

# *Triticum aestivum* sprout extract attenuates 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in mice and the expression of chemokines in human keratinocytes

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**Abstract.** Atopic dermatitis (AD) is a common chronic, recurring, inflammatory skin disease. A number of researchers have been seeking safe AD therapies for a long time. *Triticum aestivum* sprouts (TAE), known as wheatgrass, are one of the most widely used health foods worldwide. They show numerous beneficial effects, including anticancer, anti-inflammatory, anti-oxidant, anti-obesity, anti-colitis and anti-allergy effects; however, their effect on AD is unknown. In the present study, the anti-AD effects of a 70% ethanol extract of TAE were investigated in 2,4-dinitrochlorobenzene (DNCB)-treated mice with AD-like skin lesions and in tumor necrosis factor (TNF)- $\alpha$ - and interferon (IFN)- $\gamma$ -stimulated human keratinocytes (HaCaT cells). Oral administration of 200 mg/kg TAE for 10 days significantly decreased the skin thickness, transepidermal water loss and serum immunoglobulin E levels in DNCB-treated mice. In addition, TAE reduced the secretion of inflammatory chemokines via regulation of the signal transducer and activator of transcription 1 and suppressor of cytokine signaling pathways in TNF- $\alpha$ - and IFN- $\gamma$ -stimulated HaCaT cells. These results indicate that TAE may have beneficial effects in the treatment and prevention of AD and associated skin diseases.

## Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that affects ~20% of children worldwide, and the prevalence of AD increases rapidly every year (1). AD is characterized by chronic recurrence of skin inflammation, epidermal barrier dysfunction, IgE-mediated sensitization, edema, and thickened epidermis (2). Although various genetic and environmental factors have been reported to contribute to the pathogenesis and development of AD, the precise cause of AD has not yet been determined (3). Current treatments for AD include topical ointments or systemic oral administration of steroids and antihistamines to decrease inflammatory damage and itching (4). Steroids are widely used to treat AD because they alleviate atopic symptoms, while functioning as anti-inflammatory agents and promoting cell proliferation and immunosuppression. However, prolonged treatment with steroids has side effects such as the development of drug tolerance, endocrine abnormalities, increased susceptibility to infections, metabolic abnormalities, and skin atrophy that leads to the cracking of skin and bleeding (5). Therefore, there is a growing interest in AD treatments using natural materials that have fewer side effects. Natural compounds and natural extracts of various herbs have been reported as potential medicines to prevent and treat inflammatory skin diseases.

Wheat (*Triticum* sp.) is an important crop worldwide. The young grass of *Triticum aestivum*, called wheatgrass, is richer in nutrients such as vitamins, minerals, and proteins than the mature cereal plant (6). *T. aestivum* is used as a health food supplement in the form of tablets, juice, powder, and fresh produce. Many papers report that *T. aestivum* has anticancer, anti-inflammation, antioxidant (7,8), and therapeutic effects in diseases such as diabetes, colitis, allergies, and heart diseases (9-11). In previous studies, we found that *T. aestivum* sprouts are effective in treating several diseases, such as diabetes (12), obesity (13,14), liver injury (15,16), and cancer (17). Thus, *T. aestivum* sprouts represent a potential remedy for these diseases. Furthermore, the dichloromethane fraction of *T. aestivum* ameliorated allergic reaction by

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inhibition of Th2 cell differentiation in mice (18). AD is mediated by and related to allergic disease (2). However, the influence of *T. aestivum* on allergy-mediated inflammation is not clearly understood. In addition, the specific effects of *T. aestivum* sprouts in AD are not yet known.

In the present study, considering their various biological effects, we evaluated the effects of *T. aestivum* sprouts in AD. We report the anti-atopic effects of a 70% ethanol extract of *T. aestivum* sprouts (TAEЕ) *in vitro* and *in vivo*.

## Materials and methods

**Preparation of TAEЕ.** *T. aestivum* Lamarck was supplied by the National Institute of Crop Science (Jeonbuk, Korea). After germination, the seeds were grown in organic sterile peat moss at a constant temperature (average 20±2°C). The *T. aestivum* sprouts were harvested at 2 weeks after germination, lyophilized, and laboratory-scale pulverized. The pulverized *T. aestivum* sprouts (30 g) were ultrasonically extracted with 70% EtOH for 1 h and then filtered. After evaporation on a rotary vacuum evaporator (N-000; EYELA, Tokyo, Japan), the TAEЕ was obtained. For subsequent experiments, the TAEЕ was stored at 4°C and protected from light until immediately before the experiment. The TAEЕ was dissolved in purified water for use in subsequent experiments.

**Cell culture.** Human keratinocytes (HaCaT cells) were obtained from the Korean cell line bank (Seoul, Korea). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Lonza, Walkersville, MD, USA), containing 10% fetal bovine serum (FBS; Biotechnics Research, CA, USA), 100 units/ml of penicillin, and 100 µg/ml of streptomycin (both Welgene, Seoul, Korea) at 37°C in a humidified 5% CO<sub>2</sub> air atmosphere.

