

***Costaria costata* Extract Suppresses Development of Atopic Dermatitis in chloro-2,4-dinitrobenzene-treated NC/Nga Mice**

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Keywords

Costaria costata · Atopic dermatitis · Immunomodulation · NC/Nga mice · Allergic reactions

an inhibitory effect of CCE10 in AD development by means of improving the Th1/Th2 cytokine balance and anti-inflammatory effect in an in vivo model.

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Abstract

We investigated the potential effects of *Costaria costata* (CC) on atopic dermatitis (AD) development in chloro-2,4-dinitrobenzene (DNCB)-treated NC/Nga mice. CC is a brown alga distributed across the seas of Korea, China, and Japan. A total of 40 mice were randomly assigned to 5 groups with 8 mice per group: untreated Balb/c mice, AD control (0.1% w/v DNCB-treated NC/Nga mice), positive control (i.e., DNCB-treated NC/Nga mice fed a dietary supplement of 66.6 mg/kg of body weight [b.w.] of CJLP133), DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10 (CCE10 100), and DNCB-treated mice fed a dietary supplement of 300 mg/kg b.w. of CCE10 (CCE10 300) groups. The CCE10 100 and CCE10 300 treatment groups suppressed AD development including clinical and histopathological changes and a reduction in skin hydration induced by DNCB. In addition, Th2 cytokine production in primary splenocytes, serum IgE and histamine production, and mast cell infiltration into the skin were suppressed in the CCE10 300 mice compared to the CCE10 100 mice. Our finding demonstrated

Introduction

Atopic dermatitis (AD) is a chronic and inflammatory eczematous skin disease that renders the skin dry, scaly, swollen, and itchy [1]. AD usually begins in childhood, and its onset decreases substantially with age. However, it does not always disappear before adulthood and 10% of adult patients may still have AD; it appears after the age of 20 years in 2–3% of patients [2]. The cause and pathogenesis of AD are still unclear; it appears to result from complex mechanisms involving extrinsic and intrinsic factors [3]. Recent evidence suggests that AD pathogenesis is associated with not only skin tissue but also immune system abnormalities [4–6].

One of main risk factors for AD is the loss of function mutations in the filaggrin (*FLG*) gene, a skin barrier gene

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[7]. In addition, immune system abnormalities play a key role in the pathobiology and etiology of AD by the influx of lymphocytes into the dermis [8]. Increased CCL17/thymus and activation-regulated chemokine (TARC) and CCL11 (eotaxin) in the dermal cells of patients with AD are responsible for the recruitment of lymphocytes to the dermis [9, 10]. Recruited T helper (Th) cells (i.e., CD4+ T cells) are activated by dendritic cells (Langerhans cells) and secrete Th2 cytokines, such as IL-4 and IL-10, during the allergic response in AD [9–11]. IL-4 is a key Th2 cytokine critical for immunoglobulin (Ig)E production from B cells, by stimulating a switch of isotype from IgM to IgE. In the normal state, the concentration of IgE is the lowest of the immunoglobulin isotypes; during allergic reactions, its production is increased. Increased IgE binds to the high-affinity receptor for IgE and FcεRI on the surface of mast cells, which activates mast cells in the skin. Mast cell binding to IgE mediates an immediate hypersensitivity response in the development of AD by the release of histamines and mediators [11–13].

Immunosuppressive drugs, topical corticosteroids, or immune modulators that control itching and inflammation have been used for the treatment of AD. However, they are used only in severe cases and for a short period owing to their adverse effects [14]. Thus, patients have tried alternative therapies including herbal remedies [15]. In this study, we investigated the potential effects of *Costaria costata* (CC) on AD development in chloro-2,4-dinitrobenzene (DNCB)-treated NC/Nga mice. CC is a brown algae species belonging to the genus *Costaria* and the family Costariaceae, distributed across the sea of Korea, China, and Japan [16]. Although recent studies have established several biological activities of various marine algae [17, 18], only a few studies have reported the biological effects of CC in cancer and CCL4-induced liver injury [19, 20]. In our previous study, CC extract suppressed the production of proinflammatory cytokines and Th2 cytokines in HaCaT cells, and primary splenocytes and histamine in MC/9 cells [21]. In this study, we expected CC to inhibit AD development by improving the Th1/Th2 cytokine balance and anti-inflammatory effect in an in vivo model.

