Journal of Food and Nutrition Research, 2022, Vol. 10, No. 6, 415-424
Available online at http://pubs.sciepub.com/jfnr/10/6/5
Published by Science and Education Publishing
DOI:10.12691/jfnr-10-6-5

Orostachys japonicus Suppresses IFN-γ and TNF-α Induced Proinflammatory Chemokines in Atopic Dermatitis Relating HaCaT Cells via Down-regulation of Expression and Translocation of NF-κB

Yoo Jung Jeon[1,#], Sung Hyun Kim[2,#], Dong Seok Lee[1,2,3,*]

[1]Institute of Digital Anti-aging Healthcare, Graduate School, Inje University, Gimhae 50834, Republic of Korea
[2]Department of Smart Foods and Drugs, Graduate School, Inje University, Gimhae 50834, Republic of Korea
[3]Department of Biomedical Laboratory Science, Inje University, 197 Inje-ro, Gimhae 50834, Gyeongsangnam-do, Republic of Korea
[#]These authors contributed equally to this work.
*Corresponding author: mbdslee@inje.ac.kr

Received May 08, 2022; Revised June 11, 2022; Accepted June 21, 2022

Abstract To investigate the anti-atopic dermatitis (AD) activity of dichloromethane (DCM) fraction of ethanol extracts from *Orostachys japonicus* (OJ) in AD relating human keratinocytes (HaCaT cells) through down-regulation of a key transcription factor NF-κB involved in skin inflammatory responses. The DCM fraction was obtained from OJ ethanol extracts and used for this study. For anti-AD activity, HaCaT cells that have been induced with IFN-γ and TNF-α at the same concentration to produce proinflammatory chemokines were used. As analysis methods, WST assay, chemokines measurement, antibody array, nuclear/cytoplasmic protein separation, and western blotting were used. WST assay showed no toxicity to cell viability. The production levels of chemokines such as MDC, RANTES, IL-8, and TARC decreased in a concentration-dependent manner. Immunoassay through the antibody array showed that the DCM fraction down-regulated the NF-κB (p65) expression. In addition, as a result of western blotting, it was revealed that the translocation of NF-κB (p65) from the cytoplasm into the nucleus decreased. The DCM fraction from OJ ethanol extracts showed significant anti-AD activity through suppression of proinflammatory chemokines causing AD in HaCaT cells via down-regulation of expression and translocation of NF-κB (p65) into the nucleus.

Keywords: *Orostachys japonicas, atopic dermatitis, keratinocyte, chemokine, HaCaT, NF-κB (p65)*

Cite This Article: Yoo Jung Jeon, Sung Hyun Kim, and Dong Seok Lee, "*Orostachys japonicus* Suppresses IFN-γ and TNF-α Induced Proinflammatory Chemokines in Atopic Dermatitis Relating HaCaT Cells via Down-regulation of Expression and Translocation of NF-κB." *Journal of Food and Nutrition Research*, vol. 10, no. 6 (2022): 415-424. doi: 10.12691/jfnr-10-6-5.

1. Introduction

Atopic dermatitis (AD) is a chronic recurrent skin inflammatory disease characterized by inflammation, itching, redness, blistering on the dry and rough skin. Extreme itching leads to scratching behavior, which is an important symptom of AD. AD is primarily affected by genetic, environmental, and immunological factors [1,2,3]. Patients with AD often carry a personal or familial history of other allergic diseases such as asthma and allergic rhinitis, and many of them possess the genetic mutations in the genes associated with defective epidermal differentiation and skin barrier formation. Current treatment for AD includes topical application of moisturizers, anti-inflammatory agents, and phototherapy [4]. In AD, the filtration of eosinophils, mast cells, macrophages, and other inflammatory cells into the skin barrier increases [5,6,7]. Keratinocytes are associated with an inflammatory skin response based on the production of proinflammatory chemokines which are quite high in the skin of people with AD [8]. The expression of various proinflammatory chemokines produced by keratinocytes is an important aspect of the development of AD. T helper (Th2) type immune responses and T cell mediated delayed hypersensitivity reactions are known to be involved in AD [9,10].

Orostachys japonicus (Wasong) is a perennial medicinal plant mainly found in Korea, China, and Japan. It blooms in september and grows up to 20-40 cm tall on sunny rocks in mountains and tiled roofs. *O. japonicus* (OJ) tastes sour and bitter, and its nature is cool and non-poisonous. It has antipyretic, detoxification, and hemostatic effects, and is known to treat eczema, swelling, hemorrhoids, and burns. Also it has anti-cancer,

anti-inflammatory, anti-oxidant, and anti-bacterial effects. Many flavonoids, phenolic acids, triterpenoids, and sterols have been previously reported as medicinal components of OJ. The ingredients of OJ identified so far include kaempferol, quercetin, afzelin (kaempferin, or kaempferol 3-rhamnoside), astragalin (kaempferol 3-glucoside), quercitrin (quercetin 3-rhamnoside), isoquercitrin (quercetin 3-glucoside) derived from ethyl acetate (EtOAc) fraction, kaempferol 3-rhamnoside-7-glucoside, kaempferol 3,7-diglucoside, and cynaroside (luteolin 7-glucoside) from n-butanol (BuOH) fraction, campesterol glucoside and β-sitosterol glucoside from dichloromethane (DCM) fraction, and friedelin, epifriedelanol, glutinone, glutinol, β-amyrin, taraxerone, campesterol, and β-sitosterol along with ten kinds of fatty acid methyl esters from n_hexane (hexane) fraction. And polysaccharides and oligosaccharides-based components are also present in hot water (H_2O) fraction [11-24].

Activation of the NF-κB pathway is associated with an allergic inflammatory response through the phosphorylation and degradation of Iκ-B and the translocation of NF-κB into the nucleus, which regulates the expression of target genes for proinflammatory mediators and chemokines. MAPK and STAT1 signaling controls the activation of NF-κB through the phosphorylation of Iκ-B via prephosphorylation of Iκ-B kinase (IKK), and the NF-κB is known as a key transcription factor to undertake the expression of proinflammatory genes. This pathway promotes the production of proinflammatory chemokines such as MDC, RANTES, IL-8, and TARC in IFN-γ and TNF-α induced AD relating HaCaT cells. In normal conditions, NF-κB exists in the cytoplasm bound to the NF-κB inhibitor Iκ-B. The activation of NF-κB causes the degradation of Iκ-B from the complex of Iκ-B and NF-κB p65/p50 heterodimer, allowing translocation of NF-κB into the nucleus. After translocation, NF-κB localizes to its target sites and mediates expression of proinflammatory factors and chemokines. Therefore, inhibition of activation, translocation, or expression of NF-κB alleviates the production level of proinflammatory mediators and chemokines [25,26,27].

In this study, we focused on the investigation of anti-AD activity of DCM fraction containing campesterol glucoside and β-sitosterol glucoside obtained from ethanol extracts of OJ in AD relating HaCaT cells.

2. Materials and Methods

2.1. OJ Material and Preparation of Solvent Fractions Including DCM Fraction

OJ provided by Wasong farm in Miryang (Gyeongsangnam-do, Korea) was dried and crushed. 200 g of OJ powder were dissolved in 95% ethanol and extracted by boiling thrice for 3 h each time. After cooling the ethanol extracts at room temperature, they were filtered. The ethanol extracts were concentrated and dried by rotary evaporation at 40°C. After the concentrates were suspended in water, each fraction was yielded using following each solvent in the course of hexane, DCM, EtOAc, BuOH, and H_2O. All solvent fractions were concentrated by rotary evaporation at 40°C and then, dried and stored at -20°C until use. Figure 1 shows the procedure for the preparation of solvent fractions including DCM fraction from ethanol extracts of OJ.