**Cell Counting kit-8 (CCK-8) assay.** The HaCaT cell proliferation rate was evaluated using a CCK-8 (Dojindo, Kumamoto, Japan), according to the manufacturer's instructions. Briefly, HaCaT cells were seeded at 5×10<sup>3</sup> cells/well in 96-well plates. After incubation for 24 h, the cells were treated with different TAEЕ concentrations (0–400 µg/ml), and then incubated for another 24 or 48 h. The cells were washed with phosphate-buffered saline (PBS), the CCK-8 solution was added, and the cells were incubated for 1.5 h. The absorbance of cells was measured at 450 nm using a microplate reader (Synergy HTX Multi-Mode Reader; BioTek, Winooski, VT, USA).

**Animals and treatment.** Female BALB/c mice (4 weeks old) were purchased from Samtako Bio Korea (Osan, Korea). To induce AD-like skin lesions in mice, 2,4-dinitrochlorobenzene (DNCB) was used. The mice were divided into five groups: i) Normal control group, not treated with DNCB; ii) AD group, treated with DNCB; iii) TAEЕ 100 group, treated with DNCB and administered 100 mg/kg TAEЕ p.o.; iv) TAEЕ 200 group, treated with DNCB and administered 200 mg/kg TAEЕ p.o.; and v) dexamethasone p.o. After the mice were acclimatized in the facility for one week, the dorsal skin hairs of the mice were removed using an electronic clipper and hair removal cream, and the skin was allowed to heal for 24 h. A 1% (w/v) DNCB solution was prepared with an acetone and olive oil mixture (4:1, v/v), and the solution

was applied to the back of the mice once a day for 3 days from the start of the experiment. Afterwards, a 0.5% DNCB solution was applied once every 2 for 10 days. TAEЕ or dexamethasone were administered for 10 days from day 4 to day 14 of the experiment. The design of the experiment is summarized in Fig. 1A. The research was conducted in accordance with the ethical regulations of the Animal Experiment Ethics Committee of Chonbuk National University and with their approval (approval no. CBNU 2017-0002).

**Histological analysis.** Dorsal skin of the mice was sampled, fixed in 4% formaldehyde solution at room temperature for 24 h, and embedded in paraffin. Each paraffin block was serially sectioned into five 4-µm sections (n=5). Each tissue section was deparaffinized with xylene and stained with hematoxylin for 1 min and with eosin for 3 min. Other section was stained with toluidine blue for determining the number of mast cells. After staining, each tissue section was dehydrated, sealed with mounting solution, and examined under an optical microscope (CX21; Olympus, Tokyo, Japan).

**Analysis of dorsal skin moisture content.** The moisture content of the dorsal skin was analyzed using the TS-skin diagnosis system (Aram Huvis Co., Ltd., Seongnam, Korea), which measures the moisture content (%) based on the electrical capacitance of the skin surface. According to the manufacturer's instructions, three different regions of the dorsal skin were measured for 10 sec each.

**Serum IgE measurement.** Blood was collected from the mice using 23 G syringes and centrifuged at 3,000 rpm for 10 min to separate the serum. Total serum IgE was analyzed using sandwich enzyme-linked immunosorbent assay (ELISA), performed using a mouse IgE ELISA kit (BD Biosciences, San Jose, CA, USA). After incubation overnight at 4°C with 250 µl of diluted capture antibodies in 0.1 M sodium carbonate (pH 9.5), 200 µl of assay diluent was added to each well and blocked for 1 h at room temperature. The serum and serially diluted standard solutions were dispensed at 100 µl per well and allowed to react at room temperature for 2 h. Diluted detection antibodies and streptavidin-horseradish peroxidase (HRP; 100 µl) were then added into each well and incubated at room temperature for 1 h. Between each step, the wells were washed with 0.05% PBS-Tween-20. After the final wash, 100 µl of the substrate solution was dispensed and allowed to react for 30 min in the dark. To stop the reaction, 50 µl of 2 N H<sub>2</sub>SO<sub>4</sub> was added to each well, and the absorbance was measured at 450 nm using a microplate reader (Synergy HTX Multi-Mode Reader; BioTek).

