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 $3 \times 10^3$ 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 nM 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.
| 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'        |
| Primer name | Sequences |
|-------------|-----------|
| **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 $3 \times 10^5$ 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 CO2 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 nM) 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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References

1. Boehncke WH, Schon MP: **Psoriasis.** *Lancet* 2015, **386**(9997):983-994.

2. Yasukawa S, Miyazaki Y, Yoshii C, Nakaya M, Ozaki N, Toda S, Kuroda E, Ishibashi K, Yasuda T, Natsuki Y *et al.* An ITAM-Syk-CARD9 signalling axis triggers contact hypersensitivity by stimulating IL-1 production in dendritic cells. *Nature communications* 2014, **5**:3755.

3. Madison KC: **Barrier function of the skin: "la raison d'être" of the epidermis.** *The Journal of investigative dermatology* 2003, **121**(2):231-241.

4. Proksch E, Brandner JM, Jensen JM: **The skin: an indispensable barrier.** *Experimental dermatology* 2008, **17**(12):1063-1072.

5. Dinarello CA: **Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock.** *Chest* 1997, **112**(6 Suppl):321S-329S.

6. Briganti S, Picardo M: **Antioxidant activity, lipid peroxidation and skin diseases. What's new.** *Journal of the European Academy of Dermatology and Venereology : JEADV* 2003, **17**(6):663-669.

7. Lee JL, Mukhtar H, Bickers DR, Kopelovich L, Athar M: **Cyclooxygenases in the skin: pharmacological and toxicological implications.** *Toxicology and applied pharmacology* 2003, **192**(3):294-306.

8. Palladino MA, Bahjat FR, Theodorakis EA, Moldawer LL: **Anti-TNF-alpha therapies: the next generation.** *Nature reviews Drug discovery* 2003, **2**(9):736-746.

9. Ku JM, Hong SH, Kim SR, Choi HS, Seo HS, Jang BH, Ko SG, Shin YC: **Anti-allergic effects of So-Cheong-Ryong-Tang in ovalbumin-induced allergic rhinitis model.** *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies* 2016, **273**(1):123-131.

10. Ku JM, Hong SH, Kim SR, Choi HS, Kim HI, Kim DU, Oh SM, Seo HS, Kim TY, Shin YC *et al.* The prevention of 2,4-dinitrochlorobenzene-induced inflammation in atopic dermatitis-like skin lesions in BALB/c mice by Jawoongo. *BMC Complement Altern Med* 2018, **18**(1):215.

11. Oikarinen A, Haapasaari KM, Sutinen M, Tasanen K: **The molecular basis of glucocorticoid-induced skin atrophy: topical glucocorticoid apparently decreases both collagen synthesis and the**
corresponding collagen mRNA level in human skin in vivo. The British journal of dermatology 1998, 139(6):1106-1110.

12. Lin CT, Chen CJ, Lin TY, Tung JC, Wang SY: Anti-inflammation activity of fruit essential oil from Cinnamomum insularimontanum Hayata. Bioresource technology 2008, 99(18):8783-8787.

13. Lee DY, Choi G, Yoon T, Cheon MS, Choo BK, Kim HK: Anti-inflammatory activity of Chrysanthemum indicum extract in acute and chronic cutaneous inflammation. Journal of ethnopharmacology 2009, 123(1):149-154.

14. Lim YY, Lim TT, Tee JJ: Antioxidant properties of several tropical fruits: A comparative study. Food Chem 2007, 103(3):1003-1008.

15. Ku JM, Hong SH, Kim HI, Seo HS, Shin YC, Ko SG: Effects of Angelicae dahuricae Radix on 2, 4-Dinitrochlorobenzene-Induced Atopic Dermatitis-Like Skin Lesions in mice model. BMC Complement Altern Med 2017, 17(1):98.

16. Barrett NA, Austen KF: Innate cells and T helper 2 cell immunity in airway inflammation. Immunity 2009, 31(3):425-437.

17. Kawakami T, Ando T, Kimura M, Wilson BS, Kawakami Y: Mast cells in atopic dermatitis. Current opinion in immunology 2009, 21(6):666-678.

18. Jung MR, Lee TH, Bang MH, Kim H, Son Y, Chung DK, Kim J: Suppression of thymus- and activation-regulated chemokine (TARC/CCL17) production by 3-O-beta-D-glucopyanosylspinasterol via blocking NF-kappaB and STAT1 signaling pathways in TNF-alpha and IFN-gamma-induced HaCaT keratinocytes. Biochemical and biophysical research communications 2012, 427(2):236-241.

19. Bernard FX, Morel F, Camus M, Pedretti N, Barrault C, Garnier J, Lecron JC: Keratinocytes under Fire of Proinflammatory Cytokines: Bona Fide Innate Immune Cells Involved in the Physiopathology of Chronic Atopic Dermatitis and Psoriasis. Journal of allergy 2012, 2012:718725.

20. Park JH, Kim MS, Jeong GS, Yoon J: Xanthii fructus extract inhibits TNF-alpha/IFN-gamma-induced Th2-chemokines production via blockade of NF-kappaB, STAT1 and p38-MAPK activation in human epidermal keratinocytes. Journal of ethnopharmacology 2015, 171:85-93.

