Cnidium officinale Makino Promotes Skin Health via Anti-Inflammation Processes in Various Skin Cell Lines

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Research

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Abstract

Background Dermatitis is a worldwide health problem that is associated with quality of life. The skin continuously protects the body from the noxious environment. *Cnidium officinale* Makino (CM) is an herb used in traditional medicine to treat skin diseases.

Methods This study aimed to investigate whether CM exerted antioxidant and anti-inflammatory effects and to describe the effect of CM on the moisturizing and whitening of human mast cells (HMC-1), keratinocytes and melanocyte cells. Antioxidant activity was measured by a DPPH free radical assay. The mRNA expression of hyaluronan synthases 1, 2, and 3, Filaggrin, Claudin-4 and Aquaporin3 was measured by RT-PCR. Microphthalmia-associated transcription factor (MITF), tyrosinase, TRP1, TRP2, AKT, Erk and NF-kB protein levels were evaluated by Western blotting analysis.

Results We found that the levels of the DPPH free radical were decreased by CM treatments. CM exhibited anti-inflammatory activities, including the suppression of inflammation-associated molecules. We found that the levels of whitening-related proteins (MITF, tyrosinase, TRP1, and TRP2) were increased with CM treatment compared with α-MSH stimulation in B16F10 cells. CM induced the upregulation of hyaluronan synthases 1, 2, and 3, Filaggrin, Claudin-4 and Aquaporin3 mRNA expression in keratinocytes.

Conclusions These findings indicate that CM reduced several inflammatory responses. CM exhibited antioxidant, skin-moisturizing and whitening activity, indicating that CM might be a useful drug for combating inflammation and in skin care.

Background

Dermatitis is a worldwide health problem that is associated with quality of life. Millions of people worldwide suffer from inflammatory skin disorders [1, 2]. The skin is the largest organ of the human body, and it is extensively exposed to the external environment. Additionally, it functions as the necessary interface between the internal and the external environment. The skin continuously protects the body from the noxious environment. The poor appearance of the skin resulting from dermatitis affects not only the body but also the mental condition of the patient. Therefore, general skin condition is an important indicator of health [3, 4].

Inflammation is a response to stimuli such as infections and tissue injury and leads to inflammatory cell migration, cytokine, prostaglandin, and leukotriene production and proinflammatory molecule release. During inflammation, infiltrating neutrophils and cytokines are released [5–7]. As a result, uncontrolled or sustained inflammation induces several pathophysiological conditions, such as bacterial sepsis, rheumatoid arthritis, rhinitis, and skin inflammation [8–10]. Recently, moisturizers, antihistamines and corticosteroids have been used to treat skin inflammation, repair altered skin barrier function and reduce itching, but the use of steroids causes skin atrophy by reducing the amount of collagen [11]. A new therapy and intensive studies are needed. Previous studies have shown that various herbal medicines exert antioxidative, anti-inflammatory, and antimicrobial effects in animal models, thus increasing their

use for therapeutic purposes [12, 13]. Thus, herbal medicine is emerging as a novel alternative source of antioxidative and anti-inflammatory agents in food and cosmetics [14, 15].

Mast cells are activated by IgE through the high-affinity IgE receptor, and activated mast cells secrete inflammatory mediators, histamine, leukotrienes, prostaglandin E2, cytokines and chemokines. As a result, mast cells mediate various immune responses and regulate allergic inflammation, including atopic dermatitis [16, 17]. Additionally, keratinocytes play a pivotal role in the pathogenesis of inflammatory skin diseases, and activated keratinocytes induce skin inflammation by secreting Th2-related cytokines and chemokines [18, 19]. These Th2-related cytokines and chemokines stimulate the infiltration of immune cells, including mast cells, into the site of inflammation on the skin and cause skin dermatitis [20, 21]. Thus, inhibitors of these inflammatory mediators can be used for the treatment of inflammatory skin disease.

The root of *Cnidium officinale* Makino (CM) is a perennial plant in the Umbelliferae family and is extensively cultivated in Korea, China and Japan. CM is traditional herbal medicine called “Chunkung” in Korea and has been used as a medicinal plant for a long time in Asia [22–25]. CM contains many volatile phthalide derivatives that have been shown to have diverse pharmacological activities, including sedative, antianemia, antifungal, smooth muscle relaxing, and anticomplementary properties [26, 27]. In folk medicine, the dried rhizomes of CM are used to treat pain, inflammation, menstrual disturbance, vitamin deficiency, headache, and rheumatic arthralgia, and CM acts as a depressant of blood pressure [26]. In addition, there are several reports suggesting that CM inhibited tumor metastasis and angiogenesis; CM has been reported to have anticancer effects on liver cancer [28], colorectal cancer [29], multiple myeloma, and oral cancer [30]. Additionally, CM has potential roles in the chemoprevention of DNA damage and apoptosis induced by ultraviolet B radiation and reduces the content or impact of ROS [31].

Therefore, CM has a number of potential uses in various health-related fields, including the food processing, pharmaceutical, and cosmetics industries. However, the effect of CM on inflammation of the skin has not yet been elucidated. In the present study, we investigated the effects of the CM on skin inflammation.

Methods

Preparation of CM

CM was supplied by Han-poong Pharm Co., Ltd. (Jeonjoo, Republic of Korea). CM powder was dissolved in distilled water to a concentration of 20 mg/ml.

Cell Culture

Human mast cells (HMC-1) were obtained from the American Type Culture Collection (ATCC), and human keratinocyte HaCaT cells and mouse melanoma B16F10 cells were obtained from Korea Cell Line Bank (KCLB, Seoul, Korea). HMC-1 and B16F10 were maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and 100 U/mL antibiotic-antimycotic (Invitrogen). HaCaT cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic-antimycotic. Cells were maintained at 37 °C in a humidified incubator with 5% CO2.

