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Chondrus ocellatus Holmes ethanol extract suppresses the atopic dermatitis in DNCB-induced BALB/c mice

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Atopic dermatitis (AD) is a common skin disease, and it is a chronically relapsing and inflammatory skin disease accompanied by itching. Many red algae are being actively conducted, studies on the physiological activity of *C. ocellatus* Holmes are rarely conducted. To investigate whether *Chondrus ocellatus* Holmes ethanol extract (COHEE) inhibits AD progression in animal models. COHEE significantly decreased Th cytokines in ConA-stimulated splenocytes in a dose-dependent manner. AD-like skin lesions were induced by 2,4-dinitrochlorobenzene (DNCB) in BALB/c mice, and COHEE was applied to DNCB-induced mice to study the effect of COHEE on AD. COHEE lowered the number of WBCs in the blood and the spleen weight of mice. COHEE significantly decreased the secretion of IL-4 and IL-5, whereas the level of IFN-γ was increased in splenocytes. In addition, the secretion of IgE and TNF-α was significantly suppressed in the serum, and the IL-10 was increased. In conclusion, the present study indicates that COHEE has an inhibitory effect on AD and is useful for drug development and treating AD.

Keywords: *Chondrus ocellatus* Holmes, Atopic dermatitis, T-lymphocytes, Cytokine, 2,4- dinitrochlorobenzene (DNCB)

Atopic dermatitis (AD) is a common disease characterized by itching and dry skin and is a chronic inflammatory dermatitis that affects 1-2% of adults and 20% of children worldwide. Although the exact cause of AD is not known, it is a complex disease caused by interactions among various factors such as genetic predisposition, environmental factors, and abnormal immune responses of the immune system. In AD lesions, the Th2, which secretes IL-4, 5, 6, and 13, is activated, and the Th1, which secretes IFN-γ and IL-2, is inactivated, resulting in a Th1/Th2 imbalance. Cytokine secretion such as IL-4, 5, 6, and 13 from activated Th2 cells plays a central role in the etiology, which induces the activation and penetration of other inflammatory mediators, eosinophils, mast cells, and B cells into skin lesions [1 2]. In addition, cytokine secreted from Th2 cells stimulates B cells to increase the production of IgE, and excessively produced IgE intensifies damage to the skin barrier through activation and penetration of immune cells such as mast cells and eosinophils into skin tissues and inflammatory response[2]. Therefore, inflammation in AD skin lesions is characterized by a predominance of Th2 cells, and increased IgE level in serum is one of the representative features of AD [2-3]. Cytokines (IL-1β, IL-6, TNF-α) produced by inflammatory cells of skin lesions play a crucial role in deepening AD on the epithelial surface. Elevated levels of IL-1β, IL-6, and TNF-α were confirmed in the serum of patients with AD, which were found to be directly correlated with the severity of AD[4]. Based on this AD mechanism, in treating AD, the symptoms of the disease are alleviated using drug therapy that regulates or inhibits the expression of IgE and Th2 cytokines[5]. For the treatment of AD, synthetic drugs such as topical steroids, anti-histamines, and immune-suppressants are mainly used. Anti-histamines have a side effect of increasing itching, and steroids have side effects such as skin atrophy and toxicity, making them unsuitable for long-term treatment, and the possibility of recurrence of AD is very high when drug treatment is stopped. So, it is necessary to develop a treatment for AD without side effects, and

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research to find safe and effective functional substances from natural products is actively being conducted [6-7].

Meanwhile, seaweed has been consumed as food since ancient times because it is rich in dietary fiber, minerals, polysaccharides, and vitamins compared to low-calorie content. Marine organisms contain various compounds and are not actively studied compared to terrestrial organisms, so there is a high possibility of discovering new physiologically active substances. Until today, tannins, catechins, and phenols have been found to be physiologically active substances in seaweed extracts[8], and studies on anti-atopic effects using these seaweed extracts include *Chondrus canaliculatus*[9], *Sargassum horneri*[10], *S. serratifolium*[11], etc. have been developed, but an innovative treatment for AD using them has not been developed. *Chondrus ocellatus* Holmes is a red alga family Gigartinaceae, which grows widely on the east coast of Korea and is edible but rarely consumed and not used industrially[12]. Red algae contain many amino acids, inorganic substances, phenolic compounds and flavonoids and thus have antioxidant and nitrite scavenging activity. Although studies on many red algae species are being actively conducted, studies on the physiological activity of *C. ocellatus* Holmes are rarely conducted. Based on the anti-inflammatory activity of *C. ocellatus* Holmes ethanol extract (COHEE) confirmed in a previous study[13], it was intended to confirm anti-atopic activity. In this study, COHEE was prepared, and the inhibitory activity of AD-related cytokines was confirmed *via* the screening process. We studied the possibility of developing medicine for AD treatment by confirming the effect of suppressing AD symptoms in mice induced with AD-like dermatitis with DNCB.