Methods

Preparation of Extracts

CC was obtained from the Marine Biotechnology Research Center (Wando, Korea). It was extracted using 6 volumes of 10% ethanol at 80–100 °C for 3 h in a reflux apparatus and filtered (Whatman No. 6; Whatman, Maidstone, UK). The extracts were

concentrated in a rotary evaporator under reduced pressure and lyophilized. This 10% ethanol extract of CC (CCE10) was stored at –20 °C until used.

Animals

Six-week-old male NC/Nga mice and age-matched Balb/c mice were purchased from SLC, Inc. (Kotoh-cho, Hamamatsu, Japan) and housed in wire mesh-bottomed individual cages at 24 ± 1 °C in 55 ± 55% humidity with a 12-h light/dark cycle. All mice were acclimatized for 7 days before the experiment, fed standard pellet chow, and provided fresh water ad libitum. All the experiments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committees of Dan Kook University (DKU-16–013).

Induction of AD-Like Skin Lesions in NC/Nga Mice and Oral Administration

For the positive control (PC), we chose to administer CJLP133 which has been proved to be beneficial in the treatment of AD [22]. A total of 40 mice were randomly assigned to 5 groups with 8 mice per group: untreated Balb/c mice, AD control (0.1% w/v DNCB-treated NC/Nga mice), PC (DNCB-treated NC/Nga mice fed a dietary supplement of 66.6 mg/kg of birth weight [b.w.] of CJLP133), DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10 (CCE10 100), and DNCB-treated NC/Nga mice fed a dietary supplement of 300 mg/kg b.w. of CCE10 (CCE10 300). Mice were fed their experimental diets based on the AIN93G diet. After 1 week, AD-like symptoms were induced by the topical application of 0.1% w/v DNCB (Sigma-Aldrich, St Louis, MO, USA) in olive oil and acetone solution (1:3) twice a week for 2 weeks along with the diet supplement. The mice were then fed the diet supplement for 2 weeks more without DNCB treatment. At the end of 5 weeks, all mice were killed by anesthesia. The spleens and dorsal skin tissue of all the mice were dissected and blood samples were collected for assay.

Evaluation of Epidermal Hydration

Epidermal hydration was measured with a Tewameter CM825 (Courage & Khazaka GmbH, Cologne, Germany) in the dorsal skin surface at the end of 5 weeks under standardized conditions of 22–24 °C and 55–60% humidity.

Histopathological Observation of Skin in Mice

Dorsal skin tissues were fixed in 10% buffered formalin, embedded in paraffin, and sliced into 5-μm sections. The sections were stained with hematoxylin and eosin (HE) and toluidine blue for the observation of mast cells by light microscopy.

T Cell and B Cell Proliferation in Primary Splenocytes

Mice were euthanized and their spleens were aseptically isolated. Each spleen was minced with a 40-mesh strainer in culture medium (RPMI 1640, 10% FBS, 2 mM glutamine, and 1/10⁵ units/L penicillin/streptomycin). To remove any red blood cells, the cells were resuspended in a lysis buffer (144 mM NH₄Cl in 1.7 mM Tris base, pH 7.4) and placed on ice for 10 min. The cells were then washed twice with PBS (pH 7.4) and resuspended in complete RPMI. The splenocytes were cultured at a density of 1 × 10⁶ cells/well in 96-well tissue culture dishes, and treated with concanavalin A (ConA; 5 μg/ml) to induce T cell proliferation and with lipopolysaccharide (LPS; 5 μg/ml) to induce B cell proliferation. After 48 h of incubation at 37 °C, 10 μL of EZ-CyTox was added to each well,

Table 1. Food efficiency rate and organ weight of mice treated with dietary supplement of *C. costata* extract

	Untreated Balb/c mice	NC/Nga mice			
		AD control	PC	CCE10 100	CCE10 300
FER ¹	7.09±1.05 ^b	14.41±2.22 ^a	16.52±3.71 ^a	13.58±3.16 ^a	16.71±2.52 ^a
Spleen, g	0.094±0.005 ^b	0.116±0.020 ^a	0.074±0.006 ^c	0.078±0.008 ^c	0.068±0.007 ^c
Kidney, g	0.42±0.01 ^a	0.39±0.02 ^b	0.37±0.02 ^b	0.38±0.03 ^b	0.39±0.03 ^b
Liver, g	1.09±0.04 ^b	1.14±0.07 ^a	1.07±0.08 ^b	1.12±0.10 ^b	1.04±0.01 ^b

Data represent the means ± SD ($n = 8$). Different letters indicate ($a > b > c$) a significant difference among groups at $p < 0.05$, as determined by the Duncan multiple range test. AD, atopic dermatitis; FER, food efficiency rate; untreated Balb/c mice, no-treatment group; AD control, 0.1% w/v DNCB-treated NC/Nga mice; PC, positive control (DNCB-treated mice fed a dietary supplement of 66.6 mg/kg b.w. of CJLP133); CCE10 100, DNCB-treated mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, DNCB-treated mice fed a dietary supplement of 300 mg/kg b.w. of CCE10.

