

RESEARCH ARTICLE

Protective Effects of *Opuntia humifusa* Cladode Extracts against Biostir AD-induced Atopic Dermatitis in NC/Nga Mice

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Abstract The abilities of water and ethanol extracts of *Opuntia humifusa* cladode powder to protect against atopic dermatitis in NC/Nga mice were investigated for application of *O. humifusa* cladodes as functional food materials. Water extract-treated group (OHW) and ethanol extract-treated group (OHE) mice exhibited protective effects against skin lesions, such as reduced hemorrhaging and epidermolysis. The number of mast cells in skin tissues of OHE group mice was 9.3 ± 2.7 units, which was significantly ($p < 0.05$) decreased compared with other group mice. The serum level of IgE in OHE group mice ($1,156 \pm 35.19$ ng/mL) was significantly ($p < 0.05$) decreased, compared with other group mice. The serum histamine concentrations in OHW and OHE groups mice were significantly ($p < 0.05$) decreased, compared with control group mice. Overall, 75% ethanol extracts provided greater protection than water extracts. *O. humifusa* cladode extracts have potential for future use as functional materials.

Keywords: *Opuntia humifusa* cladode, atopic dermatitis, protective, immunoglobulin E, histamine

Introduction

Atopic dermatitis is an immune disorder caused by continuous exposure to external antigens (1) and manifested as characteristic symptoms of severe pruritus, edema, erythema,

exosmosis, scabs, scales, and moisture loss. Moreover, atopic dermatitis can undergo repetitive relapse to become a chronic disease (2). Although the pathogenesis and precise causes of atopic dermatitis are not yet known, genetic, environmental, psychological, pharmacologic, and immunologic factors have all been proposed (3). Steroids, moisturizers, antihistamine agents, antibiotics, and other approaches have all been used to treat atopic dermatitis. Steroid medications may cause side effects of growth retardation, skin atrophy, and hypertension when used for long-term periods. Moreover, continuous use of antihistamines is problematic because these medications can result in tolerance, hypotension, digestive disorders, and other unwanted effects (4). Thus, alternative natural agents that are suitable for long-term treatment courses are urgently needed to reduce the side effects of atopic dermatitis treatment.

Opuntia humifusa (Cheonnyuncho) is a perennial plant that can be cultivated for several years and has long been used as either a food or a food substitute (5). *O. humifusa* can survive severe cold (-20°C), and harsh environmental conditions and contains abundant nutrients, including calcium, mineral matter, and amino acids, in addition to antioxidant flavonoids, polyphenols, vitamin C, and α -tocopherol (which is related to vitamin E) (6). *Opuntia* has proved efficacious against geriatric diseases, diabetes, hypertension, arthritis, gastritis, and constipation. Moreover, *Opuntia* has also been shown to be effective for improvement of appetite and activation of bowel movements (7).

Studies of the physiological mechanisms of activation have been conducted for antioxidant and antibiotic effects against microorganisms (8) and growth inhibition effects against cancer cells (9). Although studies have examined effects of *Opuntia* extracts on the lipid metabolism of diabetic mice (10) and potential use as an antidiabetic and antioxidant agent (11), few studies have examined whether

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treatment with *O. humifusa* can improve atopic dermatitis. Herein, extracts of *O. humifusa* cladodes were examined for protective effects against atopic dermatitis in NC/Nga mice with the ultimate aim of use as a functional material.

Materials and Methods

Materials *O. humifusa* cladodes harvested in November of 2012 in Sunchang were purchased from supermarkets in Sunchang, Korea in December of 2012. The cladodes were lyophilized (PVTFD100R; Ilshin Biobase, Dongducheon, Korea), powdered (HR2860; Philips, Eindhoven, Netherlands), and strained using a 500- μ m sieve (C8.10043; Cheonggye Sanggongsa, Seoul, Korea).

Animals Seven-week-old NC/Nga mice, a model for atopic dermatitis, were used in this study. Mice were obtained from Japan SLC, Inc. (Shizuoka, Japan) (12) and 20 male mice (15–20 g) were used. Mice were given free access to drinking water and fed a standard solid diet until the first experimental day and were maintained at a temperature of $22\pm 2^\circ\text{C}$, a humidity level of $55\pm 15\%$, and a light-dark cycle of 12 h for 1 week. Five mice were included in each experimental group. All institutional and national guidelines for the care and use of laboratory animals were followed.