Materials and Methods

Animals

Five-week-old male BALB/c mice were purchased from Orient Bio Inc. (Seongnam, Korea) and used in the experiment. The mice were stabilized for one week in an animal breeding room maintained at a temperature of 20±2℃ and a humidity of 50±10%, with a 12 h light/dark cycle. Animal experiments were performed under the rules and regulations of the Animal Experiment Ethics Committee of Pukyong National University (PKNUIACUC-2022-14).

Plant materials and extraction

C. ocellatus Holmes was collected from Cheongsapo, Busan, Korea, in 2014. The *C. ocellatus* Holmes was washed with fresh water and then freeze-dried to remove moisture. The freeze-dried *C. ocellatus* Holmes was vacuum-packed in powder form and stored at -20℃ until use in the experiment. *C. ocellatus* Holmes was extracted with tenfold 95% ethanol for 24 h with a stirrer (H-0820, Dongwon Science Co., Korea). After 24 h , the extracts were centrifuged at 3,000 rpm for 10 min to take a supernatant, and the residue was additionally extracted twice using the same method. The supernatant was separated by filtration under reduced pressure and then concentrated at 35℃ with a vacuum concentrator (RE200, Yamoto Co., Japan), dried at room temperature, and stored at 20℃ for use in the experiment.

DNCB-induced AD-like mouse model

Six-week-old male BALB/c mice were randomly classified into 4 groups (n=5). After shaving the dorsal skin and ears of mice, they left for 24 h to recover from micro-wound. After 24 h , 200 μL 1% DNCB (dissolved in a mixture of acetone and olive oil (3:1)) was challenged to the dorsal skin and ears three times a week. From the second week, 200 μL 0.3% DNCB was topically applied to the dorsal skin and ears once a day, and COHEE (30 mg/mL dissolved in 2% Tween 80) or dexamethasone (0.01% dissolved in 2% Tween 80) was applied to the same area once a day every 12 h for 2 weeks. On day 22, the mice were sacrificed for further study (Fig. 1).

Clinical severity evaluation of skin dermatitis

An evaluation was started from 0 days after the DNCB application. According to the commonly used clinical visual evaluation method for evaluating the severity of atopic dermatitis (SCORing AD, SCORAD), five items were divided into erythema, edema/papulation, oozing/crusts, excoriations,

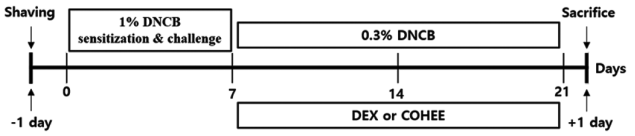

Fig. 1 — Schematic diagram of the application experiment. After dorsal hair removal, 1% DNCB was challenged 3 days for a week to sensitize. One week after, 0.3% DNCB was applied every day for 2 weeks. During that same period, 30 mg/mL COHEE or 0.01% dexamethasone was applied to mice 12 h after DNCB application. A day after the last application, Animals were sacrificed for further study.

lichenification. Each item was scored and summed as None (0), Weak (1), Moderate (2), and Severe (3) to give a score between a minimum of 0 and a maximum of 15 points per day. The total score for a week was summed up to show the change in the total score for three weeks.

Blood collection and analysis

One day after the end of the experiment, mice were anesthetized with diethyl ether and sacrificed, and approximately 1.0 mL of blood was collected from the aorta using a disposable syringe (Sungshim Medical Co., Ltd., Bucheon, Korea). The collected blood was placed in an EDTA tube, shaken for 10 min, and then analyzed with a Hemavet HV950 Multispecies Hematological Analyzer (Drew Scientific, CT, USA). The serum was separated by centrifugation at 10,000 rpm for 10 min at 4℃, and then stored at -20℃ and used for an experiment.

Spleen index measurement

One day after the end of the experiment, the spleen of the mouse was removed aseptically, and the weight and index of the tissue were calculated. The spleen index was calculated by the formula of the spleen weight (mg)/mouse weight.

Splenocyte isolation and culture

The collected spleen was washed with an RPMI 1640 medium (Gibco, NY, USA). The washed spleen was disrupted using a tissue grinder, and cells were released using a cell strainer. After cleaning the lysates with an RPMI 1640 medium, RBC lysis buffer (Tris-buffered ammonium chloride; 0.87% (w/v) NH_4Cl, *p*H 7.2) was added to remove red blood cells and left in ice for 10 min. The RBC lysis buffer was removed by centrifugation at 1,800 rpm for 5 min at 4℃, and the lysates were washed with RPMI 1640 medium with 10% fetal bovine serum (FBS). After then, the isolated cells were seeded at a concentration of 2×10^6 cells/mL with 10% FBS-RPMI 1640 medium and incubated at 37℃ for 72 h in a 5% CO_2 incubator (Sanyo, Osaka, Japan). For the *in vitro* experiment, splenocytes of normal mice were isolated in the same method and treated with COHEE (0.1, 1, 10, 50, and 100 μg/mL) with 3 μg/mL ConA (Wako Pure Chemical Industries, Osaka, Japan) for 72 h. After 72 h, the supernatant was collected by centrifugation at 2,000 rpm for 10 min at 4℃ and used for the experiment.