¹ Weight gain in grams/food intake consumption in grams × 100.

followed by incubation for 4 h at 37°C. The absorbance at 340 nm was measured using a Versamax ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Determination of Cytokines in Primary Splenocytes

The splenocytes were cultured at a density of 1×10^6 cells/well in 96-well tissue culture dishes and stimulated with ConA 5 µg/ml to induce IL-2, IFN-γ, IL-4, and IL-10 production; other splenocytes were stimulated with LPS 5 µg/ml to induce TNF-α and IL-6 production. After 24 h of incubation, the supernatants were collected and analyzed for IL-2, IL-4, IL-10, TNF-α, and IL-6 concentrations. After 72 h of incubation, the supernatants were collected and analyzed for IFN-γ concentration. Cytokine concentration was analyzed using the DuoSet ELISA development kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Determination of Immunoglobulin Concentration

Blood samples were collected from the mice after sacrifice and centrifuged at 1,500 g for 20 min at 4°C. Ig levels in serum were quantified using a mouse IgE ELISA kit, mouse IgG₁ ELISA kit, and mouse IgG₂ ELISA kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions.

Determination of Serum Histamine Concentration

Blood samples were collected from the mice after sacrifice and centrifuged at 1,500 g for 20 min at 4°C. Histamine levels were measured using a mouse histamine ELISA kit (LDN, Nordhorn, Germany).

RNA Isolation from Skin Tissue and Real-Time PCR of Chemokines

Dorsal skin tissue was homogenized using rotor-stator homogenizers in the presence of RLT buffer (lysis buffer; Qiagen, Valencia, CA, USA), including β-mercaptoethanol. Total RNA was extracted from dorsal skin tissue lysate using the RNeasy Mini Kit (Qiagen). Complementary DNA was synthesized from 2 µg of purified RNA in 20 µL of reaction buffer using the iScriptTM cDNA synthesis kit (BioRad, Hercules, CA, USA). Real-time PCR (Applied Biosystems, Foster City, CA, USA) was performed using the cDNA (500 ng/µL)

with the SYBR Green PCR Master Mix (iQ SYBR Green Supermix; BioRad). The cDNA was amplified for 40 cycles of denaturation (95°C for 15 s), annealing (58°C for 30 s), and extension (72°C for 45 s) using the following primers: GAPDH forward primer 5'-CAT GGC CTT CCG TGT TCC TA-3', reverse primer 5'-GCG GCA CGT CAG ATC CA-3'; eotaxin forward primer 5'-GAC CAG GTT GGG CAA AGA GA-3', reverse primer 5'-GGC ATC CTG GAC CCA CTT CT-3'; and TARC forward primer 5'-TCC AGG GCA AGC TCA TCT GT-3', reverse primer 5'-TCT GAT GGC CTT CTT CAC ATGT-3'. Analysis of real-time RT-PCR data and calculation of the relative quantitation were performed using the 7500 System SDS software v1.3.1 (Applied Biosystems).

Statistical Analysis

All data are expressed as means ± SD. All data were analyzed by the Student t test or one-way ANOVA using SPSS statistical software for Windows (SPSS PASW v22.0; SPSS Inc., Chicago, IL, USA). The Duncan multiple range test was used for examining differences among the groups. A p value of <0.05 was considered statistically significant.