2.2. Cell Line and Reagents

HaCaT cells are human keratinocyte cell lines. It was purchased from the German Cancer Research Center. The cells were cultured in Dulbeco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% 5,000 unit/mL penicillin-5,000 μg/mL streptomycin at 37°C under CO_2 conditions. DMEM, FBS, penicillin, and streptomycin for subculture were purchased from Hyclone (Logan, UT). Monoclonal antibodies (NF-κB, Iκ-B, IKKβ, and lamin) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The Goat anti-rabbit IgG-HRP-conjugated polyclonal secondary antibody was purchased from Enzo Life Sciences, Inc. (Enzo Life Sciences, Farmingdale, New York). All reagents used were of the highest grade.

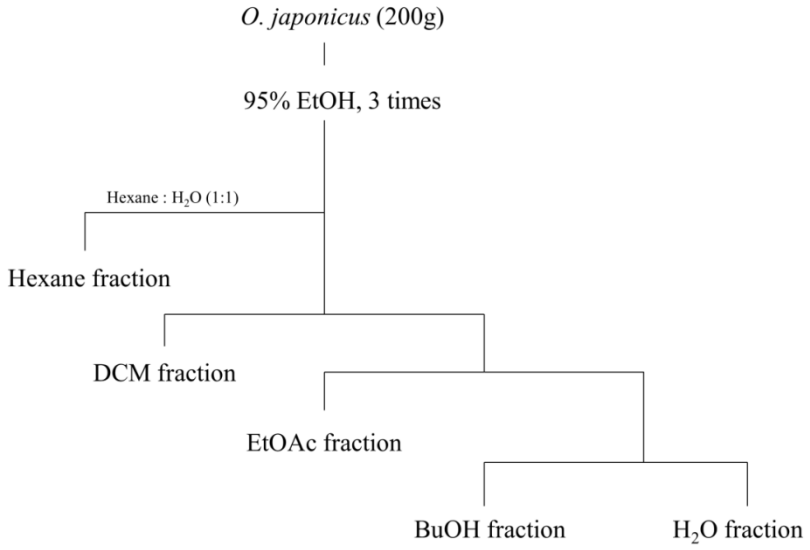

Figure 1. Preparation of DCM fraction from *Orostachys japonicus*

2.3. Cell Culture

Human keratinocyte cell line HaCaT cells were cultured at 37°C with 5% CO_2 in DMEM containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. When the cells were more than 80% confluent in the plate, they were subcultured at a ratio of 1:3. The medium was changed every 24 or 48 h. The cells were cultured for 36 to 48 h (until approximately 80% confluency). HaCaT cells were treated with vehicle, control (0.1% DMSO) or various concentrations of DCM fraction in serum free medium for 2 h. The inflammation response was induced by treating the cells with IFN-γ and TNF-α at each concentration of 10 ng/mL. The cultured cells were visually observed every day through an optical microscope.

2.4. Cell Viability Assay

The cell stability was measured using the EZ-CYTOX non-radioactive cell viability, proliferation and cytotoxicity assay kit according to the manufacturer's protocol. Briefly, the cells were seeded in 96-well plates, pre-incubated at 37°C for 21 h, and then treated with DCM fraction at the concentration of 10, 20, 40, or 80 μg/mL. After incubation for 18 h, the medium was aspirated, serum free medium was added, and then 10 μL of WST EZ-Cytox reagent was applied to each well. The cells were incubated without light for 3 h and the absorbance was measured at 450 nm using a microplate reader (PowerWaveXS, BioTek, VT, USA). This assay was repeated three times with three different samples for each measurement.

2.5. Measurement of Chemokines Production

IL-8 production was determined using a BD Biosciences OptEIA. The production of MDC, RANTES, and TARC was determined using a R&D quantikine enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. The cell culture medium supernatant was harvested. Particulate materials were immediately removed by centrifugation. 100 μL of assay diluent RD 1-45 was added to each well. And then 100 μL of standard or sample was added to each well, and incubated at 2-8°C for 2 h. Each well was aspirated, washed, and this process was repeated three times for a total of four washes. 200 μL of human MDC, RANTES, IL-8, and TARC conjugates were dispensed into each well. Each well was aspirated and washed and this process was repeated three times for a total of four washes. 200 μL of substrate solution was dispensed into each well and incubated without light for 30 min at room temperature. 50 μL of stop solution was added into each well. The optical density of each well was determined within 30 min using a microplate reader set at 450 nm (PowerWaveXS, BioTek, VT, USA). This assay was repeated three times with three different samples for each measurement [28-34].

2.6. Antibody Array

Antibody array analysis was determined using the R & D proteome profilerTM array human NF-κB pathway array kit according to the manufacturer's instructions. The cells were rinsed with PBS and the remaining PBS was removed before adding lysis buffer. 2 mL of array buffer was dispensed into each well of a 4-well multi-dish to be used. Each membrane to be used was removed from between the protective sheets, placed in each well of the 4-well multi-dish, and incubated for 1 h. Array buffer was aspirated from the well of the 4-well multi-dish. The prepared samples were added and the lid was put on the 4 well multi-dish, and incubated overnight at 2-8°C. Each membrane was removed and 20 mL of 1x wash buffer was dispensed into individual plastic container. The 4-well multi-dish was washed with deionized or distilled water and dried completely. Each membrane was rinsed by stirring for 10 min with 1x wash buffer. 1.5 mL per well of the diluted detection antibody cocktail was dispensed into the 4-well multi-dish. Each membrane was removed from the wash container. The membranes were replaced on the 4-well multi-dish having the diluted detection antibody cocktail and incubated for 1 h at room temperature. After each array was washed, 2 mL of Streptavidin-HRP diluted with array buffer was dispensed into each well of the 4-well multi-dish and incubated for 30 min. After each array was washed, 1 mL of the prepared chemical reagent mixture was evenly distributed onto each membrane and incubated for 1 min. A chemiluminescence detector WSE-6200 LuminoGraph II (ATTO KOREA, Daejeon, Republic of Korea) was used for analysis of antibody array.

2.7. Separation of Cytoplasmic and Nuclear Proteins

Cytoplasmic/nuclear proteins of the cells were extracted with a NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific Inc. USA) according to the manufacturer's instructions. The cells harvested with trypsin were centrifuged for 5 min at 1,500 rpm and transferred to a new tube. The collected cells were lysed in CER I containing protease inhibitors, vortexed for 15 sec, and kept on ice for 10 min. CER II reagent was added to the tube, vortexed for 15 sec, and kept on ice for 1 min. The cell supernatants (cytoplasmic extracts) centrifuged at 16,000 g for 5 min at 4°C immediately were transferred to a new clean pre-chilled tube. NER reagent was added to the remaining cell precipitates. And the mixture was centrifuged at 16,000 g for 5 min at 4°C. The resulting supernatants (nuclear extracts) were immediately transferred to a new clean pre-chilled tube. The protein concentration was measured using the BCA protein assay (Pierce, Rockford, IL, USA) with a microplate reader (PowerWaveXS, BioTek, VT, USA) at 520 nm.

2.8. Western Blotting Analysis

The cultured cells on the plate were rinsed twice with PBS (pH 7.4) and collected. Cytoplasmic/nuclear proteins of the cells were extracted with a NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific Inc. USA). Protein concentrations were determined using BCA protein assay (Pierce, Rockford, IL, USA). Equal amounts of protein were mixed with 2×Laemmli loading buffer and preheated at 95°C for 5 min. After boiling, they were put on ice and kept for 5 min. Samples were added onto each well of a 10%

SDS-polyacrylamide gel and then were electrophoresed. After that, they were transfered onto a PVDF membrane using a transfer system (Bio-Rad, Philadelphia, PA, USA) for 90 min at room temperature. Membranes were blocked with 5% skin milk in PBST with 10% Tween 20 for 1 h at room temperature and then incubated overnight with primary antibodies. After attaching the primary antibodies, the membranes were washed for 10 min 3 times with PBST. They were subsequently incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature and washed for 5 min 3 times with PBST. Final detection was performed with western blotting luminol reagent (Santa Cruz, CA, USA) [35-40].