Cell viability assay

The splenocytes (1×10^7 cells/mL) were seeded on a 96-well plate (Corning, NY, USA). After incubation at 37℃ for 70 h in a 5% CO_2 incubator, 20 μLl of 5 mg/mL MTT reagent was added and further incubated for 2 h. This was centrifuged at 2,000 rpm, 4℃, and 10 min, and then the supernatant was removed. Then 100 μL of DMSO was dispensed and shaken for 20 min in a dark state. The optical density (OD) was measured at 540 nm using a microplate reader (Molecular Devices Inc., CA, USA). Cell viability was calculated by the following equation: Proliferation index (%)=Sample OD/Control OD × 100

Secretion of IgE and cytokine in serum and splenocytes

The total IgE and cytokine levels in mouse sera and cultured splenocytes were measured by ELISA using the Mouse ELISA set (BD Bioscience, CA, USA). IL-4, IL-5, and IFN-γ levels in cultured splenocytes and total IgE, TNF-α, and IL-10 levels in mouse sera were detected by the mouse ELISA kit. ELISA was performed according to the manufacturer's protocol. Then, the absorbance was measured and quantified at 490 nm using a microplate reader.

Statistical analysis

Data were presented as mean ± standard deviation. Statistical analysis for all experiments was performed using a one-way ANOVA test with Statistical Analytical System V8.2 (SAS Institute Inc., NC, USA). The significance between samples was confirmed at $P<0.05$ level using Duncan's multiple range test.

Results

Effect of COHEE on the levels of T lymphocyte cytokine in ConA-stimulated splenocytes

In order to confirm the immune-regulatory effect of CHOEE *in vitro*, the effect of COHEE on Th cytokine production in splenocytes stimulated by ConA was confirmed. Th1 cells induce a late immune response by activating macrophages by secreting IL-2 and IFN-γ, and Th2 cells secrete IL-4, 5, 6, and 13 to increase the production of IgE and induce hypersensitivity reactions by inducing differentiation of mast cells and eosinophils[14]. As shown in Fig. 2, IL-4, 5, and IFN-γ were significantly increased in ConA only, and IL-4, a Th2 cytokine, was decreased in COHEE (0.1, 1, 10, 50, and 100 μg/mL) treatment in a dose-dependent manner by 67, 25, 47, 27 and 18%, respectively (Fig. 2A). IL-5 showed a significant decrease by 93, 77, 69, 30 and 23% (Fig. 2B), IFN-γ, a Th1 cytokine, also decreased by 67, 47, 47, 5 and 1%, especially showed