Cell Viability Assay

An MTS assay was performed to determine cell viability. In this assay, cells (HMC-1, HaCaT and B16F10 cells) were seeded in a 96-well plate at a density of 3×10^3 cells per well and treated 24 h later with varying concentrations of CM (5–1000 μg/mL) for an additional 24 h. HaCaT cells were treated with 1 μg/mL LPS, and B16F10 cells were treated with 100 ηM α-melanocyte-stimulating hormone (α-MSH) in the presence or absence of various concentrations of CM. Ten microliters of a solution of tetrazolium salt (WST) was added to each well of the plate, which was incubated in the dark at 37 °C for another 1 h. Optical density was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (Versa Max; Molecular Devices LLC, Sunnyvale, CA, USA).

RT-PCR

RNA was isolated using an Easy-Blue RNA Extraction Kit (iNtRON Biotech, Republic of Korea). In brief, we harvested cells (HMC-1, HaCaT and B16F10 cells), and 1 mL of R&A-BLUE solution was added to each well. Next, 200 μL of chloroform was added to the lysate, and the mixture was vigorously vortexed for 10 seconds. Then, the lysate was centrifuged at 13,000 rpm for 10 min at 4 °C. We then transferred the appropriate volume of the aqueous phase into a clean tube, added 400 μL of isopropanol and thoroughly mixed the solution by inverting the tube 5 times. After centrifuging the tube at 13,000 rpm for 10 min, the supernatant was carefully removed without disturbing the pellet. Then, 1 mL of 75% ethanol was added, and the solution was thoroughly mixed by inverting the tube 4–5 times. The mixture was then centrifuged for 1 min at room temperature, and the supernatant was carefully discarded without disturbing the pellet. Finally, the remaining RNA pellet was dried and then dissolved in 20–50 μL of RNase-free water. The concentration of the isolated RNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA). We treated each sample with DNase. Two micrograms of total cellular RNA from each sample was reverse-transcribed using a cDNA synthesis kit (TaKaRa, Otsu, Shinga, Japan). PCR was conducted in a 20-μL reaction mixture composed of a DNA template, 10 pM of each gene-specific primer, 10x Taq buffer, 2.5 mM of dNTP mixture, and 1 unit of Taq DNA polymerase (Takara, Otsu, Shinga, Japan). PCR was performed using the specific primers listed in Table 1.

Table 1
PCR primer sequences

	Primer name		Sequences
Human	Aquaporin3	Forward	5′-AGA CAG CCC CTT CAG GAT TT-3′
		Reverse	5′-TCC CTT GCC CTG AAT ATC TG-3′
Human	Claudin 4	Forward	5′-ACT TTG ATA ACT GCT CCT CTG AC-3′
		Reverse	5′-TTC GTG TCC AGC AGA GTA CC-3′
Human	Filaggrin	Forward	5′-AGT GCA CTC AGG GGG CTC ACA-3′
		Reverse	5′-CCG GCT TGG CCG TAA TGT GT-3′
Human	Hyaluronan synthase 1	Forward	5′-GGC TTG TCA GAG CTA CTT C-3′
		Reverse	5′-GCC ACG AAG AAG GGG AA-3′
Human	Hyaluronan synthase 2	Forward	5′-ATG CAT TGT GAG AGG TTT CT-3′
		Reverse	5′-CCA TGA CAA CTT TAA TCC CAG-3′
Human	Hyaluronan synthase 3	Forward	5′-CTT AAG GGT TGC TTG CTT GC-3′
		Reverse	5′-GTT CGT GGG AGA TGA AGG AA-3′
Human	IL-4	Forward	5′-TGC CTC CAA GAA CAC AAC TG-3′
		Reverse	5′-CTC TGG TTG GCT TCC TTC AC-3′
Human	IL-6	Forward	5′-AAC CTT CCA AAG ATG GCT GAA-3′
		Reverse	5′-CAG GAA CTG GAT CAG GAC TTT-3′
Human	IL-8	Forward	5′-TCA GTG CAT AAA GAC ATA CTC C-3′
		Reverse	5′-TGG CAT CTT CAC TGA TTC TTG-3′
Human	IL-13	Forward	5′-GGT CAA CAT CAC CCA GAA CC-3′
		Reverse	5′-TTT ACA AAC TGG GCC ACC TC-3′
Human	IL-17	Forward	5′-TCA TCC ATC CCC AGT TGA TT-3′
		Reverse	5′-GAG GAC CTT TTG GGA TTG GT-3′
Human	TNF-α	Forward	5′-TGA GCA CTG AAA GCA TGA TCC-3′
		Reverse	5′-ATC ACT CCA AAG TGC AGC AG-3′
Human	GAPDH	Forward	5′-CGT CTT CAC CAC CAT GGA GA-3′
		Reverse	5′-CGG CCA TCA CGC CAC AGT TT-3′

Western Blot Analysis

Cells (HMC-1, HaCaT and B16F10 cells) were lysed with cell lysis buffer (50 mM Tris-Cl at pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, and protease inhibitor). Twenty micrograms of protein was separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Protran nitrocellulose membrane, Whatman, UK). The membrane was blocked with 5% nonfat milk and BSA, probed with specific primary antibodies, incubated with HRP-conjugated secondary IgG antibodies (Calbiochem, San Diego, CA, USA), and visualized using an enhanced chemiluminescence detection system (Amersham ECL kit, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). The antibodies against COX-2 (#4842), p-AKT (S473) (#9271), total AKT (#9272) and phospho-NF-κB p65 (Ser536) (#3033) were obtained from Cell Signaling Technology (Danvers, MA, USA). The antibodies against actin (sc8432), microphthalmia-associated transcription factor (MITF) (sc-71588), p-Erk (sc-7383), total Erk (sc-1647), total NF-κB (sc-8008), TRP1 (sc-136388), TRP2 (sc-74439) and tyrosinase (sc-20035) were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). The tubulin (T5168) antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA).