3. Results

3.1. Cytotoxic Effects of OJ DCM Fraction on HaCaT Cells

Cytotoxicity was measured using WST assay of HaCaT cells pretreated with various concentrations of DCM fraction (10, 20, 40, 80, and 100 μg/mL) for 18 h. When the DCM fraction was applied at the concentration of 80 μg/mL, the cell viability was more than 90%, indicating no toxicity to the cells (Figure 2).

3.2. Effects of DCM Fraction on the Induction of Production of Proinflammatory Chemokines by IFN-γ and TNF-α in HaCaT Cells

The proinflammatory chemokines were measured using the immunoassay. HaCaT cells were cultured in six well plates for 18 h. The cells were treated with vehicle, control (0.1 % DMSO), 10, 20, 40, or 80 μg/mL of DCM fraction in serum free medium for 2 h, and then inflammation was induced by treating the cells with 10 ng/mL of IFN-γ and TNF-α for 18 h. After collecting the culture medium, the supernatant yielded was used to assay the quantities of chemokines produced. The results showed that DCM fraction reduced the production levels of MDC, RANTES, IL-8, and TARC (Figure 3). DCM fraction pretreatment dose-dependently suppressed the induction of production of AD related chemokines by IFN-γ and TNF-α in HaCaT cells.

3.3. Antibody Array Screening for NF-κB Pathway by DCM Fraction

The levels of expression of the NF-κB pathway proteins associated with AD were measured in HaCaT cells. HaCaT cells were treated with DCM fraction to assess the levels of expressed NF-κB pathway related proteins. To measure the level of protein expression the immunoassay was used. HaCaT cells were pretreated with DCM fraction for 2 h and then induced by treating the cells with IFN-γ and TNF-α at the concentration of 10 ng/mL. Culture medium was collected and then the protein concentration was measured. The expression levels of NF-κB pathway associated proteins were increased in the group induced by IFN-γ and TNF-α without pretreatment with DCM fraction. In the group pretreated with DCM fraction and then induced by IFN-γ and TNF-α, the expression levels of related proteins were lower than in the group induced by IFN-γ and TNF-α without pretreatment with DCM fraction (Figure 4). In the DCM fraction pretreated cells the quantities of NF-κB1 (p105), NF-κB2 (p100), RelA/p65, RelA/p65 (pS529), and c-Rel decreased. These results suggest that DCM fraction suppress the phosphorylation of RelA/p65 as well as the expression of NF-κB1, NF-κB2, RelA/p65, and c-Rel.

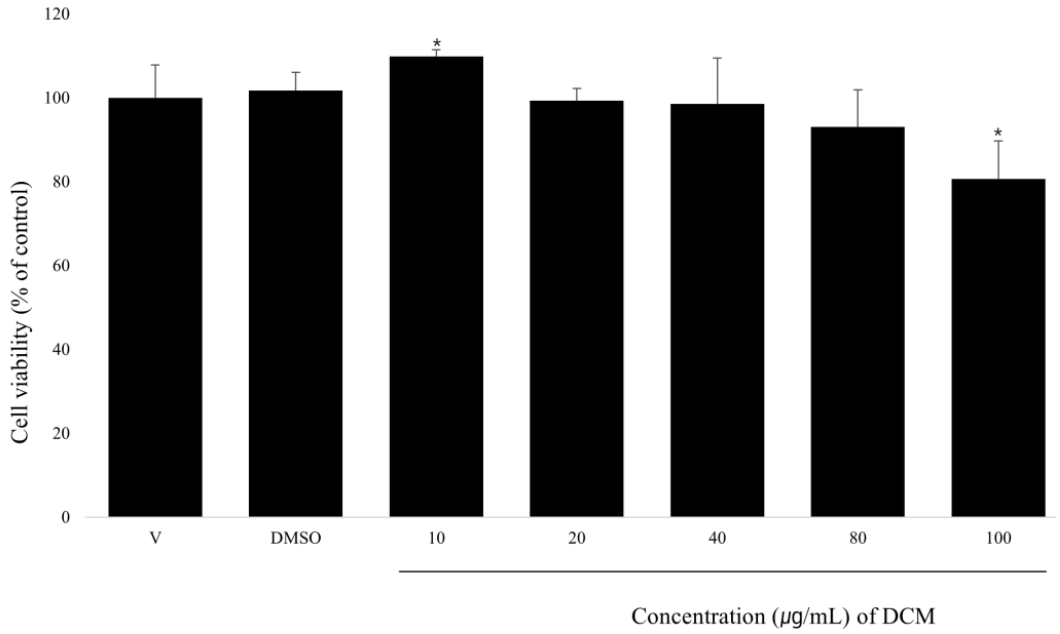

Figure 2. Effect of DCM fraction on cell viability for 18 h in HaCaT cells. The cells were treated with the 10^5 cell volume and indicated concentrations (vehicle, 0.1% DMSO, 10, 20, 40, 80, and 100 μg/mL) of DCM fraction for 18 h. The cell viabilities were determined using WST assay. The values are expressed as the means ± S.D. ∗p < 0.06 versus control

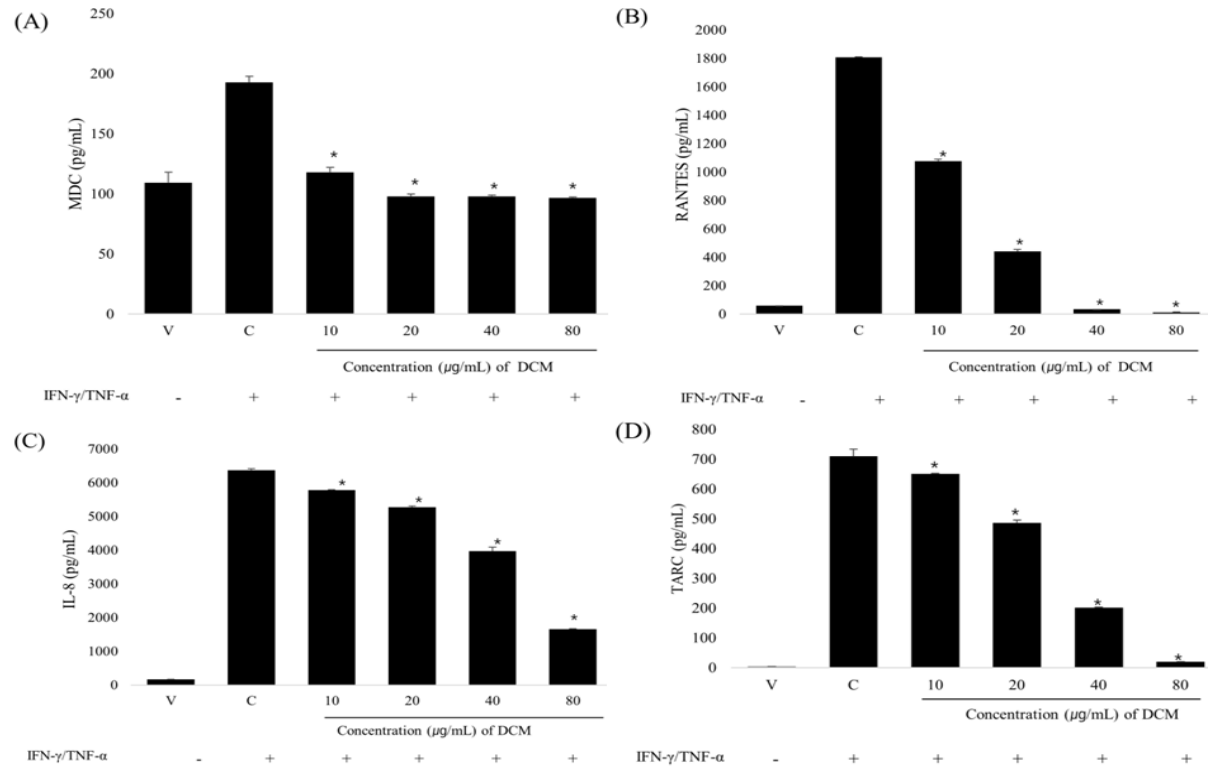

Figure 3. The production levels of (A) MDC, (B) RANTES, (C) IL-8, and (D) TARC were measured using the culture supernatant of IFN-γ and TNF-α stimulated HaCaT cells. The cells were pretreated with various concentrations of DCM fraction (0, 0.1% DMSO, 10, 20, 40, and 80 μg/mL) for 2 h and then induced with IFN-γ and TNF-α (each 10 ng/mL) for 18 h. V, vehicle (serum free medium), C, control (DMSO+IFN-γ/TNF-α). The data are presented as the means ± SEM of three experiments. ∗$p < 0.06$ versus control