21. Ju SM, Song HY, Lee SJ, Seo WY, Sin DH, Goh AR, Kang YH, Kang IJ, Won MH, Yi JS et al: Suppression of thymus- and activation-regulated chemokine (TARC/CCL17) production by 1,2,3,4,6-penta-O-galloyl-beta-D-glucose via blockade of NF-kappaB and STAT1 activation in the HaCaT cells. Biochemical and biophysical research communications 2009, 387(1):115-120.

22. Choi HS, Kim MSL, Sawamura M: Constituents of the essential oil of cnidium officinale Makino, a Korean medicinal plant. Flavour Frag J 2002, 17(1):49-53.

23. Bae KE, Choi YW, Kim ST, Kim YK: Components of rhizome extract of Cnidium officinale Makino and their in vitro biological effects. Molecules 2011, 16(10):8833-8847.

24. de la Cruz JF, Lee WS, Hwang SG: Immunomodulatory effect of Cnidium officinale Makino extract in murine peritoneal macrophages and splenocytes. Faseb Journal 2013, 27.

25. Ramalingam M, Yong-Ki P: Free radical scavenging activities of Cnidium officinale Makino and Ligusticum chuanxiong Hort. methanolic extracts. Pharmacognosy magazine 2010, 6(24):323-330.
26. Tahara E, Satoh T, Toriizuka K, Nagai H, Nunome S, Shimada Y, Itoh T, Terasawa K, Saiki I: Effect of Shimotsu-to (a Kampo medicine, Si-Wu-Tang) and its constituents on triphasic skin reaction in passively sensitized mice. *Journal of ethnopharmacology* 1999, **68**(1-3):219-228.

27. Wang JD, Narui T, Kurata H, Takeuchi K, Hashimoto T, Okuyama T: Hematological studies on naturally occurring substances. II. Effects of animal crude drugs on blood coagulation and fibrinolysis systems. *Chemical & pharmaceutical bulletin* 1989, **37**(8):2236-2238.

28. Hong H, An JC, de La Cruz JF, Hwang SG: Cnidium officinale Makino extract induces apoptosis through activation of caspase-3 and p53 in human liver cancer HepG2 cells. *Experimental and therapeutic medicine* 2017, **14**(4):3191-3197.

29. de la Cruz J, Kim DH, Hwang SG: Anti cancer effects of Cnidium officinale Makino extract mediated through apoptosis and cell cycle arrest in the HT-29 human colorectal cancer cell line. *Asian Pacific journal of cancer prevention: APJCP* 2014, **15**(13):5117-5121.

30. Lee KE, Shin JA, Hong IS, Cho NP, Cho SD: Effect of methanol extracts of Cnidium officinale Makino and Capsella bursa-pastoris on the apoptosis of HSC-2 human oral cancer cells. *Experimental and therapeutic medicine* 2013, **5**(3):789-792.

31. Jeong JB, Ju SY, Park JH, Lee JR, Yun KW, Kwon ST, Lim JH, Chung GY, Jeong HJ: Antioxidant activity in essential oils of Cnidium officinale makino and Ligusticum chuanxiong Hort and their inhibitory effects on DNA damage and apoptosis induced by ultraviolet B in mammalian cell. *Cancer epidemiology* 2009, **33**(1):41-46.

32. Jameel F, Phang M, Wood LG, Garg ML: Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. *Lipids in health and disease* 2014, **13**:195.

33. Hosoi J, Abe E, Suda T, Kuroki T: Regulation of melanin synthesis of B16 mouse melanoma cells by 1 alpha, 25-dihydroxyvitamin D3 and retinoic acid. *Cancer Res* 1985, **45**(4):1474-1478.

34. D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME: Signaling Pathways in Melanogenesis. *International journal of molecular sciences* 2016, **17**(7).

35. Lee WS, Shin JS, Jang DS, Lee KT: Cnidilide, an alkylphthalide isolated from the roots of Cnidium officinale, suppresses LPS-induced NO, PGE2, IL-1beta, IL-6 and TNF-alpha production by AP-1 and NF-kappaB inactivation in RAW 264.7 macrophages. *Int Immunopharmacol* 2016, **40**:146-155.

36. Tomoda M, Ohara N, Shimizu N, Gonda R: Characterization of a novel heteroglucan from the rhizome of Cnidium officinale exhibiting high reticuloendothelial system-potentiating and anti-complementary activities. *Biological & pharmaceutical bulletin* 1994, **17**(7):973-976.

37. Ozaki Y, Sekita S, Harada M: [Centrally acting muscle relaxant effect of phthalides (ligustilide, cnidilide and senkyunolide) obtained from Cnidium officinale Makino]. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan* 1989, **109**(6):402-406.

38. Kim JM, Lee P, Son D, Kim H, Kim SY: Falcarindiol inhibits nitric oxide-mediated neuronal death in lipopolysaccharide-treated organotypic hippocampal cultures. *Neuroreport* 2003, **14**(15):1941-1944.