Production of water and ethanol extracts For investigation into the ability of ethanol extracts to protect against atopic dermatitis, 0, 25, 50, 75, and 95% ethanol extracts were generated. A preliminary experiment revealed that a 75% ethanol extract provided the strongest activity (data not shown). Thus, animal diets were supplemented with either a 75% ethanol extract or a water extract. To obtain extracts, 100 mL of each solution was applied to 10 g of lyophilized powder as described in Materials, obtained using a reflux cooling system. Then, extraction was performed at 80°C for 3 h. Resultant extracts were passed through filter paper (Whatman No. 2; Springfield Mill, Maidstone, Kent, UK) by a vacuum filtration. Residual insoluble material was subjected to extraction using the same solution and this process was repeated twice under the same conditions. Soluble extracts were combined and clarified using centrifugation at $10,000\times g$ for 10 min in a Hitachi centrifuge (CR22G; Hitachi Koki Co., Ltd., Tokyo, Japan). Extracts were then lyophilized (PVTFD100R; Ilshin Biobase) and powdered (HR2860; Philips). The powder was filtered using a 500- μ m sieve (C8.10043; Cheonggye Sanggongsa) and used in subsequent experiments.

Experimental groups and diet supplementation Mice were divided into 4 different groups of 1) a normal group,

Table 1. Composition of experimental diets (unit: %, w/w)

Ingredients	Normal	Control	OHW ¹⁾	OHE ²⁾
Casein	20	20	20	20
Sucrose	10	10	10	10
Dextrose	13.2	13.2	13.2	13.2
Corn starch	39.7486	39.7486	39.7486	39.7486
Cellulose	5	5	5	5
Soybean oil	7	7	7	7
Water extract of OH ³⁾	-	-	10	-
Ethanol extract of OH	-	-	-	10
Mineral mix ⁴⁾	3.5	3.5	3.5	3.5
Vitamin mix ⁵⁾	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3
Choline bitartrate	0.25	0.25	0.25	0.25
TBHQ	0.0014	0.0014	0.0014	0.0014

¹⁾Diets supplemented with water extracts (10%) of *Opuntia humifusa* cladodes.

²⁾Diets supplemented with ethanol extracts (10%) of *Opuntia humifusa* cladodes.

³⁾OH, *Opuntia humifusa* cladodes.

⁴⁾The compositions of mineral mixtures were based on AIN-93G purified rodent chow.

⁵⁾The compositions of vitamin mixtures were based on AIN-93G purified rodent chow.

2) a control group, 3) a water extract-treated (OHW) group, and 4) an *O. humifusa* ethanol extract-treated (OHE) group (Table 1). Each experimental group included 5 mice. The normal group was fed a diet consisting of AIN 93G purified rodent chow (13). The skin of mice in the control, OHW, and OHE groups was treated for 3 weeks with the natural mite inducer Biostir AD (Biostir Inc., Kobe, Japan) in order to induce atopic dermatitis. Control group mice were fed the same 93G purified rodent chow as the normal group. OHW and OHE group mice were maintained on modified diets in which water extract powder and *O. humifusa* ethanol extract powder, respectively, were added as supplements to the AIN 93G purified rodent chow diet for 3 weeks.

Induction of atopic dermatitis The atopic dermatitis-inducing reagent Biostir AD (Biostir Inc.) was applied to the skin of mice for 3 weeks (14). This reagent is produced using allergens from *Dermatophagoides farina* and has been found to be effective in clinical applications because it induces a form of atopic dermatitis that is similar to human atopic dermatitis. For a first treatment, fur was removed from the back of mice to the upper auricle. Next, residual hair was removed using a hair removal cream (RaseraTM hair removal gel; Reckitt Benckiser Group plc, Slough, UK). Residual cream was then washed off using water and 100 mg of Biostir AD ointment was evenly applied using a flat stick from the back to the auricle on mice of all groups with the exception of the normal group. For a second treatment, 150 μ L of a 4% sodium dodecyl

sulfate solution was applied to the skin in order to remove surface fats and destroy cuticle barriers. The skin was allowed to dry for 2–3 h, then the same amount of Biostir AD ointment was applied to the skin of each mouse as in the first treatment. The Biostir AD ointment was applied a total of 6 times, twice a week (Monday and Thursday) for 3 weeks.

Observation of skin lesions A handscope (X50; AT Trading, Seoul, Korea) was used to observe detailed changes of the skin surface. Observations were made over the course of 3 weeks with a 1 week interval. Clinical skin scores (15) were measured for experimental and control group mice. Representative images were also captured using a handscope (X50; AT Trading). In the final week of the experimental period, clinical evaluation was performed with the unaided eye using the Yamamoto *et al.* (15) method, which is a standard method for evaluation of atopic dermatitis. Skin was evaluated according to the 4 categories of erythema/hemorrhage, dryness/scaling, edema, and excoriation/erosion. Final results were obtained as sums of individual scores. Each item was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Thus, final scores ranged from 0 to 12. The average clinical skin score in control group mice and other experimental group mice was assigned by 6 before implementation of the experimental protocol in order to obtain consistent and meaningful results.