ELISA

The levels of IL-4 (BD 555194), IL-6 (BD 555220), IL-8 (BD 555244) and tumor necrosis factor (TNF) (BD 555212) were assessed using a Duoset ELISA system (BD Biosciences, USA) according to the manufacturer's instructions. In brief, to assess the levels of IL-4, IL-6, IL-8 and TNF in HMC-1 cells treated with CM, phorbol myristate acetate (PMA) and A23187, 96-well plates were coated with capture antibody in ELISA coating buffer and incubated overnight at 4 °C. The next day, the plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T). Then, the plates were subsequently blocked with 10% FBS in PBS for 1 h at room temperature. Serial dilutions of standard antigen or sample in dilution buffer (10% FBS in PBS) were added to the plates, and the plates were incubated for 2 h at room temperature. After the plates were washed, biotin-conjugated anti-mouse IgE and streptavidin-conjugated horseradish peroxidase (SAv-HRP) were added to the plates, and the plates were incubated for 1 h at room temperature. Finally, the tetramethylbenzidine (TMB) substrate was added to the plates, and after 20 min of incubation in the dark, 50 µL of 2 N H2SO4 was added to stop the reaction. The optical density was measured at 440 nm on an automated ELISA reader. (Versa Max, Molecular Devices, CA, USA).

Dpph Free Radical Scavenging Activity Assay

The 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of garlic extract (GF) was evaluated using a method modified from Blois [32]. Briefly, a freshly prepared 100 μL of DPPH solution (0.2 mM DPPH in 95% ethanol) was added to 100 μl of GF. After shaking, the mixture was incubated for 45 min in darkness. The absorbance was then measured at 520 nm using an ELISA plate reader (Versa Max; Molecular Devices LLC, Sunnyvale, CA, USA).

Cell Migration

The human keratinocyte HaCaT cells were incubated at 3×10^5 cells/mL for 24 h in a cell culture incubator. Next, the cell monolayers were scratched with a 200-μL yellow tip and washed once with PBS. Then, the cell monolayers were treated with different concentrations of GF and cultured in a CO_2 incubator for 24 h. Cell motility was assessed 24 h later, using a photomicroscope, and the scratched area was measured. Measurements were taken to determine the distance traveled in the 24 h period by measuring the scratched area by light microscopy (Olympus, Tokyo, Japan).

Melanin Content Assay

The melanin content assay was performed as previously described with some modifications [33]. The mouse melanocyte B16F10 cells were treated with α-MSH (100 ηM) for 24 h and further treated with different concentrations of GF for another 24 h. After the treatments, the cells were detached by incubation with trypsin and subsequently centrifuged at 5000 xg for 5 min. Then, the cell pellets were solubilized in 1N NaOH at 60 °C for 60 min. The melanin content was assayed at 420 nm by an ELISA plate reader (Versa Max; Molecular Devices LLC, Sunnyvale, CA, USA).

Statistical Analysis

All experimental results are expressed as the mean ± SEM of at least three separate tests. Statistical significance was determined at $P < 0.05$, $P < 0.01$ and $P < 0.001$ and is indicated with different symbols in the figures. Statistical analyses (ANOVA) were performed using PRISM software (GraphPad Software Inc., La Jolla, CA, USA).

Results

CM Inhibited Agonist-Induced Inflammatory Cytokine Production in HMC-1 Cells

To investigate whether CM affects cytokine expression in HMC-1 cells, we stimulated HMC-1 cells with A23187 and PMA before treatment with varying concentrations of CM. No significant effect on cell viability was observed in the HMC-1 cells treated with CM alone or in combination with A23187 and PMA (Fig. 1A). Western blot analysis indicated that CM significantly reduced the agonist-stimulated protein expression of AKT, Erk, NF-κB and COX-2 in a dose-dependent manner (Fig. 1B). Moreover, RT-PCR analysis showed that CM dose-dependently suppressed the mRNA expression of IL-6, IL-8, IL-13, IL-17 and TNF-α that was induced by treatment with A23187 and PMA (Fig. 1C). We also demonstrated that CM inhibited the agonist-stimulated secretion of IL-6, IL-8, and TNF, as determined by ELISA (Fig. 1D).

Cm Suppressed Lps-induced Inflammatory Responses In Hacat Cells

Furthermore, we evaluated the anti-inflammatory activities of CM in keratinocytes. Similar to HMC-1 cells, HaCaT cells showed no significant effect of toxicity was observed either when treated with CM alone or in combination with LPS (Fig. 2A). Western blot analysis demonstrated that treatment with a high dose of CM reduced the levels of COX-2, p-AKT, and p-Erk and the activity of NF-KB in LPS-induced HaCaT cells (Fig. 2B). Finally, CM treatment decreased the mRNA levels of proinflammatory cytokines, including IL-6, IL-13, and TNF-α, in LPS-stimulated HaCaT cells (Fig. 2C).

Cm Promoted Dpph Radical Scavenging Activity

The antioxidant activity of CM was evaluated by measuring its ability to scavenge DPPH free radicals, and vitamin C (1–30 μg/mL) was used as a positive control. As shown in Fig. 3, CM demonstrated profound free radical scavenging activity with 65 and 90% inhibition at the concentrations of 500 and 1000 μg/ml, respectively. The free radical scavenging activities of vitamin C (10–30 μg/ml) and CM (250–1000 μg/ml) were similar.

Whitening Effect of CM via the Suppression of α-MSH-Induced Melanin Synthesis in B16F10 Cells

We investigated whether CM affects the whitening effect via the suppression of α-MSH-induced melanin synthesis in B16F10 cells. B16F10 cells were stimulated with α-MSH and then treated with varying concentrations of CM. No significant effect on cell viability was observed in B16F10 cells treated with CM alone or in combination with α-MSH (Fig. 4A). We next investigated the inhibitory effects of CM on α-MSH-induced melanin synthesis in B16F10 cells. To confirm the inhibitory effect of CM on α-MSH-induced melanin synthesis, we determined the melanin content in α-MSH-stimulated B16F10 cells in the absence or presence of CM. We demonstrated that CM suppresses the α-MSH-induced melanin accumulation in B16F10 cells (Fig. 4B). Because MITF is an essential transcription factor that regulates melanogenesis-associated gene expression through the α-MSH-PKA-CREB axis [34], we further investigated whether CM regulates these melanogenesis-associated signal transduction pathways. Western blot analysis showed that treatment with a high dose of CM decreased TRP1, TRP2, MITF and tyrosinase levels in α-MSH-induced B16F10 cells (Fig. 4C).