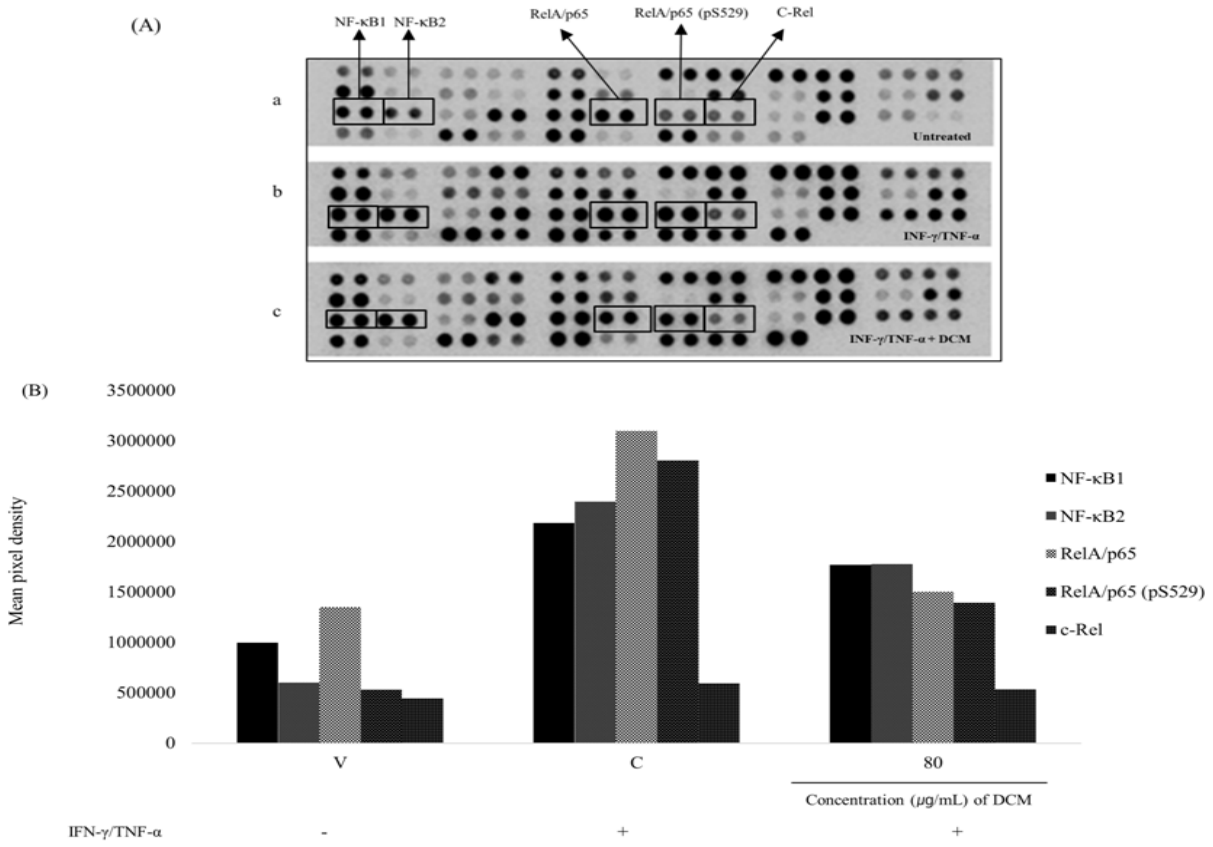

Figure 4. Effect of DCM fraction on the expression level of AD related NF-κB signaling proteins in HaCaT cells. HaCaT cells untreated (a), HaCaT cells induced by IFN-γ and TNF-α (each 10 ng/mL) without pretreatment with DCM fraction (b), HaCaT cells pretreated with DCM fraction and induced by IFN-γ and TNF-α (each 10 ng/mL) (c). HaCaT cells pretreated with vehicle, control (DMSO), or 80 μg/mL of DCM fraction for 2 h and then treated with 10 ng/mL of IFN-γ and TNF-α for 18 h. Relative abundances of proteins were calculated for NF-κB1, NF-κB2, RelA/p65, RelA/p65 (pS529), and c-Rel. V, vehicle (serum free medium), C, control (DMSO+IFN-γ and TNF-α)

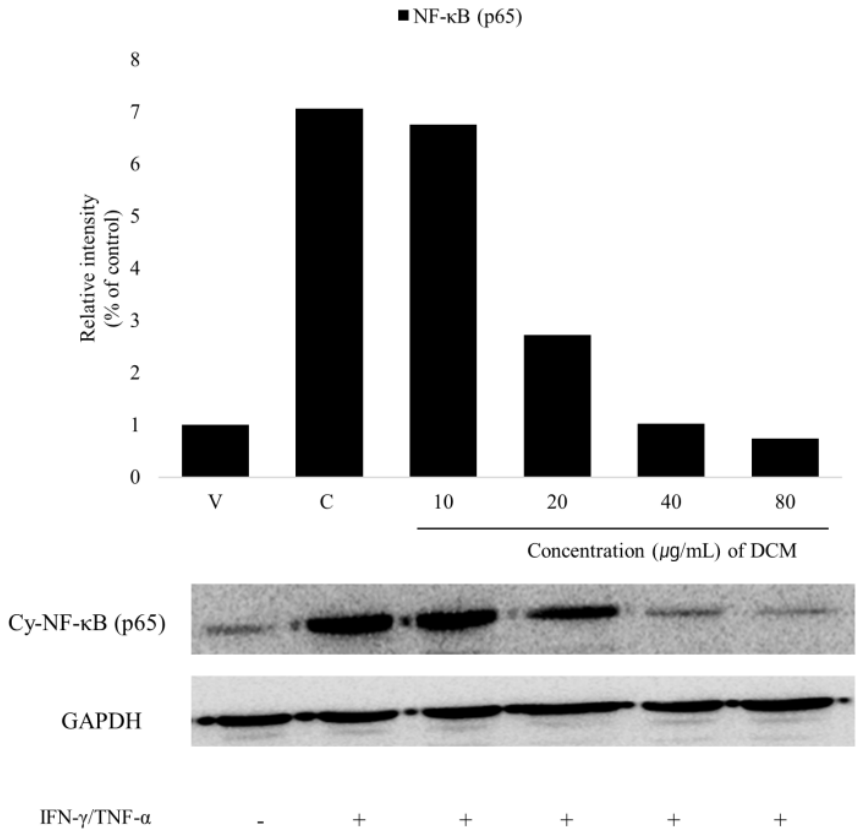

Figure 5. Cytoplasmic protein levels of NF-κB (p65) in HaCaT cells. The cells were pretreated with 0, 0.1% DMSO, 10, 20, 40, and 80 μg/mL of DCM fraction for 2 h and then stimulated with IFN-γ and TNF-α for 18 h. After separating cytoplasmic and nuclear proteins, cytoplasmic protein expression level of NF-κB (p65) were examined by western blotting. The intensity of band was quantified and presented as the bar graph. GAPDH were used as internal controls. V, vehicle (serum free medium), C, control (DMSO+IFN-γ and TNF-α)