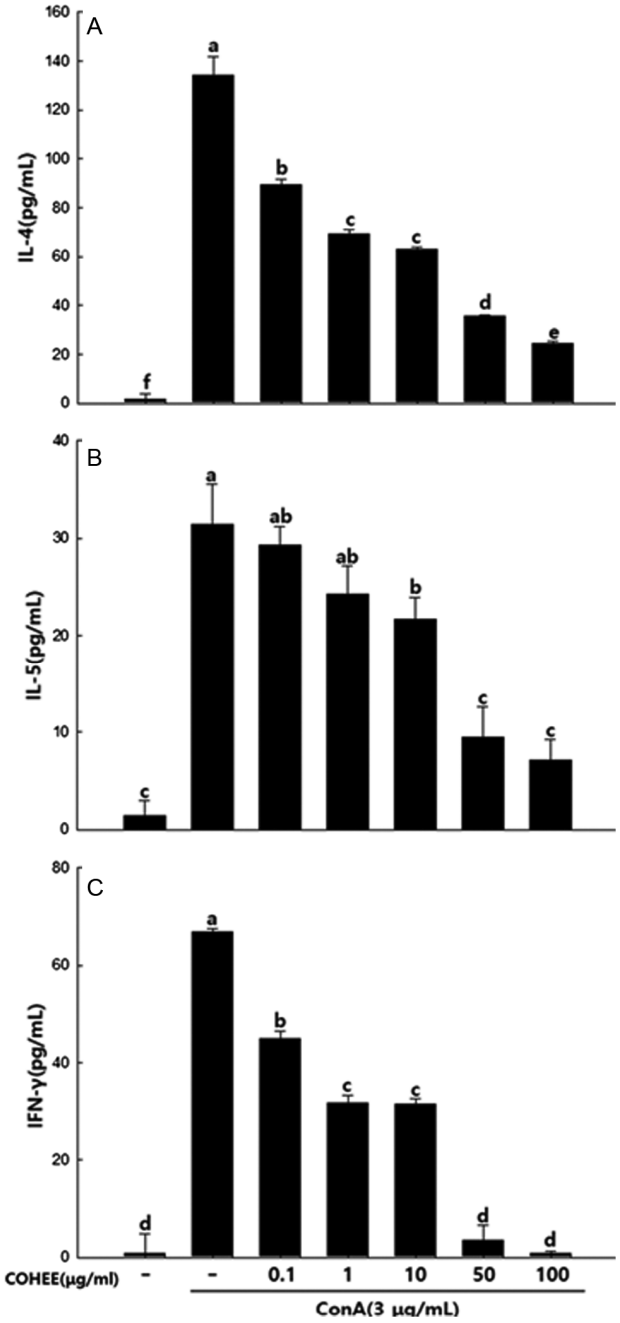

Fig. 2 — Effects of COHEE on levels cytokine production in Con A-stimulated splenocytes. (A) IL-4, (B) IL-5, and (C) IFN-γ were measured by ELISA assay.[a-f Means with different superscripts are significantly different ($P < 0.05$)]

a significant decreased at 50 and 100 μg/mL COHEE (Fig. 2C). These results suggest that COHEE inhibits the production of Th cytokines related to immune and atopic responses in splenocytes activated by antigen induction, thereby suppressing the development and aggravation of atopic diseases.

Clinical severity evaluation of skin dermatitis

The clinical dermatitis improvement effects of COHEE were observed in a DNCB-induced atopic dermatitis-like skin model (Fig. 3). As shown in Fig. 3A, erythema, dry skin, and lichenification symptoms began to be observed on the back skin of the AD-like skin mouse model except for the control group at week 1 of sensitization with DNCB. In the 2nd week, the clinical symptoms of the DNCB alone were more severe, and in the case of the DNCB with COHEE, the symptoms were suppressed compared to the DNCB alone. In the 3rd week, after applying COHEE for 2 weeks, it was confirmed that erythema and dry skin were improved, and the skin was restored to an almost normal state, such as the growth of new hair. In addition, as a result of the clinical visual evaluation score for severity evaluation, the increased score due to the application of DNCB significantly decreased at the 2nd and 3rd weeks by COHEE treatment compared to the DNCB alone (Fig. 3B).

Effect of COHEE on WBCs in mice blood

As a result of measuring the number of leukocytes and immune cells in mouse blood after the experiment, the DNCB alone showed a significant increase compared to the control group. The DNCB with DEX, a positive control, showed a significant decrease in the number of leukocytes, lymphocytes, neutrophils, and monocytes compared to the DNCB alone, and the DNCB with COHEE also showed significant values like the positive control. In addition, it was confirmed that the neutrophil, which did not show a significant decrease in the DNCB with DEX, was significantly reduced, and the number of leukocytes and monocytes was effectively reduced.

Effect of COHEE on spleen index and splenocytes proliferation

Spleen tissue is an immune organ in charge of the immune response to foreign antigens transported through the blood, and many B cells and T cells are distributed in it. Also, as an organ where the maturation and differentiation of lymphocytes occur, changes in the size and number of cells in the spleen have a significant meaning in the immune response[15]. After the experiment was completed, the spleen was collected and the changes in spleen index were measured (Fig. 4A). As a result, the spleen index increased significantly in the DNCB alone, and it increased slightly in the DNCB with COHEE compared to normal control. In the case of the