Results

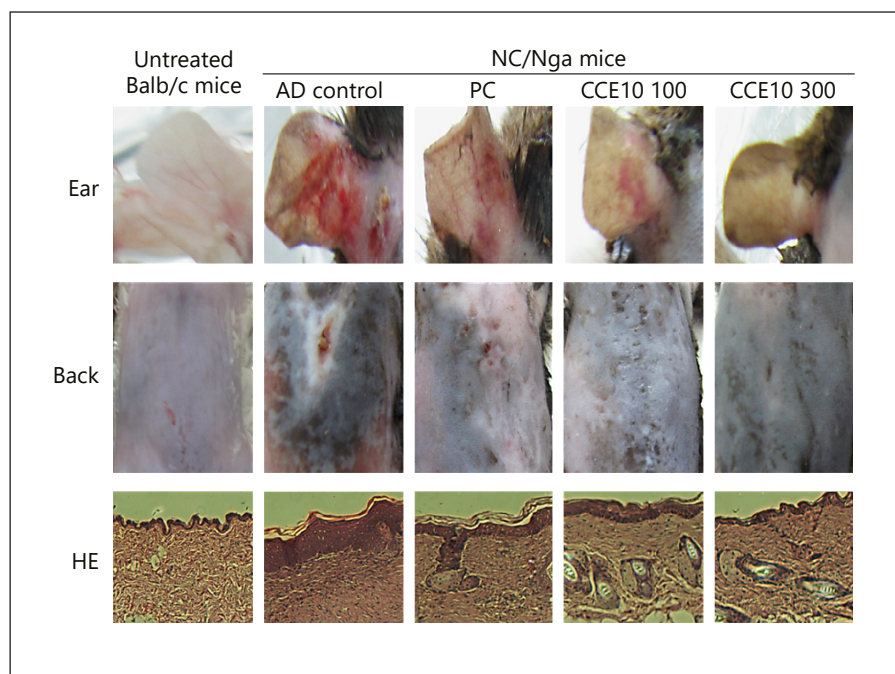
Effects of CCE10 Treatment on Food Efficiency Rate and Organ Weights

No statistically significant difference in food efficiency rate was observed across groups. However, the AD control group showed a significant increase in spleen and liver weights compared with the PC and CCE10-treated mice ($p < 0.05$) (Table 1).

Effects of CCE10 Treatment on Morphology and Histopathology

The AD control group showed AD symptoms, such as red spots, edema, and bleeding in their ears and on their

Fig. 1. Representative images of morphological and histological changes (HE staining) in skin of mice treated with dietary supplement of *C. costata* extract. Untreated Balb/c mice, no-treatment group; atopic dermatitis (AD) control, 0.1% w/v DNCB-treated mice; positive control (PC), DNCB-treated mice fed a dietary supplement of 66.6 mg/kg b.w. of CJLP133; CCE10 100, mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, mice fed a dietary supplement of 300 mg/kg b.w. of CCE10.



backs when compared with the untreated Balb/c mice. In comparison, morphological changes induced by DNCB were suppressed in mice treated with CCE10 100 and CCE10 300. HE staining revealed an increase in epidermal thickness, dead skin cell layers, and inflammatory cell infiltration in the dermis of the AD control group compared to the untreated Balb/c mice. However, CCE10 treatment suppressed the DNCB-induced increase in histopathological changes compared with the AD control group (Fig. 1).

Effects of CCE10 Treatment on Epidermal Hydration of the Dorsal Skin

Figure 2 shows epidermal hydration of the dorsal skin. Epidermal hydration significantly decreased in the AD control group (53.98 ± 3.12 AU) compared with the untreated Balb/c mice (86.22 ± 3.36 AU). CCE10 100 and CCE10 300 treatment caused a significant decrease (61.52 ± 4.20 and 80.63 ± 2.94 AU, respectively) in epidermal hydration ($p < 0.05$).

Effects of CCE10 Treatment on Chemokine Expression in the Dorsal Skin

Compared with the untreated Balb/c mice, a significant increase in the mRNA expression of eotaxin and TARC was observed in the AD control group. However, in the PC, CCE10 100, and CCE10 300 groups, a significant decrease in the mRNA expression of eotaxin and TARC was observed ($p < 0.05$) (Fig. 3).

Effects of CCE10 Treatment on T Cell and B Cell Proliferation and Cytokine Production in Primary Splenocytes

Compared with the untreated Balb/c mice, in the AD control group, T cell proliferation significantly decreased and B cell proliferation significantly increased in the mitogen-stimulated primary splenocytes. However, in the PC, CCE10 100, and CCE10 300 groups, T cell proliferation increased and B cell proliferation decreased compared with the AD control group ($p < 0.05$) (Fig. 4a).

The production of Th1 cytokines (IL-2 and IFN- γ) significantly decreased and that of Th2 (IL-4 and IL-10) and proinflammatory (TNF- α and IL-6) cytokines significantly increased in the mitogen-stimulated primary splenocytes of the AD control group mice compared with the untreated Balb/c mice. A significant increase in the production of Th1 cytokines and a significant decrease in the production of Th2 cytokines and proinflammatory cytokines were observed in the PC, CCE10 100, and CCE10 300 groups compared with the AD control group ($p < 0.05$) (Fig. 4b–d).