**RNA extraction.** Cells were seeded in 6-well plates at a concentration of 1×10<sup>5</sup> cells/ml. After overnight incubation, the cells were pretreated with TAEЕ for 2 h and incubated with 10 ng/ml tumor necrosis factor α (TNF-α) and 10 ng/ml interferon γ (IFN-γ; ProSpec-Tany TechnoGene, Rehovot, Israel) for 6 h. In addition, the dorsal skin tissue was cut at the end of the experiment. One milliliter of TRIzol solution (Ambion, Austin, TX, USA) was added to each well to extract the total RNA. The RNA was mixed with 0.2 ml of chloroform and centrifuged at 12,000 rpm at 4°C. The supernatant was collected, mixed with

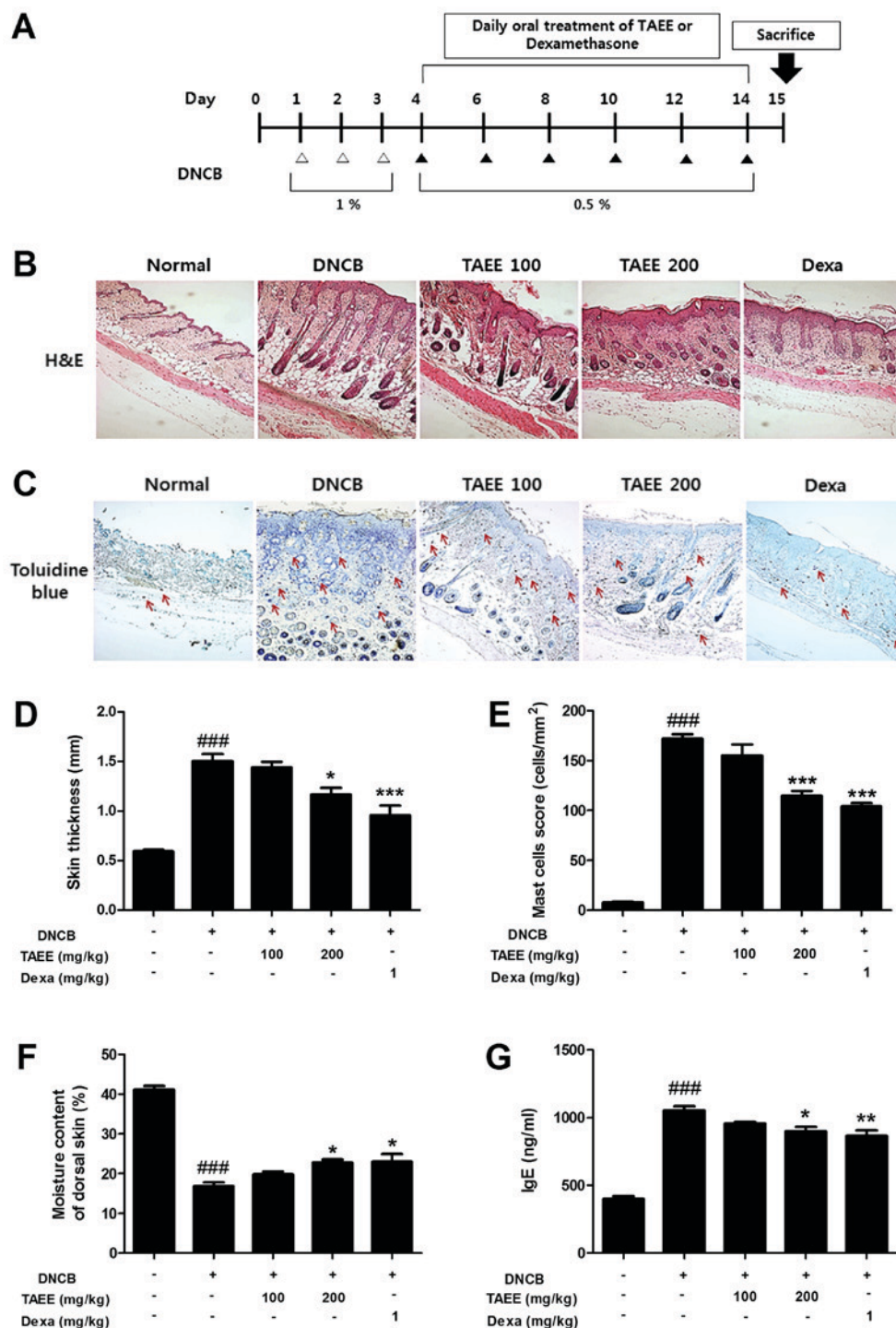


Figure 1. TAE improves clinical symptoms in DNCB-treated mice. (A) The DNCB treatment and oral administration schedule used in this experiment. Dorsal skin was removed from mice following the experiment. The histological status of the tissues was examined using (B) hematoxylin and eosin staining and (C) toluidine blue staining. Magnification,  $\times 100$ . (D) The thickness of dorsal skin tissues was measured using a thickness gauge. (E) The number of mast cells (red arrows) was counted at randomly selected locations per group. (F) Moisture content (%) was measured using a Tewameter TM 210 device. (G) The serum was collected after the mice were sacrificed and serum IgE levels were measured by ELISA. Values are presented as the mean  $\pm$  standard error of the mean. Data were analyzed by Tukey's post-hoc test. ### $P < 0.001$  vs. the normal group; \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. the DNCB-treated group. TAE, 70% EtOH extract of *T. aestivum* sprouts; DNCB, 2,4-dinitrochlorobenzene; Dexa, dexamethasone; IgE, immunoglobulin E.