CM Improves Skin Health via the Modulation of Gene Expression in HMC-1 and HaCaT Cells

We investigated whether CM affects skin health by modulating gene expression in HMC-1 and HaCaT cells. Newly activated T lymphocytes are able to produce IL-4, which is a major component of the inflammatory response in atopic dermatitis. We found that CM suppressed the levels of IL-4 mRNA and cytokines in HMC-1 cells in a dose-dependent manner (Fig. 5A, B). We next examined whether a high concentration of CM induces Aquaporin3, claudin-4, filaggrin, hyaluronan synthase (HAS)-1, HAS-2, and HAS-3 expression in cultured skin keratinocytes. CM induced the mRNA expression of Aquaporin3, claudin-4, filaggrin, HAS-1, HAS-2, and HAS-3 in keratinocytes (Fig. 5C). We investigated the effect of CM on keratinocyte migration in response to scratching. As shown in Fig. 5D, migration was increased in CM-treated keratinocytes compared to control keratinocytes in a dose-dependent manner.

Discussion

CM has been traditionally used as an anti-inflammatory agent for centuries. CM is considered an important source of various herbal medicines and is known to contain several major compounds, such as falcarindiol (FAD), 6-hydroxy-7-methoxy-dihydroligustilide, ligustilidiol, and senkyunolide H. [35–37]. Furthermore, FAD exhibited a potent inhibitory effect on the lipopolysaccharide (LPS)-induced production of nitric oxide (NO) in murine macrophages and macrophages from brain tissues [23, 38]. In the present study, we investigated the effects of CM on skin inflammation.

TNF-α is a known inflammatory factor involved in a variety of inflammatory diseases [39–41]. The activation of TNF-α induces the autocrine and paracrine activation of macrophages. As a result, an increase in the generation of inflammatory cytokines, such as IL-6, IL-17 and COX-2, can lead to a chain reaction of inflammation [42–44]. TNF-α and IL-17 are important markers of skin inflammation, and the inhibition of inflammatory cytokines, such as TNF-α and IL-17, yields positive effects on the treatment of dermatitis [45–48]. Additionally, PI3K/mTOR/Akt inhibitors are known to act as therapies for inflammatory skin diseases, such as skin atrophy [49].

We investigated whether CM affects cytokine expression in HMC-1 and HaCaT cells. CM significantly reduced agonist-stimulated AKT, Erk, NF-κB and COX-2 protein expression in a dose-dependent manner in HMC-1 and HaCaT cells. CM dose-dependently suppressed IL-6, IL-8, IL-13, IL-17 and TNF-α mRNA expression in HMC-1 cells. Additionally, CM treatment reduced the mRNA levels of proinflammatory cytokines, including IL-6, IL-13, and TNF-α, in LPS-stimulated HaCaT cells. We also demonstrated that CM inhibited the agonist-stimulated secretion of IL-6, IL-8, and TNF, as determined by ELISA. We found that CM had an anti-inflammatory effect on HMC-1 and HaCaT cells.

In the DPPH radical scavenging method, DPPH free radicals were used to determine the antioxidant (scavenging) activity of various extracts. CM demonstrated profound free radical scavenging activity in a dose-dependent manner. Additionally, the free radical scavenging activities of vitamin C (10–30 μg/ml) and CM (250–1000 μg/ml) were similar.

α-MSH is known to be released from UV-exposed keratinocytes and can stimulate melanin biosynthesis. α-MSH leads to an increase in MITF. Moreover, MITF increases the gene expression of TRP1 and TRP2 in

melanocytes [50]. We found that CM suppresses α-MSH-induced melanin accumulation in B16F10 cells. Additionally, treatment with a high dose of CM decreased TRP1, TRP2, MITF and tyrosinase levels in α-MSH-induced B16F10 cells.

There are a variety of important markers of skin health. Aquaporin3 plays a role in the moisture supply as it acts as both a water and glycerol transporter in the basal layer of the skin epidermis [51, 52]. Additionally, Claudin-4 is involved in barrier formation in keratinocytes [53]. HAS-1, HAS-2, and HAS-3, members of a class of integral membrane proteins, are the enzymes involved in hyaluronic acid synthesis [54]. The physical appearance of aged skin, including wrinkle formation and loss of skin elasticity, occurs because of a decrease in hyaluronic acid [55, 56].

The levels of IL-4 mRNA and cytokines were decreased with CM treatment in HMC-1 cells in a dose-dependent manner. Moreover, CM induced the mRNA expression of Aquaporin3, claudin-4, filaggrin, HAS-1, HAS-2, and HAS-3 in keratinocytes. We found that CM impacts keratinocyte migration in response to scratching. Taken together, our results suggest that CM regulates proinflammatory cytokine production in mast cells and keratinocytes, thereby affecting skin health.

Conclusion

Our present study demonstrates that CM treatment suppresses the production of several inflammatory cytokines and the NF-kB and MAPK pathways in HMC-1 cells and HaCaT cells. In addition, our data indicate that CM treatment decreases melanin biosynthesis in UV-exposed cells, increases DPPH radical scavenging activity, and affects several markers of skin health. Taken together, our results propose that CM might be a potentially useful drug for skin care.

Abbreviations

CM, *Cnidium officinale* Makino; BSA, bovine serum albumin; DPPH, 2.2-diphenyl-1-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; HMC-1, human mast cells; LPS, lipopolysaccharide; MITF, microphthalmia-associated transcription factor; α-MSH, α-melanocyte-stimulating hormone; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NO, nitric oxide; PMA, phorbol-12-myristate-13-acetate; TMB, tetramethylbenzidine; TNF-α, tumor necrosis factor-α; WST, tetrazolium salt.