Effects of CCE10 Treatment on Serum IgE and Histamine Production and Mast Cell Infiltration

The AD control group showed a significant increase in serum IgE and histamine production compared with the untreated Balb/c mice. However, the production of serum IgE and histamine in the PC, CCE10 100, and CCE10 300

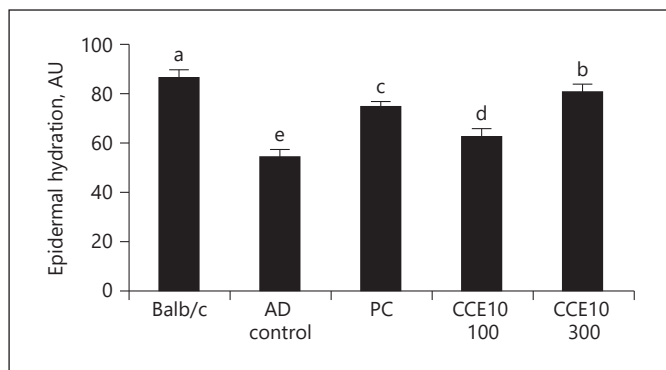


Fig. 2. Epidermal hydration of dorsal skin in mice treated with dietary supplement of *C. costata* extract. Balb/c, untreated Balb/c mice; atopic dermatitis (AD) control, 0.1% w/v DNCB-treated NC/Nga mice; positive control (PC), DNCB-treated NC/Nga mice fed a dietary supplement of 66.6 mg/kg b.w. of CJLP133; CCE10 100, DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, DNCB-treated NC/Nga mice fed a dietary supplement of 300 mg/kg b.w. of CCE10. Data represent the means $\pm$ SD ($n = 8$). Different letters indicate (a > b > c > d > e) a significant difference among groups at $p < 0.05$, as determined by the Duncan multiple-range test.

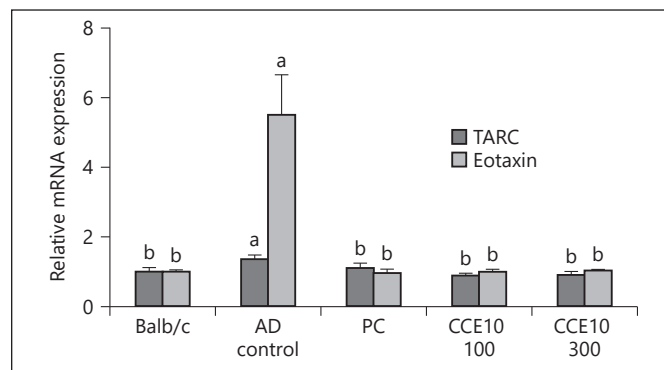


Fig. 3. RNA expression of chemokines (TARC and eotaxin) in dorsal skin of mice treated with a dietary supplement of *C. costata* extract. Balb/c, untreated Balb/c mice; atopic dermatitis (AD) control, 0.1% w/v DNCB-treated NC/Nga mice; positive control (PC), DNCB-treated NC/Nga mice fed a dietary supplement of 66.6 mg/kg b.w. of CJLP133; CCE10 100, DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, DNCB-treated NC/Nga mice fed a dietary supplement of 300 mg/kg b.w. of CCE10. Data represent the means $\pm$ SD ($n = 8$). Different letters indicate (a > b) a significant difference among groups at $p < 0.05$, as determined by the Duncan multiple-range test.