0.5 ml of 2-propanol, and centrifuged at 12,000 rpm for 10 min, after which the RNA pellets were dried. The dried RNA pellets were dissolved in RNase-free water.

**Reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA was quantified using spectrophotometry, after

which cDNA was synthesized using 2  $\mu$ g of total RNA and a PrimeScript™ II 1st strand cDNA synthesis kit (Takara Bio Inc., Otsu, Japan). RT-PCR was performed using a Real-Time™ PCR System with SYBR-Green PCR Master Mix (both Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles



of 95°C for 15 sec and 60°C for 1 min. The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was simultaneously measured for normalization. The nucleotide sequences of the primers used are shown in Table I.

**Western blot analysis.** HaCaT cells were harvested in PBS. The cells were then centrifuged at 12,000 g for 20 min at 4°C to remove the supernatant, lysed using radioimmunoprecipitation assay (RIPA) lysis buffer (Pierce Biotechnology, Rockford, IL, USA), and kept on ice for 30 min. The extracted proteins were mixed with 5X SDS sample buffer. Lysates were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h at room temperature. The membranes were then incubated with primary antibodies at 4°C overnight. After incubation, the membranes were washed with TBST buffer three times for 15 min and then incubated for 1 h at room temperature with HRP-conjugated secondary antibodies diluted to 1:5,000. Membranes were then washed four times with TBST buffer and protein signals were developed using an enhanced chemiluminescence (ECL) detection kit (Merck Millipore, Burlington, MA, USA). Images were obtained using the Fusion Fx gel documentation system (Vilber Lourmat, Marne-la-Vallee, France).

**Statistical analysis.** Results are expressed as mean values  $\pm$  standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) with Tukey's post-hoc test to determine differences between groups. All statistical analyses were performed using Graph Pad Prism software 5.0 (Graph Pad Software, Inc. La Jolla, CA, USA). \* $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effects of TAEЕ on AD-like symptoms in DNCB-treated mice.** To investigate the effect of TAEЕ on AD-like skin lesions, the DNCB-treated mice were orally administered TAEЕ for 10 days. As a positive control, the DEXA group was orally administered dexamethasone. On day 15 after the start of the experiment, the mice were sacrificed (Fig. 1A). To examine the effect of TAEЕ on skin thickness, sections of dorsal skin tissue were stained with hematoxylin and eosin (H&E) and observed under a microscope. Repeated DNCB application caused severe skin changes, including skin hypertrophy and fibrosis of the dermis in the dorsal skin tissues of DNCB-treated mice. TAEЕ treatments lead to reduced skin thickness in a dose-dependent manner. Oral administration of 200 mg/kg TAEЕ and 1 mg/kg dexamethasone markedly decreased the skin thickness (Fig. 1B and D). Furthermore, to analyze the effect of TAEЕ on the number of mast cells, sections of dorsal skin tissue were stained with toluidine blue and examined under a microscope. Repeated DNCB application increased the number of mast cells that infiltrated the dermis of the dorsal skin of DNCB-treated mice. Oral administration of TAEЕ reduced the number of infiltrated mast cells in a dose-dependent manner. Oral administration of

200 mg/kg TAEЕ and 1 mg/kg dexamethasone significantly decreased the number of mast cells (Fig. 1C and E).

**Effects of TAEЕ on transepidermal water loss (TEWL) in dorsal skin of DNCB-treated mice.** AD increases skin moisture loss and impairs the skin barrier function. Therefore, skin hydration is essential to control AD (19). We examined the effect of TAEЕ on loss of skin moisture induced by DNCB. Fig. 1F shows the moisture content of dorsal skin after 10 days of oral drug administration. We observed a marked decrease in the skin moisture content of down to 40.77% in the DNCB-treated group. Compared with the DNCB-treated group, the skin moisture content increased in a dose-dependent manner by oral administration of TAEЕ. Oral administration of 200 mg/kg TAEЕ and 1 mg/kg dexamethasone significantly recovered the TEWL to 55.61 and 55.83%, respectively.

**Effects of TAEЕ on elevation of serum IgE levels in DNCB-treated mice.** IgE binds to receptors on the surface of mast cells or white blood cells, leading to an allergic reaction. In addition, IgE antibody production is related to the T helper 2 (Th2) immune response (20). IgE plays an important role in AD occurrence and progression, and patients with AD usually have high serum IgE levels. We analyzed the effect of TAEЕ on serum levels of total IgE using ELISA. The serum IgE levels were elevated in the DNCB-treated group. Compared with the DNCB-treated group, the serum IgE levels were markedly decreased in a dose-dependent manner by oral administration of TAEЕ (Fig. 1G).