Declarations

Consent for publication

Not applicable

Availability of data and materials

All data and materials are contained and described within the manuscript.

Conflict of interest

The authors declare that there were no conflicts of interest.

Ethics approval and consent to participate

Not applicable

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Authors' contributions

JMK carried out the experiment and drafting of manuscript. SHH, HIK, MJK and KM revised the research and manuscript and assisted in the research work. YCS guided the research, revised and submitted the manuscript. SGK designed, supervised the experiments and corrected the manuscript. All the authors read and approved the final manuscript.

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Figures

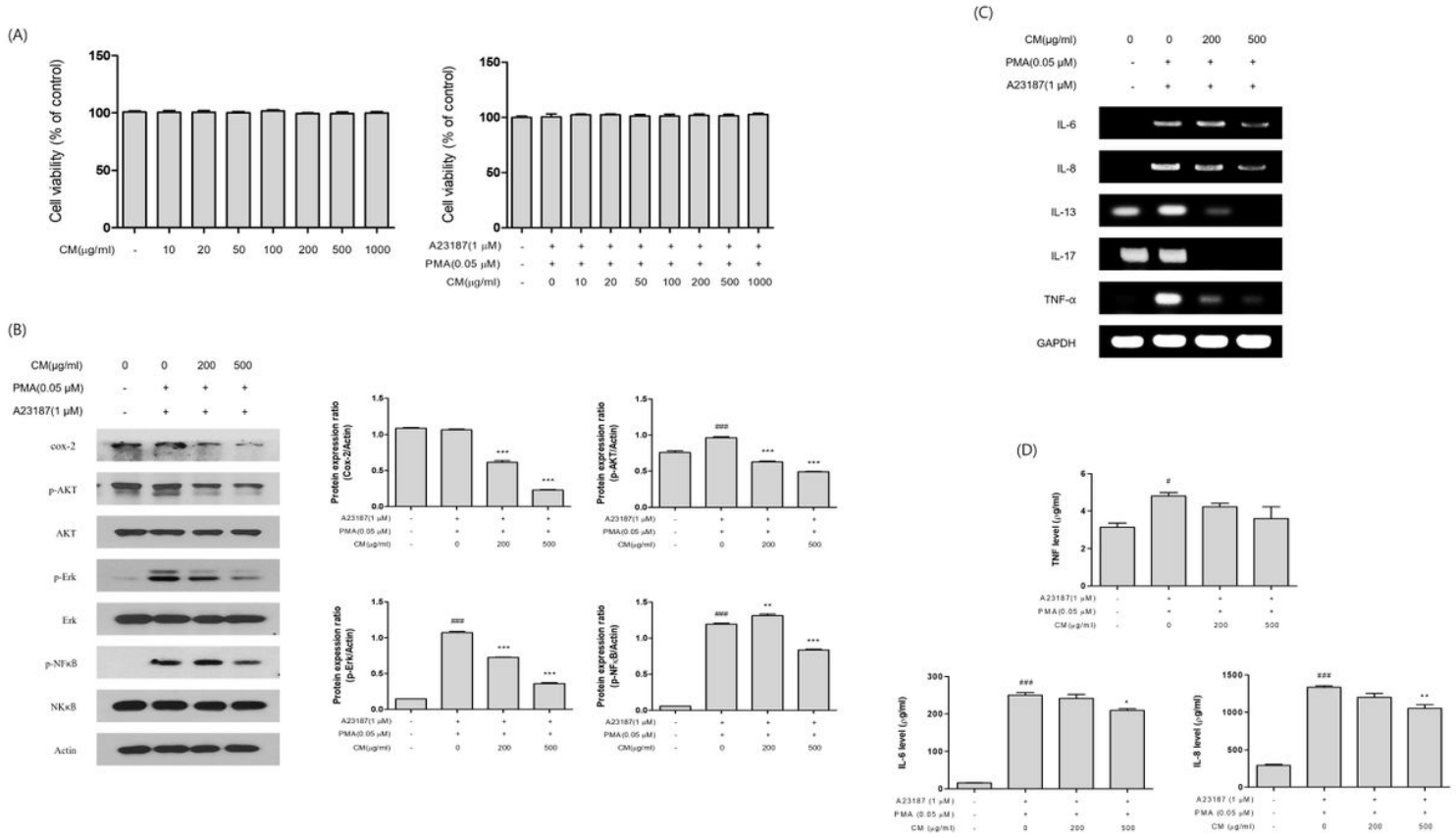

Figure 1

Effects of CM on cytokine expression in HMC-1 cells. HMC-1 cells were stimulated with A23187 (1 μg/ml) and PMA (0.05 μg/ml) and then treated with different concentrations of CM (10-1000 μg/ml) for 24 h (A). Whole cell lysates were analyzed by Western blotting (B). IL-6, IL-8, IL-13, IL-17 and TNF-α mRNA expression was measured by RT-PCR (C). The culture medium of the cells was harvested, and TNF, IL-6 and IL-8 cytokine levels were measured by ELISA (D). The data are presented as the mean ± SEM of three independent experiments. #P < 0.05, ##P < 0.01 and ###P < 0.001 compared to the normal control group. ∗P < 0.05, ∗∗P < 0.01 and ∗∗∗P < 0.001 compared to the A23187- and PMA-stimulated groups.