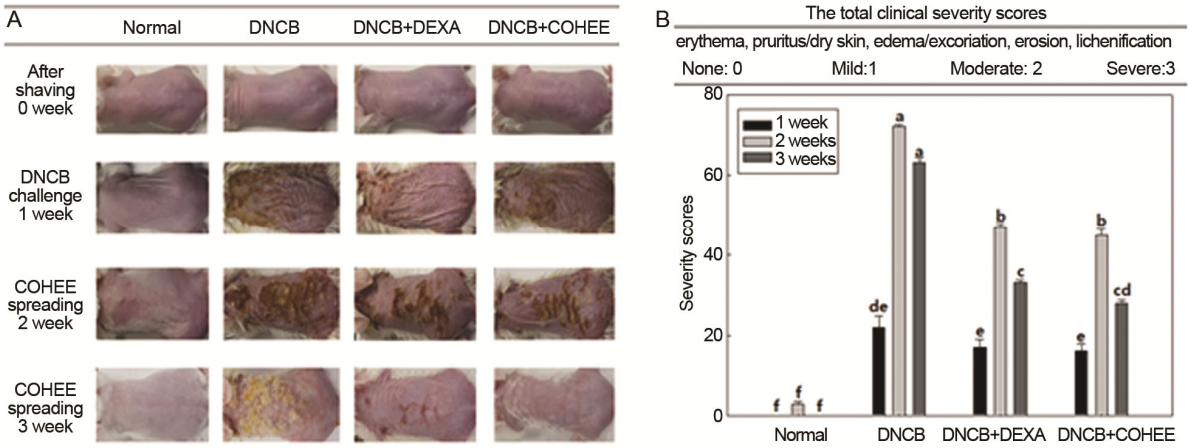

Fig. 3 — Effects of COHEE on DNCB-induced AD clinical symptoms. (A) clinical skin appearance and (B) the total clinical severity score of DNCB-induced BALB/c mice (n=5). Normal, Negative control; DNCB, DNCB with the vehicle; DNCB+DEXA, DNCB with dexamethasone; DNCB+COHEE, DNCB with COHEE.[$^{a-d}$ Means with different superscripts are significantly different ($P < 0.05$)]

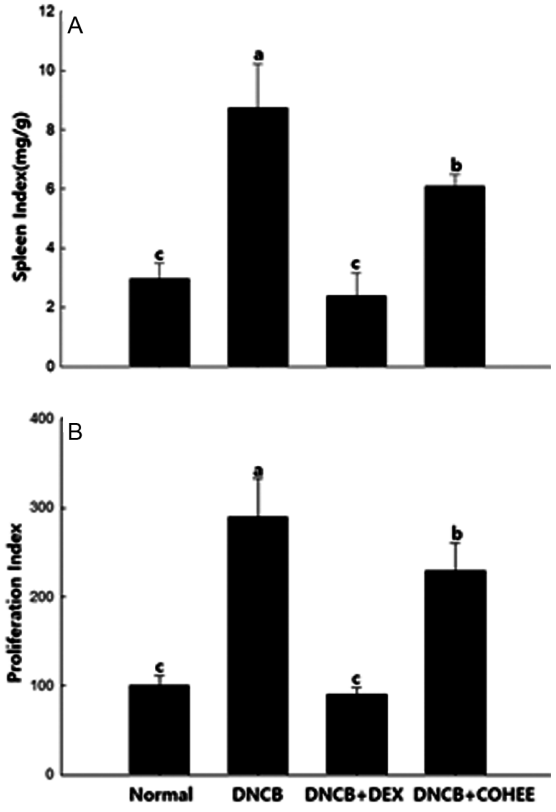

Fig. 4 — Effect of COHEE on spleen index and splenocyte proliferation(n=5). (A) Spleen index: spleen index (mg/g) = spleen weight (mg)/body weight. (B) Proliferation index (%) = Sample OD/Control OD × 100. Normal, Negative control; DNCB, DNCB with the vehicle; DNCB+DEXA, DNCB with dexamethasone; DNCB+COHEE, DNCB with COHEE.[$^{a-d}$ Means with different superscripts are significantly different ($P < 0.05$)]

comparison of splenocyte proliferative index (Fig. 4B), a clear increase was confirmed in the DNCB only, and the DNCB with DEX decreased by about 10% compared to the normal control. In the case of the DNCB with COHEE, it was confirmed that the spleen proliferative index significantly increased to about 129% compared to the normal control. These results suggest that COHEE activates immune response by inducing the proliferation of splenocytes via acting as an immune activator on splenocytes.

Effect of COHEE on the levels IgE, TNF-α and IL-10 in DNCB-induced mice sera

The effect of COHEE on the level of total IgE and cytokine in serum was confirmed (Fig. 5). In the case of the DNCB alone, the IgE contents in serum significantly increased. Compared to the DNCB alone, the DNCB with DEX, which is positive control, and DNCB with COHEE showed a decrease by about 8% and 13%, respectively (Fig. 5A). IL-10, an anti-inflammatory cytokine, was significantly decreased in the DNCB alone compared to the normal control, and the level of IL-10 increased significantly in the DNCB with COHEE (Fig. 5B). The production of TNF-α, an inflammatory cytokine, was significantly increased in the DNCB alone but decreased to a level that was not significantly different from the normal control in the DNCB with DEX and DNCB with COHEE (Fig. 5C).