**Effects of TAEЕ on expression of chemokines in DNCB-treated mice.** Keratinocytes are activated by inflammatory stimulation to produce a variety of chemokines. These chemokines include regulated upon activation, normally T-expressed, and presumably secreted (RANTES, also known as CCL5), macrophage-derived chemokine (MDC, also known as CCL22), and IFN- $\gamma$ -induced protein of 10 kDa (IP-10, also known as CXCL10) (21). To analyze the effects of TAEЕ on inflammatory chemokines in dorsal skin tissue, we assessed the mRNA levels of *RANTES*, *MDC*, and *IP-10* using real-time PCR. In skin lesions, the expression levels of *RANTES*, *MDC*, and *IP-10* were elevated in the DNCB-treated group. Oral administration of TAEЕ decreased the mRNA levels of *RANTES*, *MDC*, and *IP-10* in a dose-dependent manner. Oral administration of 200 mg/kg TAEЕ and 1 mg/kg dexamethasone considerably lowered the mRNA levels of the said chemokines (Fig. 2).

**Effects of TAEЕ on expression of chemokines in TNF- $\alpha$ - and IFN- $\gamma$ -treated HaCaT cells.** We analyzed the effect of TAEЕ on cell viability in HaCaT cells using the CCK-8 assay. The cells were pretreated with TAEЕ at doses of 0, 25, 50, 100, 200, and 400  $\mu$ g/ml for 24 and 48 h. As shown in Fig. 3A, the viability of HaCaT cells was similar at all concentrations. To investigate the effects of TAEЕ on the expression of inflammatory chemokines in HaCaT cells, we analyzed the mRNA levels of *RANTES*, *MDC*, and *IP-10* using real-time PCR. As shown Fig. 3, the expression levels of *RANTES*, *MDC*, and *IP-10* were elevated in TNF- $\alpha$ - and IFN- $\gamma$ -treated cells. TAEЕ treatment significantly decreased the mRNA levels of *RANTES*, *MDC*, and *IP-10*.

Table I. Primer sequences for reverse transcription-polymerase chain reaction.

Gene	Sense	Anti-sense	BPs
hRANTES	CGCTGTCATCCTCATTGCTA	GCACTTGCCACTGGTGTAGA	148
hMDC	TGCCGTGATTACGTCCTGTTAC	AAGGCCACGGTCATCAGAGTAG	201
hIP-10	TTGCTGCCTTATCTTTCTGACTC	ATGGCCTTCGATTCTGGATT	222
mRANTES	TGCCACGTCAAGGAGTATTTTC	AACCCACTTCTTCTCTGGGTTG	112
mMDC	GTGGCTCTCGTCCTTCTTGC	GGACAGTTTATGGAGTAGCTT	249
mIP-10	CTGAGTGGGACTCAAGGGAT	TCGTGGCAATGATCTCAACAC	151
hSOCS-1	TTTTTCGCCCTTAGCGTGA	AGCAGCTCGAAGAGGCAGTC	119
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC	226

h, human; m, mouse; RANTES, regulated on activation, normal T cell expressed and secreted; MDC, macrophage-derived chemokine; IP-10, IFN- $\gamma$ -induced protein of 10 kd; SOCS-1, suppressor of cytokine signaling; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; BPs, base pairs.

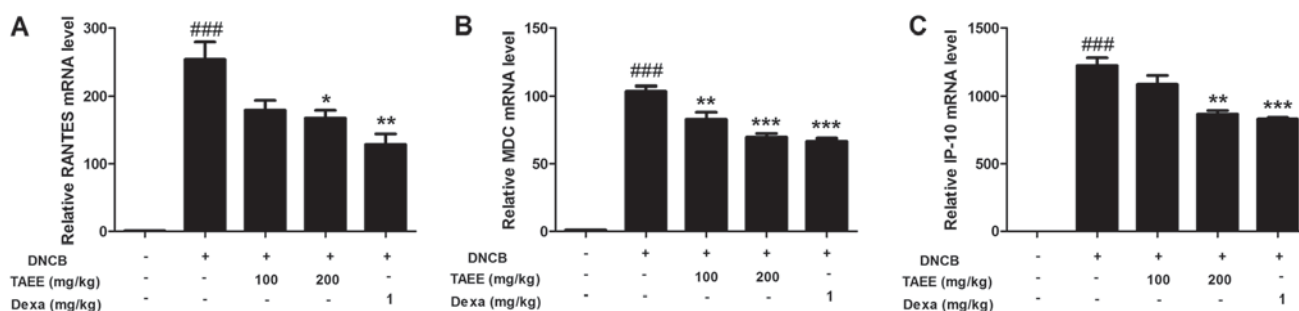


Figure 2. TAE decreases inflammatory chemokines in DNCB-treated mice. The mRNA levels of the chemokines (A) RANTES, (B) MDC and (C) IP-10 were determined using reverse transcription-polymerase chain reaction. Values are presented as the mean  $\pm$  standard error of the mean of three independent experiments. Data were analyzed by Tukey's post-hoc test.  $^{###}P<0.001$  vs. the normal group;  $^{*}P<0.05$ ,  $^{**}P<0.01$  and  $^{***}P<0.001$  vs. the DNCB-treated group. TAE, 70% EtOH extract of *T. aestivum* sprouts; DNCB, 2,4-dinitrochlorobenzene; Dexa, dexamethasone; RANTES, regulated upon activation, normally T-expressed, and presumably secreted; MDC, macrophage-derived chemokine IP-10, IFN- $\gamma$ -induced protein of 10 kDa.