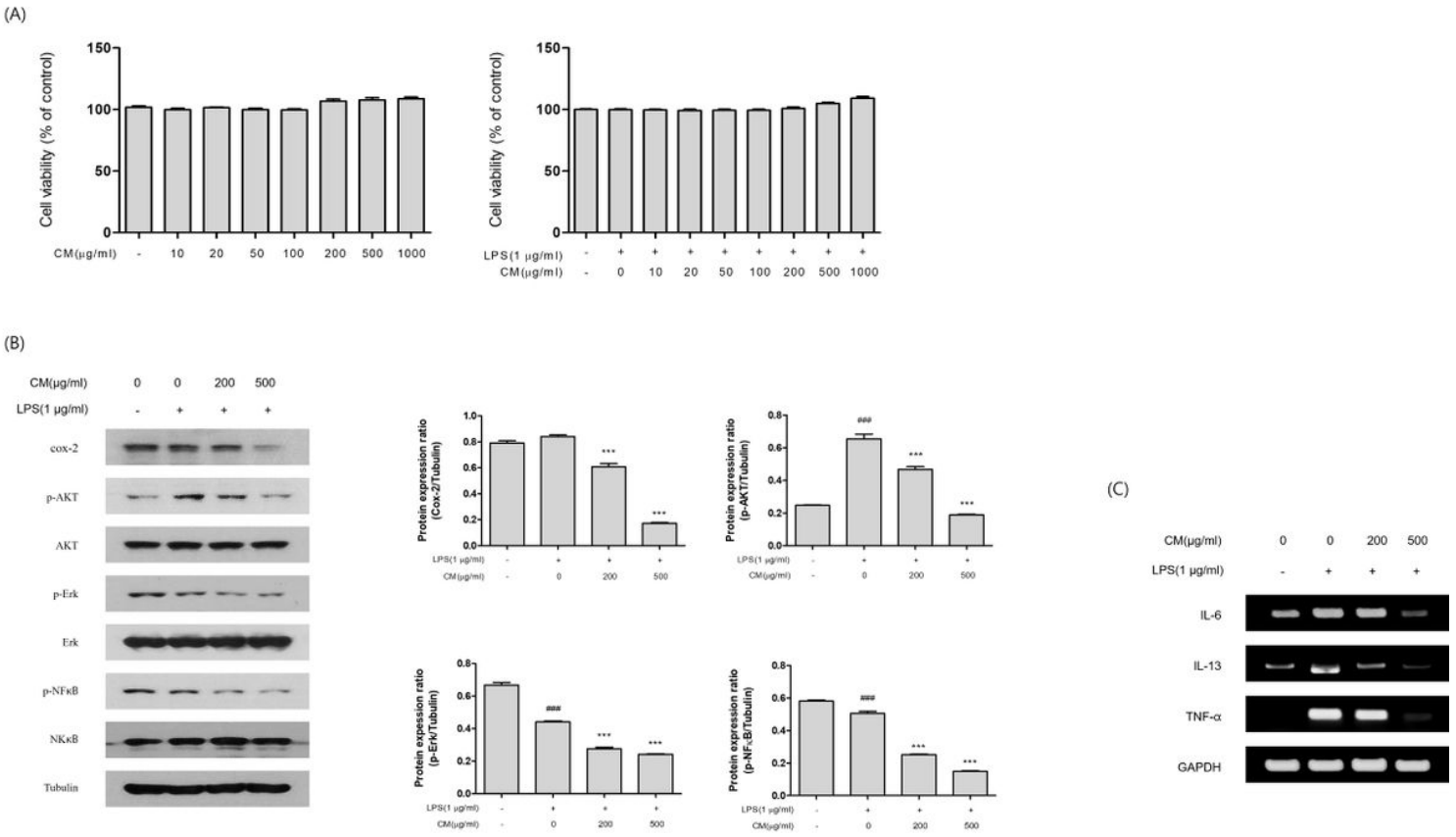

Figure 2

Effects of CM on cytokine expression in HaCaT cells. HaCaT cells were stimulated with LPS (1 μg/ml) and then treated with different concentrations of CM (10-1000 μg/ml) for 24 h (A). Whole cell lysates were analyzed by Western blotting (B). IL-6, IL-13 and TNF-α mRNA expression was measured by RT-PCR (C). The data are presented as the mean ± SEM of three independent experiments. #P < 0.05, ##P < 0.01 and ###P < 0.001 compared to the normal control group. ∗P < 0.05, ∗∗P < 0.01 and ∗∗∗P < 0.001 compared to the LPS-stimulated group.