Effect of COHEE on the secretion of cytokines in DNCB-induced mice splenocytes

The effect of COHEE on cytokine secretion in the splenocytes of the DNCB-induced mouse model was confirmed (Fig. 6). The secretion of IFN-γ was significantly decreased in the DNCB alone compared to the normal control, but the DNCB with DEX

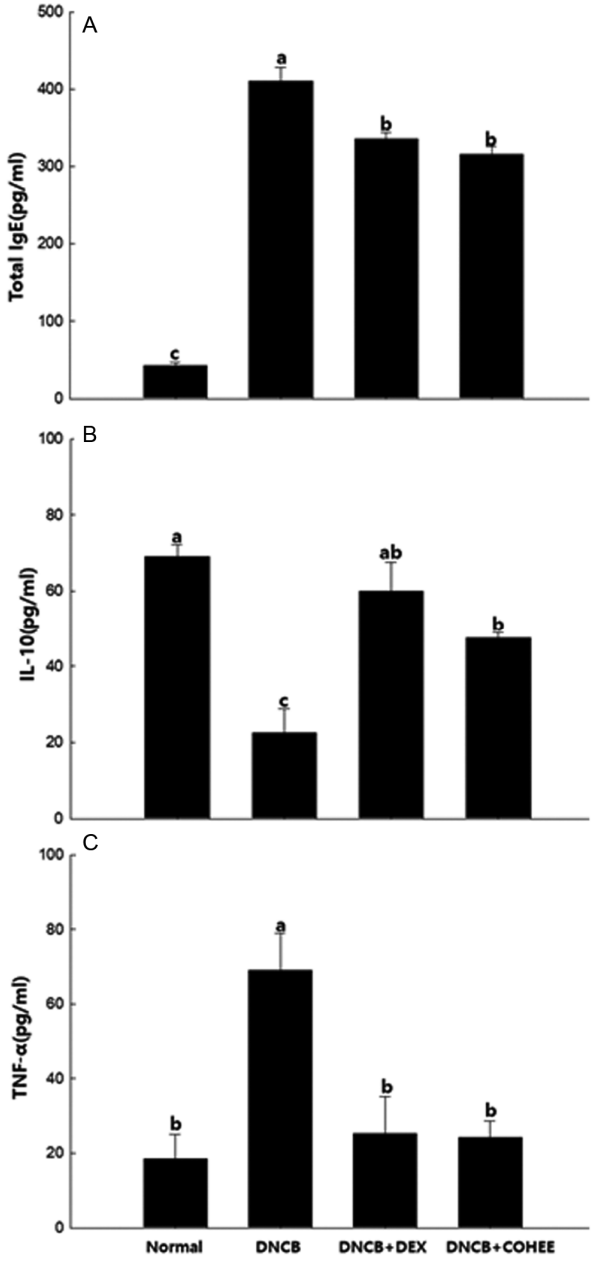

Fig. 5 — Effect of COHEE on IgE and cytokine production in mice sera. (A) IgE, (B) IL-10, and (C) TNF-α were measured by ELISA assay.[a-c Means with different superscripts are significantly different ($P < 0.05$)]

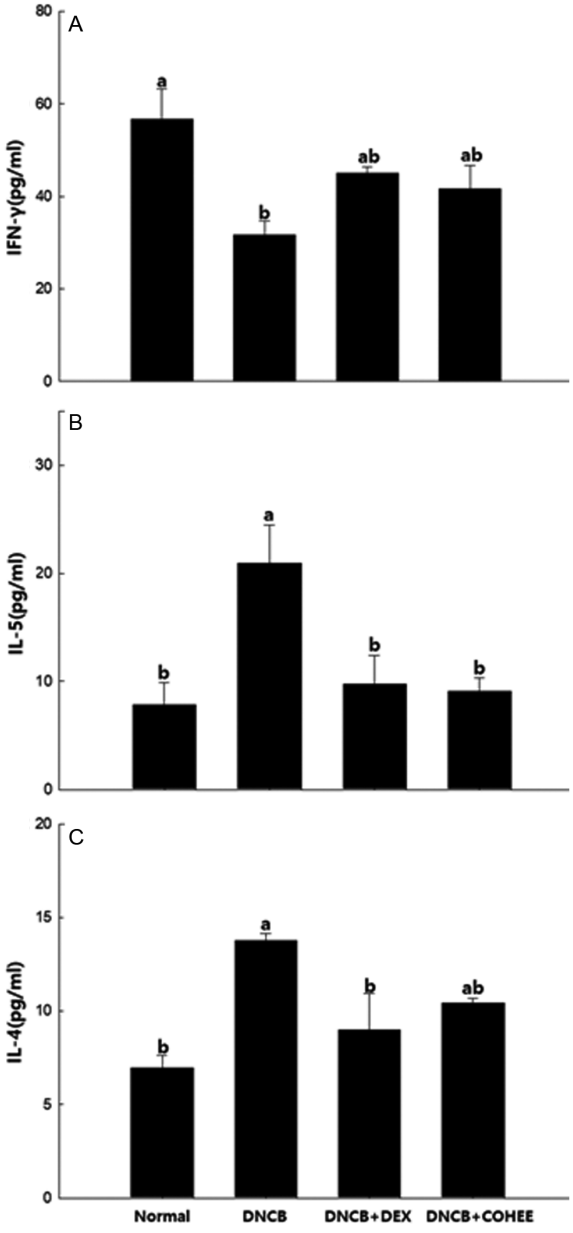

Fig. 6 — Effect of COHEE on the secretion of cytokines in DNCB-induced mice splenocytes. (A) IFN-γ, (B) IL-5, and (C) IL-4 was measured by ELISA assay.[a-b Means with different superscripts are significantly different ($P < 0.05$)]