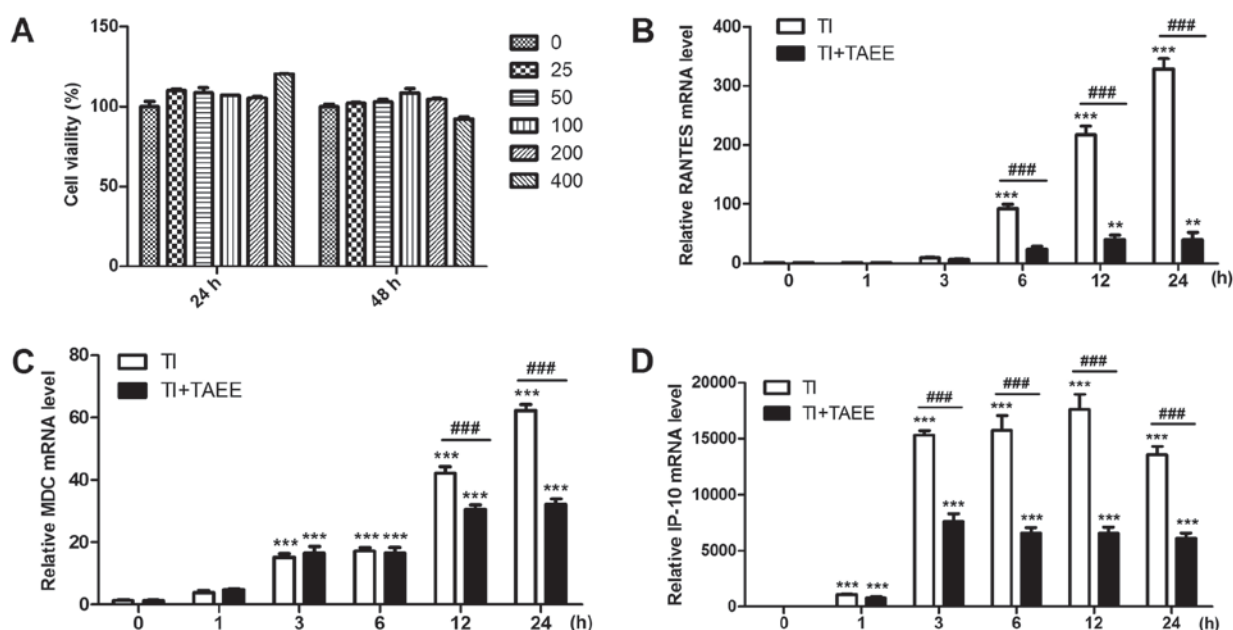


Figure 3. TAE suppresses inflammatory chemokines in TNF- $\alpha$  and IFN- $\gamma$ -stimulated HaCaT cells. (A) HaCaT cells were treated with various concentrations of TAE for 24 and 48 h and the cell viability was determined using the Cell Counting kit assay. Cells were pre-treated with TAE (50  $\mu$ g/ml) for 2 h and in the presence of TNF- $\alpha$  (10 ng/ml) and IFN- $\gamma$  (10 ng/ml) for the indicated time. The mRNA levels of (B) RANTES, (C) MDC and (D) IP-10 were examined using reverse transcription-polymerase chain reaction. GAPDH was used as the internal control. Values are presented as the mean  $\pm$  standard error of the mean of three independent experiments. Data were analyzed by Tukey's post-hoc test.  $^{**}P<0.01$  and  $^{***}P<0.001$  vs. 0 h;  $^{###}P<0.001$ . TAE, 70% EtOH extract of *T. aestivum* sprouts; TI, TNF- $\alpha$  + IFN- $\gamma$ ; TNF, tumor necrosis factor; IFN, interferon; RANTES, regulated upon activation, normally T-expressed, and presumably secreted; MDC, macrophage-derived chemokine IP-10, IFN- $\gamma$ -induced protein of 10 kDa.

