: Dimethyl sulfoxide.; FBS: Fetal bovine serum; H_2O_2: Hydrogen peroxide; HO-1: Heme oxygenase 1; IL6: Interleukin 6; IL8: Interleukin 8; KEAP1: Kelch-like ECH-associated protein 1; NF-κB: Nuclear factor kappa B; NO: Nitric oxide; NRF2: Nuclear factor erythroid 2-related factor 2; P21: Cyclin dependent kinase inhibitor 1A; P53: Tumor protein P53; PBS: Phosphate buffered saline; PI: Propidium iodide; qRT-PCR: Quantitative real-time polymerase chain reaction; ROS: Reactive oxygen species; SA-β-gal: Senescence-associated β-galactosidase; TNF-α: Tumor necrosis factor-alpha; UV: Ultraviolet; WST-1: Water-soluble tetrazolium salt

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Authors' contributions

SH did all of the research background such as the experiments, data collection, and statistical analysis as well as drafted the manuscript. The author 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 there are no competing interests.

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