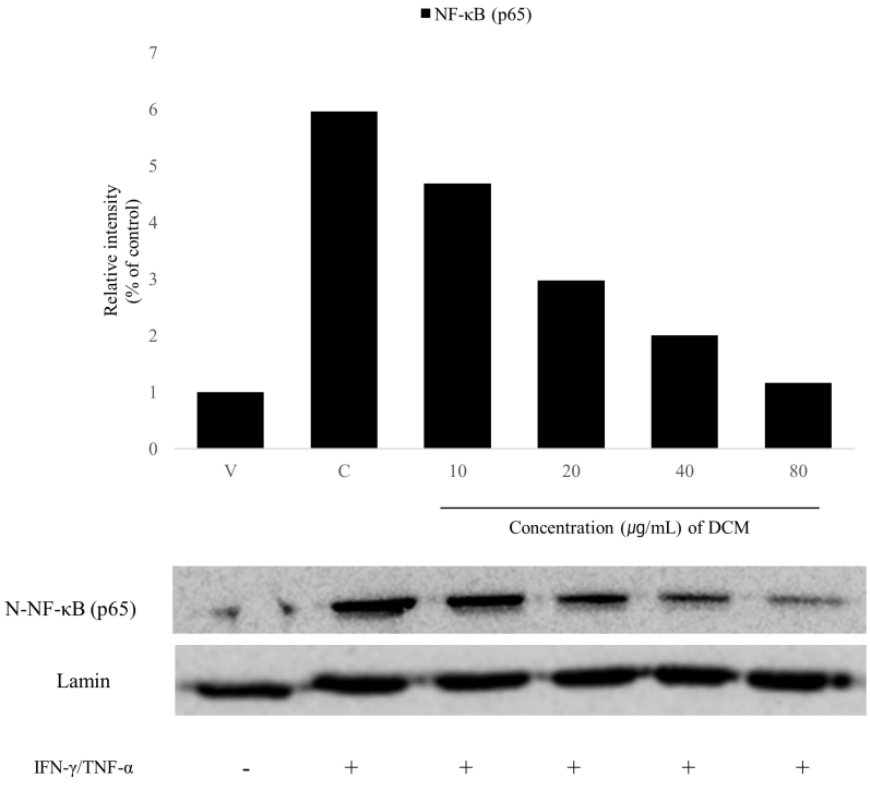

Figure 6. Nuclear protein levels of NF-κB (p65) in HaCaT cells. The cells were pretreated with 0, 0.1% DMSO, 10, 20, 40, and 80 μg/mL of DCM fraction for 2 h and then stimulated with IFN-γ and TNF-α for 18 h. After separating cytoplasmic and nuclear proteins, nuclear translocation level of NF-κB (p65) were examined by western blotting. The intensity of band was quantified and presented as the bar graph. Lamin were used as internal controls. V, vehicle (serum free medium), C, control (DMSO+IFN-γ and TNF-α)

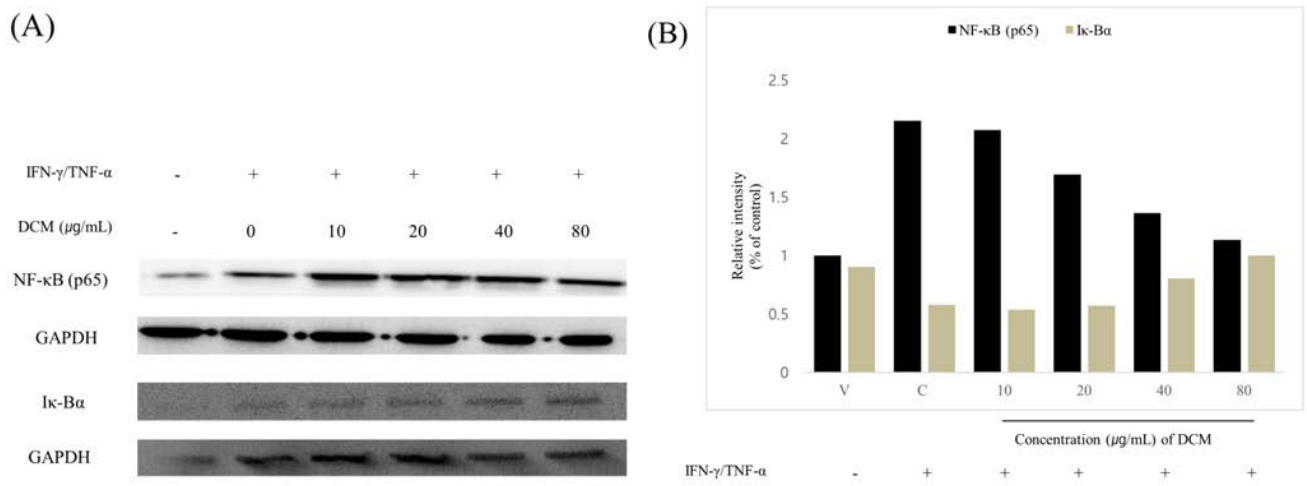

Figure 7. The protein levels of NF-κB (p65) and Iκ-Bα in HaCaT whole cells. The cells were pretreated with 0, 0.1% DMSO, 10, 20, 40, and 80 μg/mL of DCM fraction for 2 h and then stimulated with IFN-γ and TNF-α for 18 h. After lysis the cell, the level of NF-κB (p65) and Iκ-Bα in whole cells were examined by western blotting. The intensity of band (A) was quantified and presented as the bar graph (B). GAPDH were used as internal controls. V, vehicle (serum free medium), C, control (DMSO+IFN-γ and TNF-α).

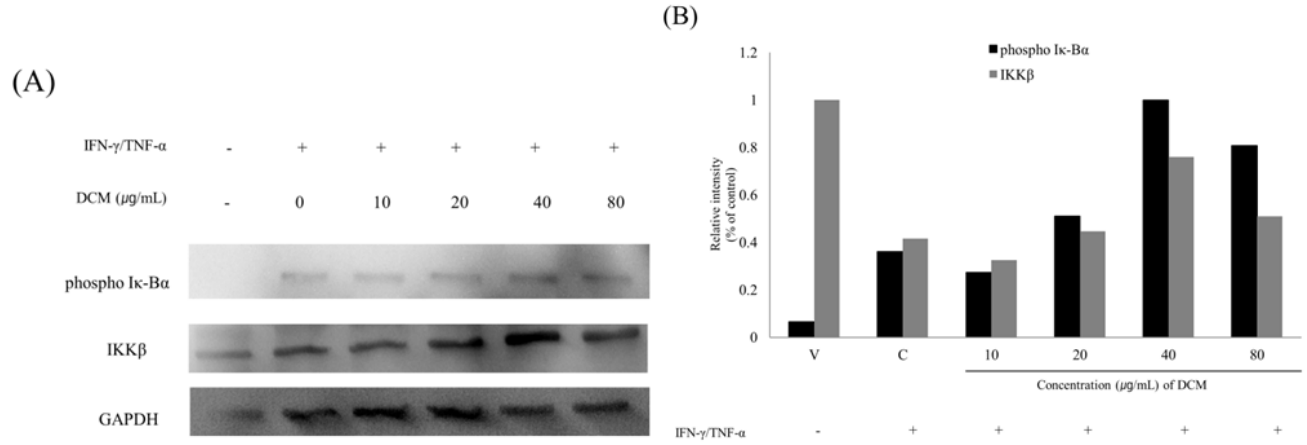

Figure 8. The protein levels of phospho Iκ-Bα and IKKβ in HaCaT whole cells. The cells were pretreated with 0, 0.1% DMSO, 10, 20, 40, and 80 μg/mL of DCM fraction for 2 h and then stimulated with IFN-γ and TNF-α for 18 h. After lysis the cell, the level of phospho Iκ-Bα and IKKβ in whole cells were examined by western blotting. The intensity of band (A) was quantified and presented as the bar graph (B). GAPDH were used as internal controls. V, vehicle (serum free medium), C, control (DMSO+IFN-γ and TNF-α).