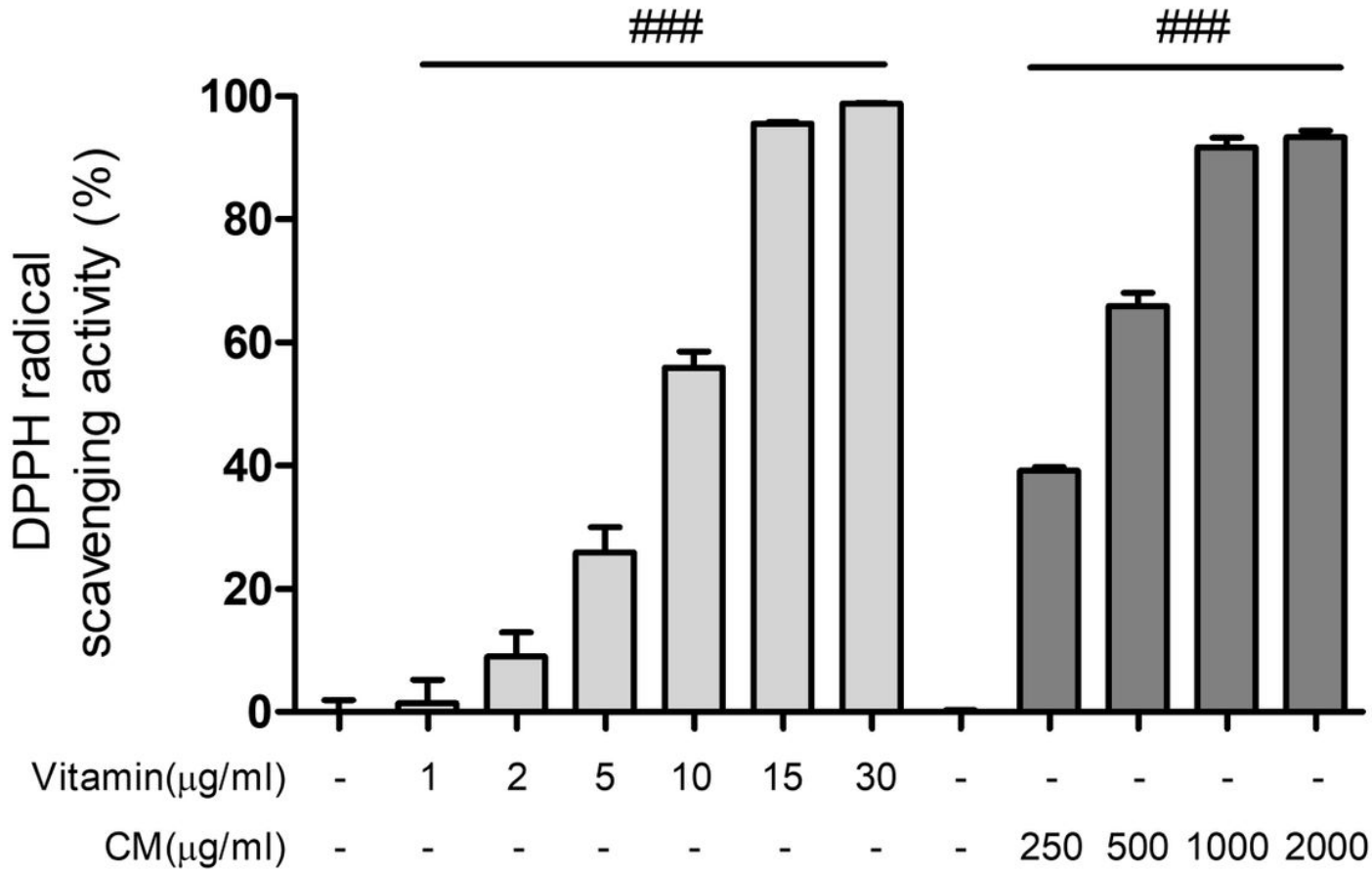

Figure 3

Comparison of the antioxidant activity of vitamin C and CM with a DPPH assay. The DPPH radical scavenging activity of CM (250-2000 μg/mL) and vitamin C (1-30 μg/mL). The data are presented as the mean ± SEM of three independent experiments. #P < 0.05, ##P < 0.01 and ###P < 0.001 compared to the normal control group.