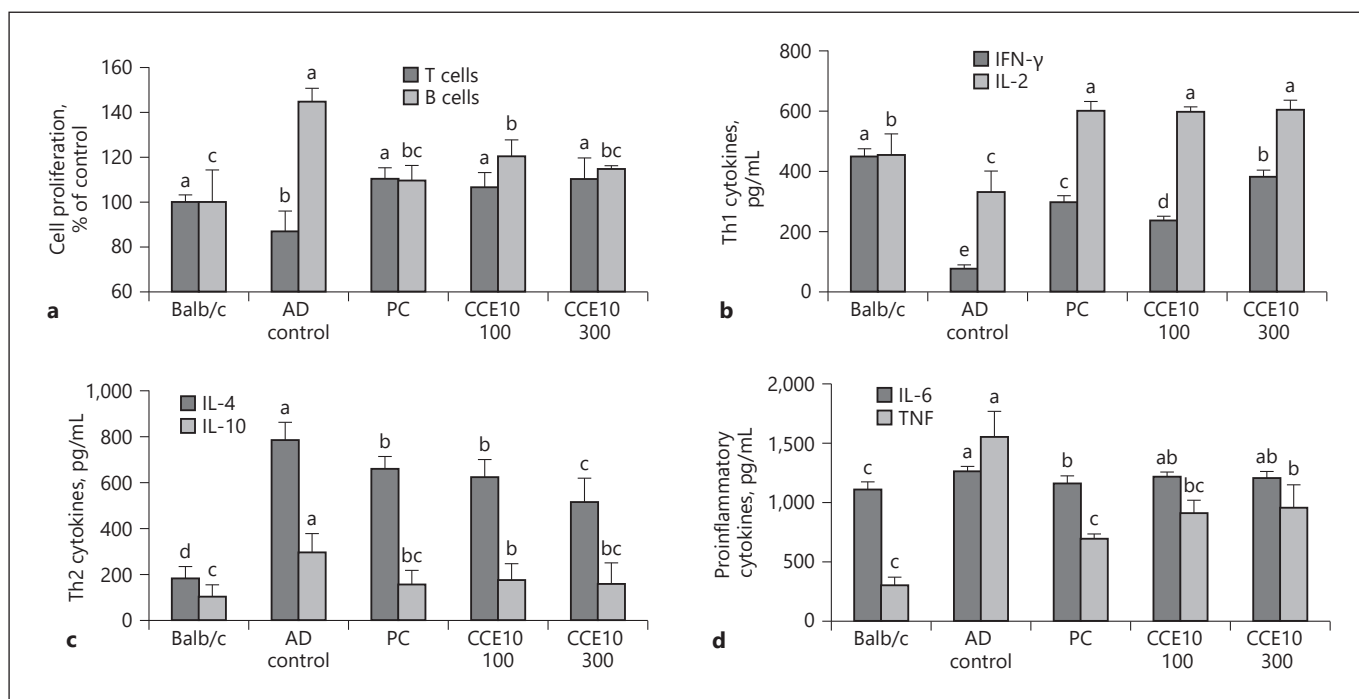


Fig. 4. Proliferation of T and B cells (a) and production of Th1 cytokines, IL-2 and IFN- γ (b), Th2 cytokines, IL-4 and IL-10 (c), and proinflammatory cytokines, TNF- α and IL-6 (d) in mitogen-stimulated primary splenocytes of mice treated with a dietary supplement of *C. costata* extract. Balb/c, untreated Balb/c mice; atopic dermatitis (AD) control, 0.1% w/v DNCB-treated NC/Nga mice; positive control (PC), DNCB-treated NC/Nga mice fed a dietary

supplement of 66.6 mg/kg b.w. of CJLP133; CCE10 100, DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, DNCB-treated NC/Nga mice fed a dietary supplement of 300 mg/kg b.w. of CCE10. Data represent the means $\pm$ SD ($n = 8$). Different letters indicate (a > b > c) a significant difference among groups at $p < 0.05$, as determined by the Duncan multiple-range test.