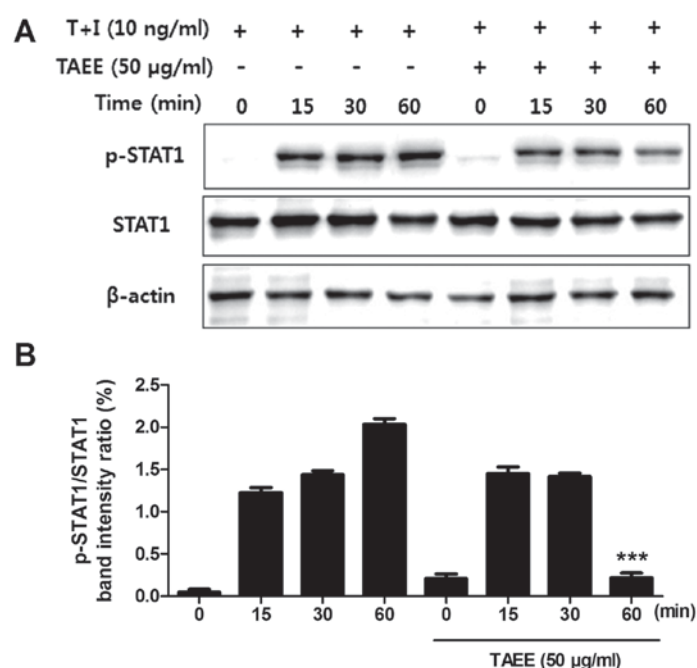


Figure 4. TAEЕ inhibits STAT-1 phosphorylation in TNF- $\alpha$ - and IFN- $\gamma$ -stimulated HaCaT cells. Cells were pre-treated with TAEЕ (50  $\mu$ g/ml) for 2 h and in the presence of TNF- $\alpha$  (10 ng/ml) and IFN- $\gamma$  (10 ng/ml) for the indicated time. (A) The protein levels of phosphorylated and total forms of STAT1 were determined by western blot analysis and (B) the data was quantified. The intensities of the bands are expressed as the mean  $\pm$  standard error of the mean of three independent experiments. Data were analyzed by Tukey's post-hoc test. \*\*\*P<0.001 vs. the TNF- $\alpha$  and IFN- $\gamma$  treatment-only group. TAEЕ, 70% EtOH extract of *T. aestivum* sprouts; T+I, TNF- $\alpha$  + IFN- $\gamma$ ; TNF, tumor necrosis factor; IFN, interferon; STAT1, signal transducer and activator of transcription 1.

*Effects of TAEЕ on TNF- $\alpha$ - and IFN- $\gamma$ -induced STAT1 phosphorylation in HaCaT cells.* Previous reports have shown that TNF- $\alpha$  and IFN- $\gamma$  activate the STAT pathway in human epidermal keratinocytes and that TNF- $\alpha$ - and IFN- $\gamma$ -induced release of chemokines involves phosphorylation of the STAT1 transcription factor (22). Therefore, we examined STAT1 phosphorylation in TNF- $\alpha$ - and IFN- $\gamma$ -treated HaCaT cells, with or without TAEЕ. The results showed that, when compared with the control, treatment with TAEЕ for 1 h effectively inhibited STAT1 phosphorylation (Fig. 4A and B).

*Effects of TAEЕ on SOCS1 expression in TNF- $\alpha$ - and IFN- $\gamma$ -treated HaCaT cells.* SOCS1 exhibits inhibitory activity against STAT1 (23,24). Therefore, we assessed the effects of TAEЕ on SOCS1. The *SOCS1* mRNA levels were examined in TNF- $\alpha$ - and IFN- $\gamma$ -treated HaCaT cells in the presence or absence of TAEЕ. As shown in Fig. 5, TAEЕ markedly enhanced *SOCS1* expression compared with the control.

## Discussion

AD is characterized by relapsing, eczematous skin lesions, skin hypersensitivity, and dry skin, caused by the interaction of Th1 and Th2 cells (2,25). Despite extensive research, the exact cause of AD and a definitive cure remain elusive. Although AD is usually treated with anti-inflammatory or immunosuppressive drugs such as steroids and antihistamines, many of these treatments have serious side effects (26,27). Currently, many patients are turning to alternative strategies that use plant-based natural products with fewer side effects. Therefore, it is essential to investigate health products and new drugs for the safe and effective prevention and treatment of AD. Many