showed a significant increase in IFN-γ secretion by about 35% and the DNCB with COHEE by about 20% (Fig. 6A). IL-5 secretion significantly increased in the DNCB only, but both the DNCB with DEX and COHEE decreased the secretion to the extent that there was no significant difference from the normal control (Fig. 6B). In the case of IL-4, the secretion increased significantly in the DNCB alone, and compared to DNCB alone, the DNCB with DEX showed a significant decrease in secretion by about 35%, and the DNCB with COHEE showed about 24%. Although the reduction efficiency was lower than DNCB with DEX, the amount of secretion was significantly reduced (Fig. 6C).

Discussion

Atopic dermatitis is a common chronic inflammatory disease characterized by itching, eczema, erythema, and lichenification. Itching in AD

leads to sleep deprivation, and apparently, dermatitis affects many aspects of patients' lives, including psychological stress and low self-esteem, leading to negative psychosocial and health outcomes[4]. In the treatment of dermatitis, topical corticosteroids are widely recommended as the primary anti-inflammatory treatment for AD. Still, long-term use causes many side effects, such as skin atrophy, reduced immune function, and pigmentation, and high treatment expenses make long-term use difficult[16]. Therefore, attempts to develop effective therapies with fewer side effects remain an important priority.

C. ocellatus Holmes is a seaweed of the red algae in Gigartinaceae family. In the case of other red algae, their physiological activities, such as antioxidant[17], anti-obesity[18], and anti-inflammatory[12], are being actively studied, but studies on *C. ocellatus* Holmes have hardly been conducted. In a previous study, the anti-inflammatory activity of the *C. ocellatus* Holmes ethanol extract (COHEE) was confirmed[13]. Because AD and inflammation have a close correlation, based on the results, the anti-atopic effect of the COHEE was confirmed, and the potential as a material for the development of therapeutic agents for AD was confirmed.

T lymphocytes switch to Th1 and Th2 depending on the type of cytokine secreted by activation of the CD+4 receptor[2]. In normal conditions, Th1 and Th2 cells interact to maintain an immune response in balance, but in atopy and asthma, the normal Th1/Th2 balance collapses. In the acute phase of AD, Th2 cell differentiation is increased, intensifying eosinophil penetration and inflammation. When AD persists and enters the chronic phase, Th1 cells also produce IFN-γ, contributing to the deepening of AD. In the chronic phase, IFN-γ, which is excessively produced due to an increase in Th1 cells, induces apoptosis of keratinocytes and the formation of eczematous lesions, which intensifies the lesions[5,19-20]. In this study, COHEE reduced secretion of Th cytokines, IL-4, IL-5, and IFN-γ in splenocytes of mice in which ConA-induced T cells were activated, resulting in potential anti-atopic effects *in vitro*.

When the clinical symptom improvement effect by applying COHEE to DNCB-induced AD-like mice was confirmed, clinical symptoms such as erythema, itching and dry skin, hematoma, and lichenification caused by AD were confirmed to be recovered by the application of COHEE. Therefore, it is considered effective for recovering the skin barrier by improving skin dryness by applying COHEE.

Leukocytes recognize the influx of external microorganisms, such as bacteria and viruses or antigens, and perform functions such as antigen presentation, phagocytosis, and antibody production[21]. For this reason, the number of immune cells increases at the site of inflammation, and excessive inflammatory cytokines and mediators released from immune cells are known to further intensify inflammation[22]. From the results of blood analysis in this study, the application of COHEE reduced the total leukocyte count, which was increased due to the induction of DNCB, especially eosinophil related to the severity of AD, monocytes related to chronic infection, and lymphocytes that play the center of acquired immunity. These results suggest that COHEE suppresses AD by reducing the number of inflammatory cells.