3.4. Suppression of Expression and Translocation of NF-κB (p65) Signaling Pathway by DCM Fraction

To further investigate the suppression of expression and translocation of NF-κB (p65) by DCM fraction, this study measured the expression and translocation level of NF-κB (p65). Western blotting was performed to measure the protein expression level. Nuclear and cytoplasmic proteins were isolated to assess the level of translocation of NF-κB (p65) into the nucleus. In order to examine the expression levels of NF-κB (p65) and Iκ-Bα in whole cell lysate, the cells were lysed to measure the whole protein concentration. NF-κB (p65) in the cytoplasmic fraction decreased in a concentration-dependent manner (Figure 5). NF-κB (p65) in the nuclear fraction decreased in a concentration-dependent manner (Figure 6). NF-κB (p65) decreased in the whole cell lysate, and Iκ-Bα increased in the whole cell lysate in a concentration-dependent manner (Figure 7). The levels of phospho Iκ-Bα and IKKβ were slightly increased in the low concentration, but decreased in high concentration (Figure 8). The DCM fraction suppresses the expression of NF-κB (p65) and the translocation of NF-κB (p65) from the cytoplasm into the nucleus while adequately modulating the levels of phospho Iκ-Bα and IKKβ.

4. Discussion

With the increasing incidence of allergic diseases around the world, the risk of skin lesions such as AD is increasing. AD is a chronic inflammatory disease that is difficult to treat. Most of the commercially available AD treatments consist of chemical components. With increasing interest in the development of therapeutic agents using phytochemicals suitable for AD, various phytochemicals are attracting attention as candidates. According to the previous studies, the physiologically active ingredient in the DCM fraction of ethanol extracts from OJ has anti-inflammatory activity. Hence DCM fraction is expected to have preventive and inhibitory effects on AD. This study aims to examine whether the AD-related inflammatory reactions are regulated by pretreating HaCaT cells with DCM fraction. In this study,

the NF-κB signaling pathway and concrete mechanism involving in suppression of production of IFN-γ and TNF-α induced proinflammatory chemokines in AD relating HaCaT cells pretreated with DCM fraction was investigated. Chemokines such as MDC, RANTES, IL-8, and TARC produced by NF-κB (p65) transcription factor stimulate various AD relating inflammatory responses [41-47]. Figure 9 represents the signaling pathway of the NF-κB (p65) and suppression mode of DCM fraction of ethanol extracts from OJ.

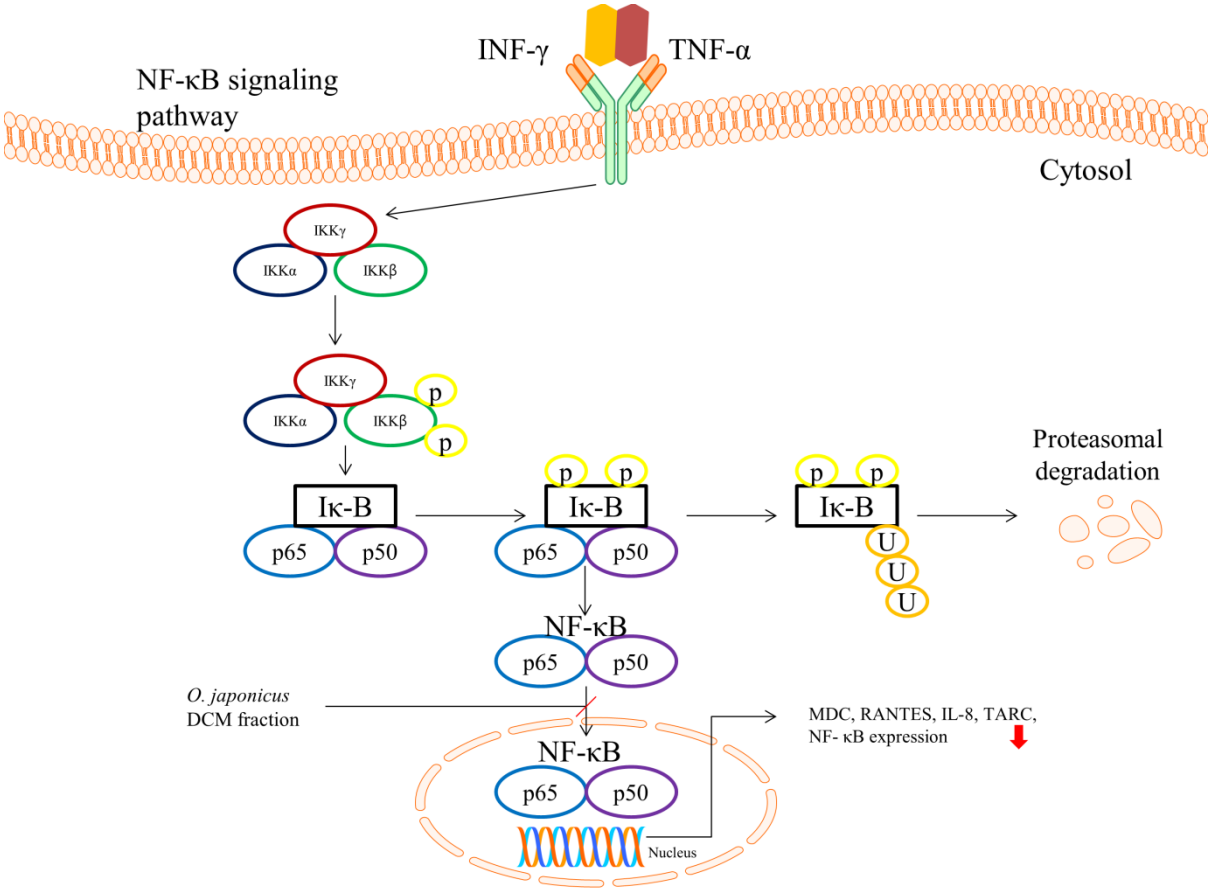

Figure 9. Signaling pathway of the NF-κB (p65) and suppression mode of DCM fraction from ethanol extracts of *Orostachys japonicus*

5. Conclusion

DCM fraction suppressed the production of IFN-γ and TNF-α induced proinflammatory chemokines such as MDC, RANTES, IL-8, and TARC in AD relating HaCaT cells through down_regulating the expression of NF-κB (p65) and the translocation of NF-κB (p65) from cytoplasm into the nucleus while modulating the levels of phospho Iκ-Bα and IKKβ. These results suggest that DCM fraction exhibit potential anti_AD activity and could be utilized to develop therapeutic or preventive agents for AD.

Conflict of Interest Statement

Authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MOE) (NRF-2017R1D1A1B03034570).

Authors' Contributions

Both YJJ and SHK were involved in designing the work, data collection, data analysis, and interpretation. DSL was involved in drafting the article.

References

[1] Ngo, H.T., Fang, M., Hwang, E., Kim, YS., Park B, Seo SA, Do N.Q., Nguyen Q.T. and Yi T.H., "Inhibitory Effects of *Urtica thunbergiana* Ethanol Extract on Atopic Dermatitis-Induced NC/Nga Mice," *Antioxidant*, 9 (3). 197-212. Mar.2020.

[2] Yoo, O.K., Choi, W.J. and Keum, Y.S., "Cardamonin Inhibits Oxazolone-Induced Atopic Dermatitis by the Induction of NRF2 and the Inhibition of Th2 Cytokine Production," *Antioxidants*, 9 (9). 834-845. Sep.2020.

[3] Kawahara, T., Tsutsui, K., Nakanishi, E. and Inoue, T., "Hamauzu Y. Effect of the topical application of an ethanol extract of quince seeds on the development of atopic dermatitis-like symptoms in NC/Nga mice," *BMC*, 17. 80-87. Jan.2017.

[4] Sung, Y.Y. and Kim, H.K., "Crocin Ameliorates Atopic Dermatitis Symptoms by down Regulation of Th2 Response via Blocking of NF-κB/STAT6 Signaling Pathways in Mice," *Nutrients*, 10 (11). 1625-1634. Nov.2018.

[5] Lee, J.H., Lim, J.Y., Jo, E.H., Noh, H.M., Park, S., Park, M.C. and Kim, D.K., "Chijabyukpi-Tang Inhibits Pro-Inflammatory

Cytokines and Chemokines via the Nrf2/HO-1 Signaling Pathway in TNF-α/IFN-γ-Stimulated HaCaT Cells and Ameliorates 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis-Like Skin Lesions in Mice," *Frontiers in Pharmacology*, 11. 1018-1028. Jul.2020.