39. Oshima H, Ishikawa T, Yoshida GJ, Naoi K, Maeda Y, Naka K, Ju X, Yamada Y, Minamoto T, Mukaida N *et al.*: TNF-alpha/TNFR1 signaling promotes gastric tumorigenesis through induction of Noxo1 and...
Gna14 in tumor cells. *Oncogene* 2014, 33(29):3820-3829.

40. Vandevyver S, Dejager L, Van Bogaert T, Kleyman A, Liu Y, Tuckermann J, Libert C: Glucocorticoid receptor dimerization induces MKP1 to protect against TNF-induced inflammation. *The Journal of clinical investigation* 2012, 122(6):2130-2140.

41. Liu J, Yan J, Jiang S, Wen J, Chen L, Zhao Y, Lin A: Site-specific ubiquitination is required for relieving the transcription factor Miz1-mediated suppression on TNF-alpha-induced JNK activation and inflammation. *Proceedings of the National Academy of Sciences of the United States of America* 2012, 109(1):191-196.

42. Krueger JG, Fretzin S, Suarez-Farinas M, Haslett PA, Phipps KM, Cameron GS, McColm J, Katcherian A, Cueto I, White T et al: IL-17A is essential for cell activation and inflammatory gene circuits in subjects with psoriasis. *The Journal of allergy and clinical immunology* 2012, 130(1):145-154 e149.

43. Merrill JC, You J, Constable C, Leeman SE, Amar S: Whole-body deletion of LPS-induced TNF-alpha factor (LITAF) markedly improves experimental endotoxic shock and inflammatory arthritis. *Proceedings of the National Academy of Sciences of the United States of America* 2011, 108(52):21247-21252.

44. Guba SC, Sartor CI, Gottschalk LR, Jing YH, Mulligan T, Emerson SG: Bone marrow stromal fibroblasts secrete interleukin-6 and granulocyte-macrophage colony-stimulating factor in the absence of inflammatory stimulation: demonstration by serum-free bioassay, enzyme-linked immunosorbent assay, and reverse transcriptase polymerase chain reaction. *Blood* 1992, 80(5):1190-1198.

45. Senra L, Mylonas A, Kavanagh RD, Fallon PG, Conrad C, Borowczyk-Michalowska J, Wrobel LJ, Kaya G, Yawalkar N, Boehncke WH et al: IL-17E (IL-25) enhances innate immune responses during skin inflammation. *The Journal of investigative dermatology* 2019.

46. Diani M, Altomare G, Reali E: T cell responses in psoriasis and psoriatic arthritis. *Autoimmunity reviews* 2015, 14(4):286-292.

47. Chong SZ, Tan KW, Wong FHS, Chua YL, Tang Y, Ng LG, Angeli V, Kemeny DM: CD8 T cells regulate allergic contact dermatitis by modulating CCR2-dependent TNF/iNOS-expressing Ly6C+ CD11b+ monocytic cells. *The Journal of investigative dermatology* 2014, 134(3):666-676.

48. Chiricozzi A, Guttman-Yassky E, Suarez-Farinas M, Nograles KE, Tian S, Cardinale I, Chimenti S, Krueger JG: Integrative responses to IL-17 and TNF-alpha in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *The Journal of investigative dermatology* 2011, 131(3):677-687.

49. Agarwal S, Mirzoeva S, Readhead B, Dudley JT, Budunova I: PI3K inhibitors protect against glucocorticoid-induced skin atrophy. *EBioMedicine* 2019.

50. Kim HJ, Yonezawa T, Teruya T, Woo JT, Cha BY: Nobiletin, a polymethoxy flavonoid, reduced endothelin-1 plus SCF-induced pigmentation in human melanocytes. *Photochemistry and photobiology* 2015, 91(2):379-386.
51. Sougrat R, Morand M, Gondran C, Barre P, Gobin R, Bonte F, Dumas M, Verbavatz JM: Functional expression of AQP3 in human skin epidermis and reconstructed epidermis. *The Journal of investigative dermatology* 2002, **118**(4):678-685.

52. Frigeri A, Gropper MA, Umenishi F, Kawashima M, Brown D, Verkman AS: Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. *Journal of cell science* 1995, **108** (Pt 9):2993-3002.

53. Kirschner N, Rosenthal R, Furuse M, Moll I, Fromm M, Brandner JM: Contribution of tight junction proteins to ion, macromolecule, and water barrier in keratinocytes. *The Journal of investigative dermatology* 2013, **133**(5):1161-1169.

54. Itano N, Kimata K: Mammalian hyaluronan synthases. *IUBMB life* 2002, **54**(4):195-199.

55. Longas MO, Russell CS, He XY: Evidence for structural changes in dermatan sulfate and hyaluronic acid with aging. *Carbohydrate research* 1987, **159**(1):127-136.

56. Ghersetich I, Lotti T, Campanile G, Grappone C, Dini G: Hyaluronic acid in cutaneous intrinsic aging. *International journal of dermatology* 1994, **33**(2):119-122.

**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 nM) 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.
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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