Morphological and histological examination of mast cells in skin tissue Mice were anesthetized by diethyl ether after the third week of receiving the *O. humifusa* ethanol extract. General histological changes in skin tissue and specific morphological changes of mast cells in skin tissue were observed. To this end, the mouse body was treated with a perfuse fixative and the skin of the back was opened in either a parallel or a perpendicular direction to the spine. The tissue was then fixed for 12 h in a Bouin solution. After fixation, a general dehydration process (16) was performed and tissues were treated with paraffin. Then, a 7 μ m slice was obtained using a knife and stained with Hematoxylin and Eosin (H&E) and toluidin blue (16).

Immunohistochemical observation of myeloperoxidase and mast cell tryptase reactivity in skin cells For investigation into the presence of myeloperoxidase in skin tissue and changes in the levels of cytokines, 7 μ m paraffin-embedded tissue sections were examined using immunohistochemistry. Endogenous peroxidases were quenched in a solution of 0.3% H_2O_2 in 100% methanol. Nonspecific binding sites were then blocked by soaking in normal serum (1:50 for 30 min). Rabbit monoclonal anti-mast cell tryptase antibodies (Santa Cruz Biotechnology, Santa Cruz,

CA, USA) (1:200) and rabbit polyclonal anti-myeloperoxidase antibodies (Abcam Inc., Cambridge, MA, USA) (1:50) were used as primary antibodies. All working dilutions were determined based on preliminary optimization experiments with appropriate positive controls (17). Sections were incubated in primary antibodies in a humidified chamber at 4°C for 24 h. Antibodies were removed by 3 washes with a 0.1 M phosphate buffer (5 min each). Sections were then treated with biotinylated anti-immunoglobulin G (IgG; Vector Laboratories, Inc., Burlingame, CA, USA) according to the method used by Hsu *et al.* (17). Incubation in secondary antibodies was performed in a humidified moisture chamber for 30 min. Unbound antibodies were removed by washing with 0.1 M phosphate buffer 3 times for 5 min, and an ABC solution containing peroxidase was added to the sections. Antibodies were then washed twice for 15 min in 0.1 M phosphate buffer saline (PBS), then sections were treated for 5 min in a diaminobenzidine solution (30 mg in 150 mL of 0.1 M PBS). Immunoreactive areas were then visualized based on addition of hydrogen peroxide (0.005% for 15 min), which resulted in formation of a brown reaction product. Tissue sections were then washed with 0.1 M phosphate buffer and clarified. Then, tissue sections were mounted using permount and observed under an optical microscope.

Measurement of serum immunoglobulin E (IgE) levels

Serum IgE levels were measured using an ELISA kit (GenWay Biotech, Inc., San Diego, CA, USA) with the sandwich ELISA method (18). Serum samples were diluted 50 $\times$ in a buffer with a 0.1 M PBS, then added to anti-mouse IgE antibody-coated 96-well microplates (100 μ L/well). Recombinant mouse IgE was used as a positive control. Plates were incubated at room temperature for 30 min, after which unbound antibodies were removed by 4 washes in a wash buffer with a 0.1 M PBS. Microplates were then treated with HRP-conjugated antibodies (100 μ L/well) in the dark at room temperature for 30 min. Unbound antibodies were removed by 4 washes in wash buffer with a 0.1 M PBS. For visualization of bound antibodies, 100 μ L of a TMB solution was added to each well, followed by keeping for 10 min. Reactions were stopped by addition of a 0.3 M H_2SO_4 solution (100 μ L/well), and resultant absorbance values were measured by using a UV spectrophotometer (UV-1650 PC; Shimadzu Co., Kyoto, Japan) at 450 nm after 10 min.

Measurement of the serum histamine concentration

Serum histamine concentrations were measured using a histamine EIA kit (Bertin Pharma, Montigny le Bretonneux, France) with an enzyme immunoassay (EIA) method (19). Briefly, 20 μ L of derivatization reagent dissolved in *N,N*-dimethylformamide (DMF) was added to a mixture

comprised of 50 μL of a derivatization buffer and 200 μL of serum. Then, 100 μL of the mixture was added to each well of histamine antibody-coated microplate. Unbound proteins were removed by 5 washes in a wash buffer. Next, a histamine-AChE tracer was added to each well (100 μL /well), except for the blank control, followed by holding at 4°C for 24 h. Unbound tracer was removed by 5 washes in a wash buffer. Finally, Ellman's reagent (200 μL /well) was added and the resultant absorbance at 414 nm was measured by using a UV spectrophotometer (UV-1650 PC; Shimadzu Co.) in the dark.