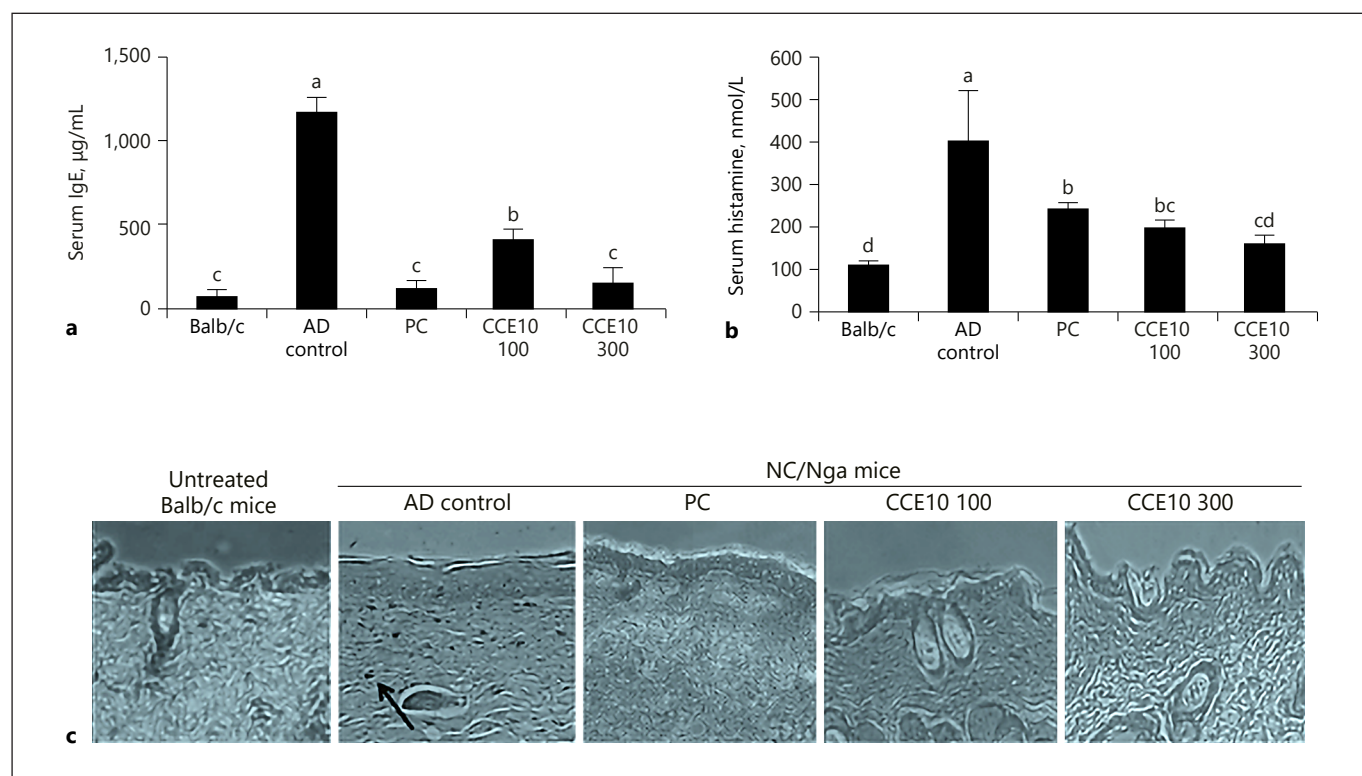


Fig. 5. Observation of serum IgE (**a**), serum histamine levels (**b**), and mast cell infiltration (**c**) by toluidine blue staining in mice treated with dietary supplement of *C. costata* extract. Balb/c, untreated Balb/c mice; AD control, 0.1% w/v DNCB-treated NC/Nga mice; PC, 0.1% w/v DNCB-treated NC/Nga mice fed a dietary supplement of 66.6 mg/kg b.w. of CJLP133; CCE10 100, 0.1% w/v

DNCB-treated NC/Nga mice fed a dietary supplement of 100 mg/kg b.w. of CCE10; CCE10 300, 0.1% w/v DNCB-treated NC/Nga mice fed a dietary supplement of 300 mg/kg b.w. of CCE10. Data represent the means $\pm$ SD ($n = 8$). Different letters indicate (a > b > c > d) a significant difference among groups at $p < 0.05$, as determined by the Duncan multiple-range test.

groups significantly decreased compared with the AD control group ($p < 0.05$) (Fig. 5a, b).

Toluidine blue staining of the dorsal skin showed that DNCB stimulation induced an increase in dermal mast cell infiltration in the AD control group. The dermal mast cell infiltration induced by DNCB was decreased by CJLP133, CCE10 100, and CCE10 300 treatment (Fig. 5c).

Discussion

In this study, we investigated the effects of dietary supplementation with CCE10 on the development of AD in DNCB-treated Nc/Nga mice. Nc/Nga mice develop dermatitis spontaneously while Balb/c mice do not under the same conditions [23]. In this study, DNCB was chosen as the sensitizing chemical for the development of AD-like lesions in Nc/Nga mice [24]. In our previous study, we found that CC suppressed eotaxin and TARC expression

induced by TNF- α and IFN- γ stimulation in the keratinocyte cell line HaCaT. Eotaxin can be released from other cell types such as eosinophils, macrophages, lymphocytes, endothelial cells, epithelial cells, chondrocytes, smooth muscle cells, and fibroblasts as well as keratinocytes. The released eotaxin causes the skin infiltration of eosinophils and contributes to the development of an inflammatory reaction in AD [9]. In addition, Shoda et al. [25] suggested that dermal cells may play a key role in the source of TARC during AD development. TARC is expressed in keratinocytes, vascular endothelial cells, T cells, and dendritic cells, and induces the infiltration of CCR4+ lymphocytes. Thus, eotaxin and TARC are useful markers for AD and play important roles in the inflammatory reaction during AD development. In this study, we demonstrated that mRNA expression of eotaxin and TARC in the skin tissue from DNCB-treated NC/Nga mice decreased with CCE10 treatment but increased in the untreated mice (Fig. 3).