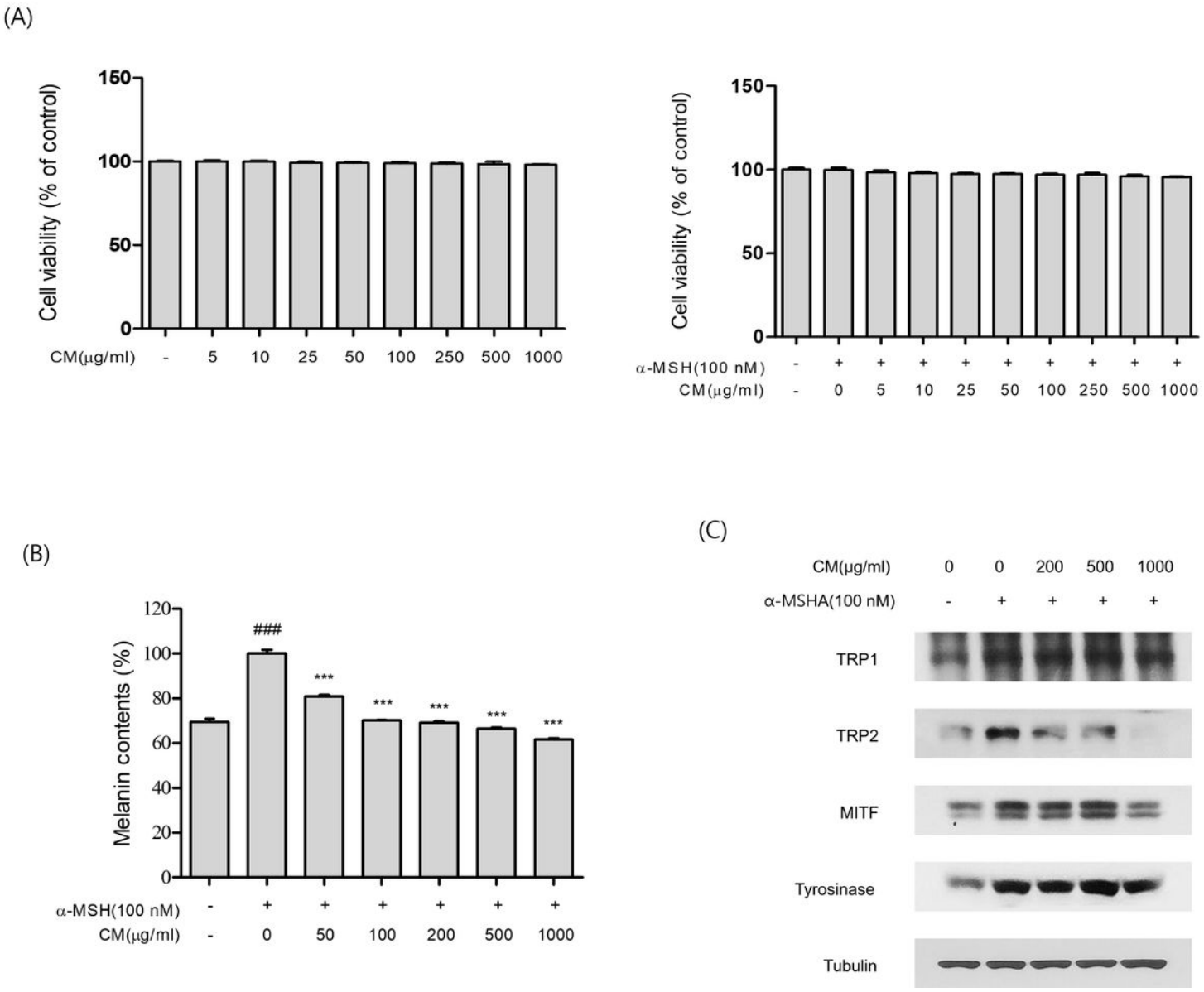

Figure 4

Effect of CM on melanin synthesis in B16F10 cells. B16F10 cells were stimulated with α-MSH (100 ηM) and then treated with different concentrations of CM (5-1000 μg/ml) for 24 h (A). B16F10 cells were cultured with α-MSH for 24 h, and then the melanin content was measured after treatment with various concentrations of CM for another 24 h (B). MITF, TRP1, TRP2 and tyrosinase mRNA expression was measured by RT-PCR (C). The data are presented as the mean ± SEM. #P < 0.05, ##P < 0.01 and ###P < 0.001 compared to the normal control group. ∗P < 0.05, ∗∗P < 0.01 and ∗∗∗P < 0.001 compared to the α-MSH-stimulated group.

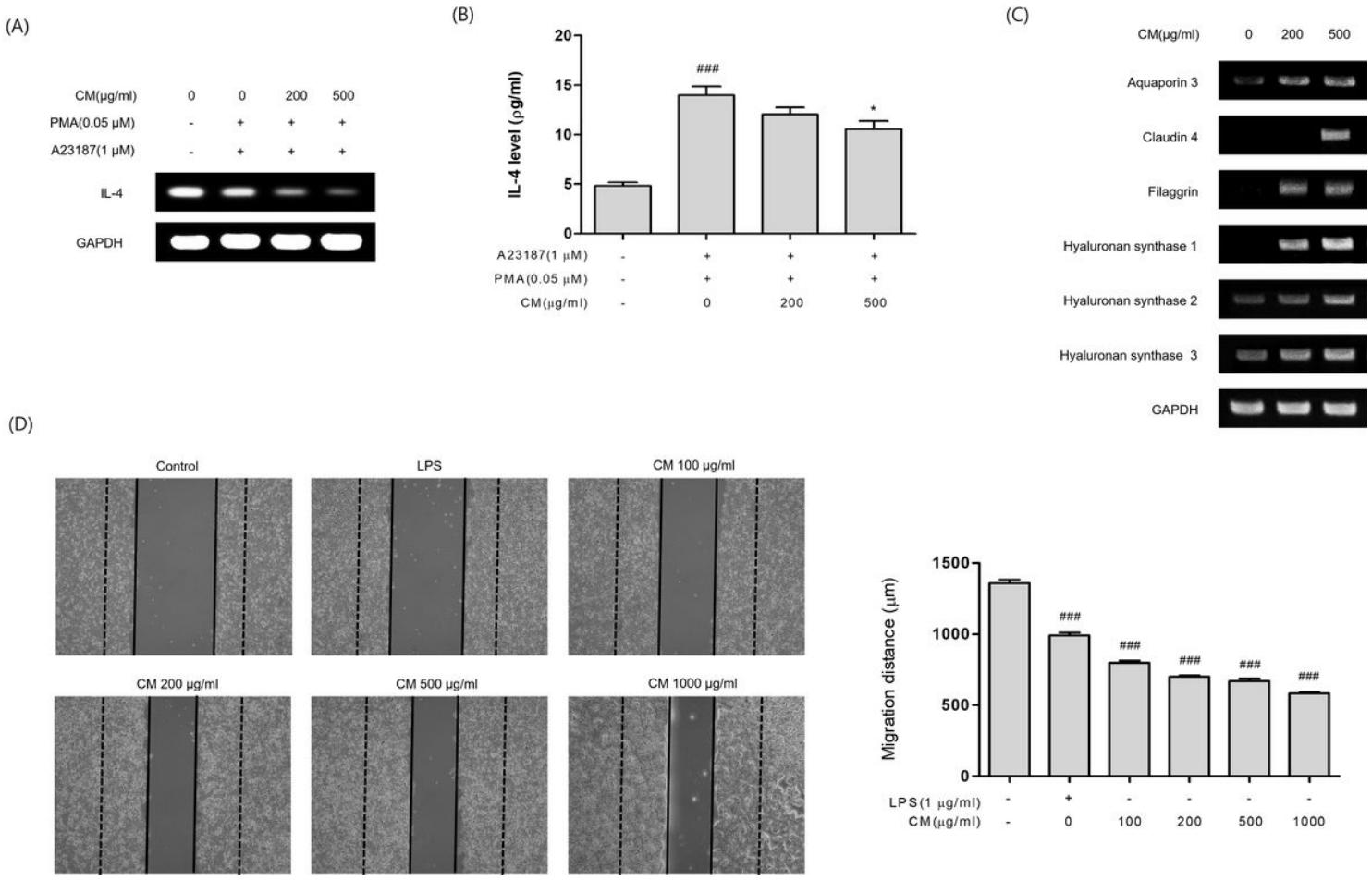

Figure 5

Effects of CM on the skin health of HMC-1 and HaCaT cells. HMC-1 or HaCaT cells were stimulated with A23187 (1 μg/ml) and PMA (0.05 μg/ml) or LPS and then treated with different concentrations of CM (200-500 μg/ml) for 24 h. IL-4 mRNA expression was measured by RT-PCR (A). The culture medium of the cells was harvested, and IL-4 cytokine levels were measured by ELISA (B). The mRNA expression of Aquaporin3, Claudin-4, Filaggrin, HAS-1, HAS-2, and HAS-3 was measured by RT-PCR (C). The migration of HaCaT was measured with a cell migration assay (D). The data are presented as the mean ± SEM. #P < 0.05, ##P < 0.01 and ###P < 0.001 compared to the normal control group. ∗P < 0.05, ∗∗P < 0.01 and ***P < 0.001 compared to the A23187- and PMA-treated or LPS-stimulated groups.

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