The spleen is an important immune organ where the maturation and differentiation of T and B lymphocytes take place, and it performs the main immunity against foreign antigens. Therefore, changes in the size of the spleen or the number of splenocytes are used as important indicators for enhancing immune activity, and when immune-stimulating substances such as antigens are generated, proliferation and differentiation of lymphocytes are induced in the spleen. Hence, the proliferation of splenocytes has an essential meaning in the immune response. T and B cells and phagocytic cells exist in the spleen. Since T lymphocytes account for more than 60% of various immune cells, splenocytes are mainly used to confirm immune activity in terms of T cell activity[15,23-24]. In the results of the spleen index and splenocyte proliferation capacity of COHEE in this study, the application of COHEE increased the spleen index and splenocyte proliferation compared to the normal control. These results are considered to be due to the activation of the immune response by the application of COHEE acting as a mitosis promoter of splenocytes. On the other hand, in the case of the application of DEX, there was no significant difference in spleen index and splenocyte proliferation ability compared to the normal control despite the application of DNCB together.

Allergen-specific IgE plays a central role in skin reactions (itching and erythema) by interacting with high-affinity receptors for mast cell and basophil activation [6]. Mast cells are the main effector of the IgE-

mediated allergic inflammatory response in diseases such as AD and allergic rhinitis. Many studies have reported that mast cells are increased in the skin lesions of AD patients, and it is known that excessively produced IgE in the blood migrates to skin lesions and activates mast cells[2]. Therefore, elevated IgE levels in the blood are one of the immunological indicators of AD, and IgE is known to be proportional to clinical severity in AD patients[25]. Mast cells activated by IgE stimulation release various cytokines such as TNF-α and IL-4 and inflammatory mediators such as histamine and β-Hexosaminidase, through which they are involved in the inflammatory process and induce penetration to the skin lesions of immune cells[2]. TNF-α is a major inflammatory cytokine produced by most cells. It plays a vital role in the influx of inflammatory cells into skin lesions by inducing the expression of inflammatory cytokines, chemokines, and adhesion molecules[2]. In addition, it inhibits the proliferation of Th1 cells, resulting in a decrease in cell-mediated immunity. Therefore, the regulation of inflammatory cytokines to control the early stages of AD is considered a reasonable approach for AD treatment[26]. On the other hand, IL-10 is a representative cytokine that has an anti-inflammatory effect. It is mainly secreted from monocytes and lymphocytes and serves to reduce the secretion and activity of inflammatory cytokines such as TNF-α. It is also known to inhibit T cell-mediated responses and suppress cell-mediated immune responses against bacterial pathogens[5,27]. The application of COHEE reduced the amount of IgE increased in bloody by DNCB, suppressed the production of TNF-α, and increased the production of IL-10. These results suggest that COHEE has the effect of suppressing excessive production of IgE and regulating the inflammatory response of AD by regulating the production of inflammatory and anti-inflammatory cytokines.

In the early stages of AD, activated Th2 cells induce the secretion of IL-4, 5, and 13, which increases epidermal thickness, inflammation, and itching[2]. IL-4 stimulates B cells to induce IgE production, and IL-5 induces an increase in eosinophils to increase their penetration into the skin, leading to aggravated AD. In addition, in acute AD, the ability to produce IFN-γ, which plays a role in suppressing the response of Th2 cells, is reduced, and cytokines secreted from Th2 cells suppress differentiation into Th1 cells, thereby inducing IgE overproduction and Th2 immune responses. This process leads to a decrease in cell-mediated immunity[14]. Meanwhile, IFN-γ, a cytokine produced in Th1 cells, increases the activity of macrophages and NK cells to enhance the cell-mediated immune responses against cancer surveillance and infection, and it has been reported to have a protective effect against allergy by strongly inhibiting the expression of IL-4 receptors in T cells[27]. In this study, the application of COHEE decreased the secretion of IL-4 and 5, which are Th2 cytokines, increased in splenocytes due to DNCB induction. In addition, by increasing the secretion of reduced IFN-γ, the possibility of enhancing cell-mediated immunity by activating Th1 cell function was shown. From the above results, COHEE has a clinical symptom improvement effect in AD, regulates the production and activity of Th1 and Th2 cytokines, suppresses the over expression of IgE, and regulates the production of inflammatory cells and cytokines, so it is believed to have excellent efficacy in improving AD.

Conclusion

This study demonstrated that *C. ocellatus* Holmes ethanol extract (COHEE) exerts immunoregulatory effects by modulating T cell cytokine production, reducing inflammatory responses, and alleviating atopic dermatitis-like symptoms in a DNCB-induced mouse model. COHEE treatment significantly reduced Th2 cytokines (IL-4, IL-5) and Th1 cytokine (IFN-γ) in ConA-stimulated splenocytes, indicating its role in immune regulation. In vivo, COHEE improved dermatitis symptoms, reduced immune cell counts in blood, and regulated spleen function by enhancing splenocyte proliferation. Additionally, it decreased serum IgE and TNF-α while increasing IL-10, supporting its anti-inflammatory effects. Overall, these findings suggest that COHEE has potential as an immune modulator with anti-inflammatory and anti-atopic properties, warranting further investigation for therapeutic applications.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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