[6] Choi, Y.A., Yu, J.H., Jung, H.D., Lee, S.Y., Park, P.H., Lee, H.S., Kwon, T.K., Shin, T.Y., Lee, S.W., Rho, M.C., Jang, Y.H. and Kim, S.H., "Inhibitory effect of ethanol extract of *Ampelopsis brevipedunculata* rhizomes on atopic dermatitis-like skin inflammation," *Journal of Ethnopharmacology*, 238. 111850-111859. Jun.2019.

[7] Abdullah, A.A., Morris, K., Lwaleed, B.A., Lau, L., Jones, K., Cooper, R. and Jenkins, R., "Honey is potentially effective in the treatment of atopic dermatitis: Clinical and mechanistic studies," Immunity *Inflammation and Disease*, 5 (2). 190-199. Jun.2017.

[8] Lee, Y.H., Choi, H.K., N'deh, K., Choi, Y.J., Fan, M., Kim, E.K., Chung, K.H. and An, J.H., "Inhibitory Effect of *Centella asiatica* Extract on DNCB-Induced Atopic Dermatitis in HaCaT Cells and BALB/c Mice," *Nutrients*, 12. 411-426. Feb.2020.

[9] Kim, O.K., Nam, D.E., Lee, M.H., Kwon, H.O., Park, J.J., You, Y.H., Kim, S.I., Lee, J. and Jun, W., "The Effects of *Costaria costata* Extracts on Atopic Dermatitis in an In Vitro Model," *J Med Food*, 19 (10). 945-951. Oct.2016.

[10] Bao, L., Zhang, H. and Chan, L.S., "The involvement of the JAK-STAT signaling pathway in chronic inflammatory skin disease atopic dermatitis," *JAK-STAT*, 2 (3). 2162-3996. Jul.2013.

[11] Jeong, J.H., Ryu, D.S., Suk, D.H. and Lee, D.S., "Anti-inflammatory effects of ethanol extract from *Orostachys japonicus* on modulation of signal pathways in LPS-stimulated RAW 264.7 cells," *BMB Reports*, 44 (6). 399-404. Jun.2011.

[12] Lee, H.S., Ryu, D.S., Lee, G.S. and Lee, D.S., "Anti-inflammatory effects of dichloromethane fraction from *Orostachys japonicus* in RAW 264.7 cells: Suppression of NF-κB activation and MAPK signaling," *Journal of Ethnopharmacology*, 140 (2). 271-276. Mar.2012.

[13] Lee, H.S., Bilehale, D., Lee, G.S., Ryu, D.S., Kim, H.K., Suk, D.H. and Lee, D.S., "Anti-inflammatory effect of the hexane fraction from *Orostachys japonicus* in RAW 264.7 cells by suppression of NF-κB and PI3K-Akt signaling," *Journal of Functional Foods*, 5 (3): 1217-1225. Jul.2013.

[14] Lee, H.S., Lee, G.S., Kim, S.H., Kim, H.K., Suk, D.H. and Lee, D.S., "Anti-oxidizing effect of the dichloromethane and hexane fractions from *Orostachys japonicus* in LPS-stimulated RAW 264.7 cells via upregulation of Nrf2 expression and activation of MAPK signaling pathway," *BMB Reports*, 47 (2). 98-103. Feb.2014.

[15] Ryu, D.S., Lee, H.S., Lee, G.S. and Lee, D.S., "Effects of the Ethylacetate Extract of *Orostachys japonicus* on Induction of Apoptosis through the p53-Mediated Signaling Pathway in Human Gastric Cancer Cells," *Biol pharm Bull*, 35 (5). 660-665. Feb.2012.

[16] Ryu, D.S., Baek, G.O., Kim, E.Y., Kim, E.Y., Kim, K.H. and Lee, D.S., "Effects of polysaccharides derived from *Orostachys japonicus* on induction of cell cycle arrest and apoptotic cell death in human colon cancer cells," *BMB Reports*, 43 (11). 750-755. Nov.2010.

[17] Ma, C.J., Jung, W.J., Lee, K.Y., Kim, Y.C. and Sung, S.H., "Calpain inhibitory flavonoids isolated from *Orostachys japonicus*," *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24 (3). 676-679. Jun.2009.

[18] Lee, J.H., Lee, S.J., Park, S.M., Kim, H.K., Jeong, W.Y., Choi, J.Y., Sung, N.J., Lee, W.S., Lim, C.S., Kim, G.S. and Shin, S.C., "Characterisation of flavonoids in *Orostachys japonicus* A. Berger using HPLC-MS/MS: Contribution to the overall antioxidant effect," *Food Chemistry*, 124 (4). 1627-1633. Feb.2011.

[19] Park, H.J., Young, H.S., Park, K.Y., Rhee, S.H., Chung, H.Y. and Choi, J.S., "Flavonoids from the Whole Plants of *Orostachys japonicus*," *Arch Pharm Res*, 14 (2). 167-171. Jun.1991.

[20] Choi, S.Y., Chung, M.J., Seo, W.D., Shin, J.H., Shon, M.Y. and Sung, N.J., "Inhibitory Effects of *Orostachys japonicus* Extracts on the Formation of N-Nitrosodimethylamine," *J Agric Food Chem*, 54. 6075-6078. Jul.2006.

[21] Lee, G.S., Lee, H.S., Kim, H.S., Suk, D.H., Ryu, D.S. and Lee, D.S., "Anti-cancer Activity of the Ethylacetate Fraction from *Orostachys japonicus* for Modulation of the Signaling Pathway in HepG2 Human Hepatoma Cells," *Food Sc. Biotechnol*, 23 (1). 269-275. Dec.2013.

[22] Lee, H.Y., Park, Y.M., Kim, J., Oh, H.G., Kim, K.S., Kang, H.J., Kim, R.R., Kim, M.J., Kim, S.H., Yang, H.J. and Oh, J., "*Orostachys japonicus* A. Berger Extracts Induce Immunity-Enhancing Effects on Cyclophosphamide-Treated Immunosuppressed Rats," *BioMed Research International*, 2019. 1-9. Jan.2019.

[23] Lee, H.S., "*Orostachys japonicus* extract inhibits the lipopolysaccharideinduced pro-inflammatory factors by suppression of transcription factors," *Food Science & Nutrition*, 8. 1812-1817. Feb.2020.

[24] Debnath, T., Lee, Y.M., Lim, J.H. and Lim, B.O., "Anti-allergic and anti-atopic dermatitis effects of *Gardenia Fructus* extract," *Food and Agricultural Immunology*, 29 (1). 665-674. Mar.2018.

[25] Choi, J.K., Jang, Y.H., Lee, S.Y., Lee, S.R., Choi, Y.A., Jin, M., Choi, J.H., Park, J.H., Park, P.H., Chio, H.K., Kwon, T.K., Khang, D.W. and Kim, S.H., "Chrysin attenuates atopic dermatitis by suppressing inflammation of keratinocytes," *Food and Chemical Toxicology*, 110. 142-150. Dec.2017.

[26] Cho, S.H., Kim, H.S., Lee, W.W., Han, E.J., Kim, S.Y., Fernando, I.S., Ahn, G. and Kim, K.N., "Eckol from *Ecklonia cava* ameliorates TNF-α/IFN-γ-induced inflammatory responses via regulating MAPKs and NF-κB signaling pathway in HaCaT cells," *International Immunopharmacology*, 82. 106146-106153. Feb.2020.

[27] Jeong, N.H., Yang, E.J., Jin, M., Lee, J.Y., Choi, Y.A., Park, P.H., Lee, S.R., Kim, S.U., Shin, T.Y., Kwon, T.K., Jang, Y.H., Song, K.S. and Kim, S.H., "Esculetin *from Fraxinus rhynchophylla* attenuates atopic skin inflammation by inhibiting the expression of inflammatory cytokines," *International Immunopharmacology*, 59. 209-216. Jun.2018.