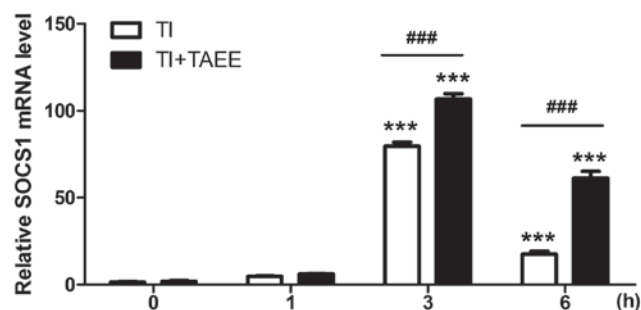


Figure 5. TAEЕ induces SOCS1 expression in TNF- $\alpha$ - and IFN- $\gamma$ -stimulated HaCaT cells. Cells were pre-treated with TAEЕ (50  $\mu$ g/ml) for 2 h and in the presence of TNF- $\alpha$  (10 ng/ml) and IFN- $\gamma$  (10 ng/ml) for the indicated time. The mRNA level of *SOCS1* was analyzed using reverse transcription-polymerase chain reaction. GAPDH was used as the internal control. Values are presented as the mean  $\pm$  standard error of the mean of three independent experiments. Data were analyzed by Tukey's post-hoc test. \*\*\*P<0.001 vs. 0 h; ###P<0.001 vs. the TNF- $\alpha$  and IFN- $\gamma$  treatment-only group. TAEЕ, 70% EtOH extract of *T. aestivum* sprouts; TI, TNF- $\alpha$  + IFN- $\gamma$ ; TNF, tumor necrosis factor; IFN, interferon; SOCS1, suppressor of cytokine signaling 1.

plant-based products have been used to treat and meliorate AD (28,29).

*T. aestivum* sprouts, known as wheatgrass, are consumed in the form of juices or dried powders, and are known as a health food. *T. aestivum* sprouts contain vitamins A, B, C, and K, calcium, potassium, iron, magnesium, sodium, amino acids, chlorophyll, and minerals (30). In our previous studies, we showed that a dichloromethane fraction isolated from *T. aestivum* sprouts attenuated the allergic immune response in ovalbumin (OVA)-sensitized mice, which indicated that *T. aestivum* sprouts might have the potential to regulate the

immune response in allergic diseases (18). However, until now, the effect of *T. aestivum* sprouts on AD, an allergic diseases, was not known. In this study, we examined whether TAEF, as a promising plant candidate compound, could attenuate AD in a DNCB-treated mouse model and in inflammatory cytokine-treated human keratinocytes.

AD patients have skin barrier dysfunctions, such as skin hyperkeratosis and increased TEWL (31). In addition, AD increases the levels of IgE antibodies. When IgE binds to cell surface receptors, mast cells become activated and secrete histamine, causing inflammation and worsening the skin condition. Thus, it is important to reduce serum IgE levels and TEWL. For this reason, we measured skin thickness and moisture content and serum IgE levels in DNCB-treated dermatitis. Oral administration of TAEF significantly reduced TEWL and serum IgE levels when compared to DNCB-treated mice. A histological section of dorsal skin tissues showed that TAEF markedly suppressed an increase in the thickness of the epidermis and dermis, as well as the infiltration of mast cells.

Keratinocytes make up to 90% of cells in the epidermis and, when activated by inflammation (e.g., by TNF- $\alpha$ , and IFN- $\gamma$ ), produce a variety of chemokines. Th2-related chemokines attract inflammatory cells to promote their infiltration into inflammatory skin lesions. These infiltrating inflammatory cells promote a switch from acute to chronic responses in AD. In chronic AD skin lesions, Th1 cells produce TNF- $\alpha$  and IFN- $\gamma$ . In HaCaT cells, Th2-related chemokines are induced by TNF- $\alpha$  and IFN- $\gamma$  stimulation. The downregulation of inflammatory chemokine production in keratinocytes may be an effective therapeutic strategy for inflammatory skin diseases.

Many studies have shown that TNF- $\alpha$  and IFN- $\gamma$  induce the production of Th2-chemokines through STAT in human epidermal keratinocytes (32,33).

The SOCS proteins are cytokine-inducible negative regulators of cytokine signaling, and their levels are increased by IFN- $\gamma$  treatment. The family consists of three proteins, of which SOCS1 and SOCS3 inhibit increased STAT1 phosphorylation in response to IFN- $\gamma$  stimulation (23,24).

In the present study, we found that TAEF suppressed the mRNA levels of chemokines such as RANTES, MDC and IP-10 in TNF- $\alpha$ - and IFN- $\gamma$ -stimulated HaCaT cells and in DNCB-treated mice. In addition, TAEF decreased STAT1 phosphorylation and increased the mRNA levels of *SOCS1*. Thus, TAEF appears to decrease the expression of chemokines by inhibiting the STAT1 pathway and increasing the level of *SOCS1*. These results suggest that TAEF exerts its protective effect in skin inflammation by regulating pro-inflammatory chemokines via phosphorylation of STAT1.

In conclusion, the results of our study demonstrate that TAEF is a natural anti-AD compound, which inhibits AD like-skin lesions and the release of inflammatory chemokines in the skin by regulating inflammation and allergy mediators. Thus, TAEF has potential as a natural treatment for inflammatory allergic responses of the skin.

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## Availability of data and materials

All data sets generated or analyzed during this article are included within the published article.

## Authors' contributions

Y-ML, D-KK and H-HK designed the study. J-HL and H-HK wrote the manuscript. H-HK collected clinical samples. J-HL reviewed and analyzed the data. All authors confirmed and approved review for this manuscript.

## Ethics approval and consent to participate

The present study was approved by the Animal Experiment Ethics Committee of Chonbuk National University (approval no. CBNU 2017-0002).

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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