The major morphological and histopathological features of AD skin are increased epidermal thickness, erythema, hemorrhage, and skin dryness, which can contribute to the skin barrier defect during AD development [26]. The skin serves as a protective barrier against exogenous chemicals, microorganisms, physical injuries, and water loss [26, 27]. Maintaining hydration in the skin is vital for skin health; however, the protective effect against dehydration is impaired in the skin of AD patients, which aggravates the symptoms of AD [27]. Thus, avoiding skin dryness can reduce itching and is an important part of AD treatment. In this study, the CCE10 treatment suppressed AD development, including morphological and histopathological changes and the reduction in skin hydration induced by DNCB in NC/Nga mice (Fig. 1, 2). These data suggest that CCE10 can suppress water loss from the skin and also the development of AD symptoms in DNCB-treated NC/Nga mice.

T lymphocytes are a major source of cytokines, which play a major role in immune functions during AD development [28]. In acute AD, activated skin-resident dendritic cells migrate to local lymph nodes and induce the polarization of Th cells into Th2 cells. The activation of Th2 cells causes the accumulation of high levels of Th2 cytokines, such as IL-4, IL-10, and IL-13, which inhibit Th1 cell polarization [11, 12, 28]. Böhm and Bauer [11] reported that Th2 cytokines play a key role in the early stage of inflammatory reactions in AD whereas Th1 cells are generated in the later stages. Increased Th2 cytokines modulate the stimulation of B cell to IgE class switching in response to hypersensitivity and allergy. Thus, an Th1/Th2 imbalance plays an important function in the immune reaction during AD development [11–13, 28]. We found that DNCB stimulation increases the production of Th2 cytokines despite a reduction in T cell proliferation in primary splenocytes. In addition, DNCB treatment in NC/Nga mice resulted in the decrease of Th1 cytokine, IFN- γ , and IL-2 production. However, CCE10 treatment suppressed the changes of Th1 and Th2 cytokine production and T cell proliferation in DNCB-treated NC/Nga mice (Fig. 4) and inhibited the production of proinflammatory cytokines induced by DNCB treatment. Jeong et al. [18] suggested that the brown alga *Hizikia fusiforme* exerts immunomodulatory effects on murine macrophages and splenocytes by inhibiting proinflammatory cytokine production. Their study and ours have thus proved the potential immunomodulatory effects of brown algae.

In the development of AD, IgE mediates mast cell activation and sensitization by binding to Fc ϵ RI on the mast

cell surface. After binding, it can be sensitized, and release granular mediators (including histamines) and synthesize prostaglandins and leukotrienes. Numerous previous studies demonstrated that AD patients have elevated serum IgE and histamine levels [29–31]. During AD, hypersensitivity responses are observed in the skin tissue due to histamine binding to histamine receptors which are expressed on vascular smooth muscle cells endothelial cells, neurons, and immune cells [31]. Thus, histamine receptor antagonism and antihistamines have been used in antiallergic treatment for a long time. Chung et al. [32] demonstrated that a low concentration of dietary histamine improved AD symptoms in a 6-year-old Korean boy. Therefore, the inhibition of histamine production and release are important for the control of AD development. In this study, we found that DNCB treatment stimulated IgE and histamine production and the infiltration of mast cells into the skin. However, CCE10 treatment suppressed IgE and histamine production and mast cell infiltration induced by DNCB treatment in NC/Nga mice (Fig. 5).

Few studies have demonstrated the possible effects of brown algae on AD development. Kang et al. [33] documented that the topical application of *Sargassum fulvellum*, a perennial brown alga, inhibited the development of AD-like skin lesions in DNCB-induced Balb/c mice. Choi et al. [34] used moisturizing creams containing *Laminaria japonica* extracts on human skin and found that hydration significantly increased compared with the placebo. In addition, we found that dietary supplement with CCE10 suppressed the development of AD. These results suggest that brown algae may have potential applications in the prevention and treatment of AD.

In conclusion, dietary supplementation with CCE10 inhibited AD-like skin lesions in DNCB-treated NC/Nga mice. Our results showed the inhibitory effect of CCE10 on immune system abnormalities mediated by Th1/Th2 cytokine imbalance and on the production of IgE and histamine in DNCB-treated NC/Nga mice. These data suggest that CCE10 is a potential candidate for application in the treatment and prevention of AD.

Acknowledgments

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Statement of Ethics

All the experiments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committees of Dan Kook University (DKU-16-013).

Disclosure Statement

The authors have no conflict of interest to declare.

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