[28] Dao, T.P., Song, K.H., Kim, J.Y. and Kim, Y.S., "Igalan from *Inula helenium* (L.) suppresses the atopic dermatitis-like response in stimulated HaCaT keratinocytes via JAK/STAT3 signaling," *Infammation Research*, 69. 309-319. Jan .2020.

[29] Cha, K.J., Im, M.A., Gu, A., Kim, D.H., Lee, D., Lee, J.S. and Kim, I.S., "Inhibitory effect of *Patrinia scabiosifolia* Link on the development of atopic dermatitis-like lesions in human keratinocytes and NC/Nga mice," *Journal of Ethnopharmacology*, 206 (12). 135-143. Mar.2017.

[30] Choi, Y.A., Yu, J.H., Jung, H.D., Lee, S.Y., Park, P.H., Lee, H.S., Kwon, T.K., Shin, T.Y., Lee, S.W., Rho, M.C., Jang, Y.H. and Kim, S.H., "Inhibitory effect of ethanol extract of *Ampelopsis brevipedunculata* rhizomes on atopic dermatitis-like skin inflammation," *Journal of Ethnopharmacology*, 238 (28). 1-9. Apr.2019.

[31] Choi, J.H., Jin, S.W., Lee, G.H., Cho, S.M. and Jeong, H.G., "*Orostachys japonicus* ethanol extract inhibits 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice and TNF-α/IFN-γ-induced TARC expression in HaCaT cells," *Toxicological Research*, 36. 99-108. Apr.2020.

[32] Ha, Y.N., Lee, Y.H., Jeong, J.W., Park, M., Ko, J.Y., Kwon, O.W., Lee, J. and Kim, Y.J., "*Pyropia yezoensis* Extract Suppresses IFN-Gammaand TNF-Alpha-Induced Proinflammatory Chemokine Production in HaCaT Cells via the Down-Regulation of NF-κB," *Nutrients*, 12. 1238-1253. Apr.2020.

[33] Hou, D.D., Di, Z.H., Qi, R.Q., Wang, H.X., Zheng, S., Hong, Y.X., Guo, H., Chen, H.D. and Gao, X.H., "Sea Buckthorn (*Hippophaë rhamnoides* L.) Oil Improves Atopic Dermatitis-Like Skin Lesions via Inhibition of NF-κB and STAT1 Activation," *Skin Pharmacol Physiol*, 30. 268-276. Sep.2017.

[34] Jin, S.E., Ha, H.K., Shin, H.K. and Seo, C.S., "Anti-Allergic and Anti-Inflammatory Effects of Kuwanon G and Morusin on MC/9 Mast Cells and HaCaT Keratinocytes," *Molecules*, 24. 265-278. Jan.2019.

[35] Yang, J.H., Hwang, Y.H., Gu, M.J., Cho, W.K. and Ma, J.Y., "Ethanol extracts of *Sanguisorba officinalis* L. suppress TNF-α/IFN-γ-induced pro-inflammatory chemokine production in HaCaT cells," *Phytomedicine*, 22 (14). 1262-1268. Nov.2015.

[36] Yang, J.H., Yoo, J.M., Lee, E., Lee, B.H., Cho, W.K., Park, K.I. and Ma, J.Y., "Anti-inflammatory effects of Perillae Herba ethanolic extract against TNF-α/IFN-γ-stimulated human keratinocyte HaCaT cells," *Journal of Ethnopharmacology*, 211 (30). 217-223. Nov.2018.

[37] Yang, J.H., Lee, E., Lee, B.H., Cho, W.K., Ma, J. and Park, K.I., "Ethanolic Extracts of *Artemisia apiacea* Hance Improved

Atopic Dermatitis-Like Skin Lesions In Vivo and Suppressed TNF-Alpha/IFN-Gamma-Induced Proinflammatory Chemokine Production In Vitro," *Nutrients*, 10. 806-816. Jul.2018.

[38] Yang, J.H., Do, H.J., Lee, E., Yim, N.H., Cho, W.K., Park, K.I. and Ma, J.Y., "Jageum-Jung improves 2,4-dinitrochlorobenzene-induced atopic dermatitislike skin lesions in mice and suppresses pro-inflammatory chemokine production by inhibiting TNF-α/IFN-γ-induced STAT-1 and NFκB signaling in HaCaT cells," *Journal of Ethnopharmacology*, 221 (15). 48-55. Apr.2018.

[39] Cha, K.J., Kashif, A., Hong, M.H., Kim, G., Lee, J.S., Kim, I.S., "Poncirus Trifoliata (L.) Raf. Extract Inhibits the Development of Atopic Dermatitis-like Lesions in Human Keratinocytes and NC/Nga mice," *International Journal of Medical Sciences*, 16 (8). 1116-1122. Aug.2019.

[40] Segawa, R., Shigeeda, K., Hatayama, T., Dong, J., Mizuno, N., Moriya, T., Hiratsuka, M. and Hirasawa, N., "EGFR transactivation is involved in TNF-α-induced expression of thymic stromal lymphopoietin in human keratinocyte cell line," *Journal of Dermatological Science*, 89. 290-298. Mar.2018.

[41] Wu, S., Yu, D., Liu, W., Zhang, J., Liu, X., Wang, J., Yu, M., Li, Z., Chen, Q., Li, X. and Ye, X., "Magnoflorine from *Coptis chinese* has the potential to treat DNCB-induced Atopic dermatits by inhibiting apoptosis of keratinocyte," *Bioorganic & Medicinal Chemistry*, 28 (2). 115093-115107. Sep.2020.

[42] Cho, K.H., Parveen, A., Kang, M.C., Subedi, L., Lee, J.H., Park, S.Y., Jin, M.R., Yoon, H.K., Son, Y.K. and Kim, S.Y., "*Pyrus ussuriensis* Maxim. leaves extract ameliorates DNCB-induced atopic dermatitis-like symptoms in NC/Nga mice," *Phytomedicine*, 48 (15). 76-83. May.2018.

[43] Cho, K.H., Kang, M.C., Parveen, A., Yumnam, S. and Kim, S., "Anti-Inflammatory Effect of Chloroform Fraction of *Pyrus ussuriensis* Maxim. Leaf Extract on 2, 4-Dinitrochlorobenzene-Induced Atopic Dermatitis in nc/nga Mice," *Nutrients*, 11. 276-287. Jan.2019.

[44] Park, S.H., An, J.E., Jang, S., Kim, J.Y., Lee, J.W. and Kim, H.K., "*Gardenia jasminoides* extract without crocin improved atopic dermatitis-like skin lesions via suppression of Th2-related cytokines in Dfe-induced NC/Nga mice," *Journal of Ethnopharmacology*, 241 (15). 112015-112023. Jun.2019.

[45] Yang, H.J., Kim, M.J., Kang, S.A., Moon, N.R., Kim, D.S., Lee, N.R., Kim, K.S. and Park, S., "Topical treatments of *Saussurea costus* root and *Thuja orientalis* L. synergistically alleviate atopic dermatitis-like skin lesions by inhibiting protease-activated receptor-2 and NF-κB signaling in HaCaT cells and Nc/Nga mice," *Journal of Ethnopharmacology*, 199 (6). 97-105. Feb.2017.

[46] Hou, D.D., Zhang, W., Gao, Y.L., Sun, Y.Z., Wang, H.X., Qi, R.Q., Chen, H.D. and Gao, X.H., "Anti-inflammatory effects of quercetin in a mouse model of MC903-induced atopic dermatitis," *International Immunopharmacology*, 74. 105676-105686. Jun.2019.

[47] Gil, T.Y., Kang, Y.M., Eom, Y.J., Hong, C.H. and An, H.J., "Anti-Atopic Dermatitis Effect of Seaweed Fulvescens Extract via Inhibiting the STAT1 Pathway," *Mediators of Inflammation*, 2019. 11-21. Mar.2019.