Statistical analysis Results were expressed as a mean $\pm$ standard deviation (SD). Statistical significance of differences between groups was determined using Student's *t*-test at $p < 0.05$ for significance (Statistical Package for the Social Sciences, version 12.0; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Observation of skin lesions For experimental group mice, the atopic dermatitis-inducing ointment Biostir AD was applied to the skin 6 times over 3 weeks. The extent of erythema, edema, scaling, eschar, and epidermolysis on the back skin of all NC/Nga mice in the control, OHW, and OHE groups was observed using the unaided eye. Clinical skin scores for control and experimental group mice were determined using a standard handscope. Individual skin scores varied in each group and the mean score was defined to be 6 for each group.

After induction of atopic dermatitis via application of Biostir AD to NC/Nga mice 6 times over 3 weeks, some mice were fed a diet supplemented with either water or ethanol extracts of *O. humifusa* cladodes (10%) for 3 weeks. The skin of each mouse was then observed using a handscope. Throughout the first week, the extent of erythema, edema, scaling, eschar, and epidermolysis varied across the control, OHW, and OHE group mice. The extent of symptoms (expressed as the total clinical score) was 5.2 ± 1.1 for control group mice, while scores of OHW and OHE group mice were 5.4 ± 1.5 and 4.6 ± 1.3 , respectively. These scores were not significantly ($p < 0.05$) different from control group mice scores (Fig. 1 and 2A).

In the second week, the extent of symptoms varied in control group mice, with a total clinical skin score of 5.4 ± 1.1 . In contrast, some mice in the OHW group exhibited reduced hemorrhaging and epidermolysis, compared with mice in the control group. Moreover, most of the mice in the OHW group exhibited only edema and scaling and the total clinical skin score of OHW group mice was 4.6 ± 1.3 . For OHE group mice, the extent of hemorrhaging and epidermolysis was reduced with most mice only exhibiting

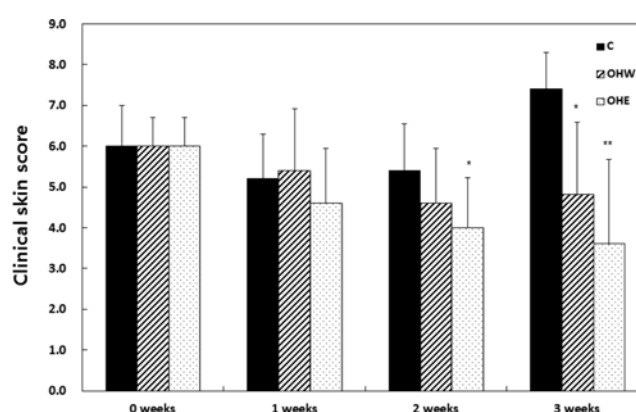


Fig. 1. Effects on the total clinical skin score of *O. humifusa* cladode extracts applied for 3 weeks to atopic dermatitis NC/Nga mice models induced using Biostir AD. C; control group, OHW; Diets supplemented with water extracts (10%) of *O. humifusa* cladodes, OHE; Diets supplemented with ethanol extracts (10%) of *O. humifusa* cladodes. Values are significantly different from control group ($n=5$); ** $p < 0.01$; * $p < 0.05$.

scaling. The clinical score of OHE group mice was 4.0 ± 1.2 , which was significantly ($p < 0.05$) lower, compared with control group mice (Fig. 1 and 2B).

In the third week, mice in the control group exhibited severe erythema, edema, scaling, eschar, and epidermolysis. The total clinical skin score for mice of this group was 7.4 ± 0.9 . In contrast, some mice in the OHW group exhibited reduced hemorrhaging and epidermolysis, compared with mice in the control group. Most mice in the OHW group only exhibited edema and scaling. The total clinical skin score of OHW group mice was 4.8 ± 1.8 , which was significantly ($p < 0.05$) decreased, compared to mice of the control group. Mice in the OHE group exhibited significantly ($p < 0.05$) reduced hemorrhaging and epidermolysis, with most of the mice exhibiting only scaling. The total clinical skin score of OHW group mice was 3.6 ± 2.0 , which was also significantly ($p < 0.01$) decreased, compared with control group mice (Fig. 1 and 2C).

Skin lesions were also measured using the Samwhang-sejegami extract method as described by Hwang *et al.* (20). The strongest effect was observed with the highest extract concentration in week 12, at which the total skin score was 1.4. In this study, the total skin score of OHE group mice reached 3.6 by the third week. Thus, results obtained in this study were promising, considering that extracts were only applied for 3 and not 12 weeks. Even more effective results could probably be obtained with prolonged extract treatment periods.

Effects on mast cells and histological changes in skin tissue Mast cells are the major initiations of inflammatory reactions, such as acute allergic reactions. These reactions are stimulated by secretion of histamine after coupling of

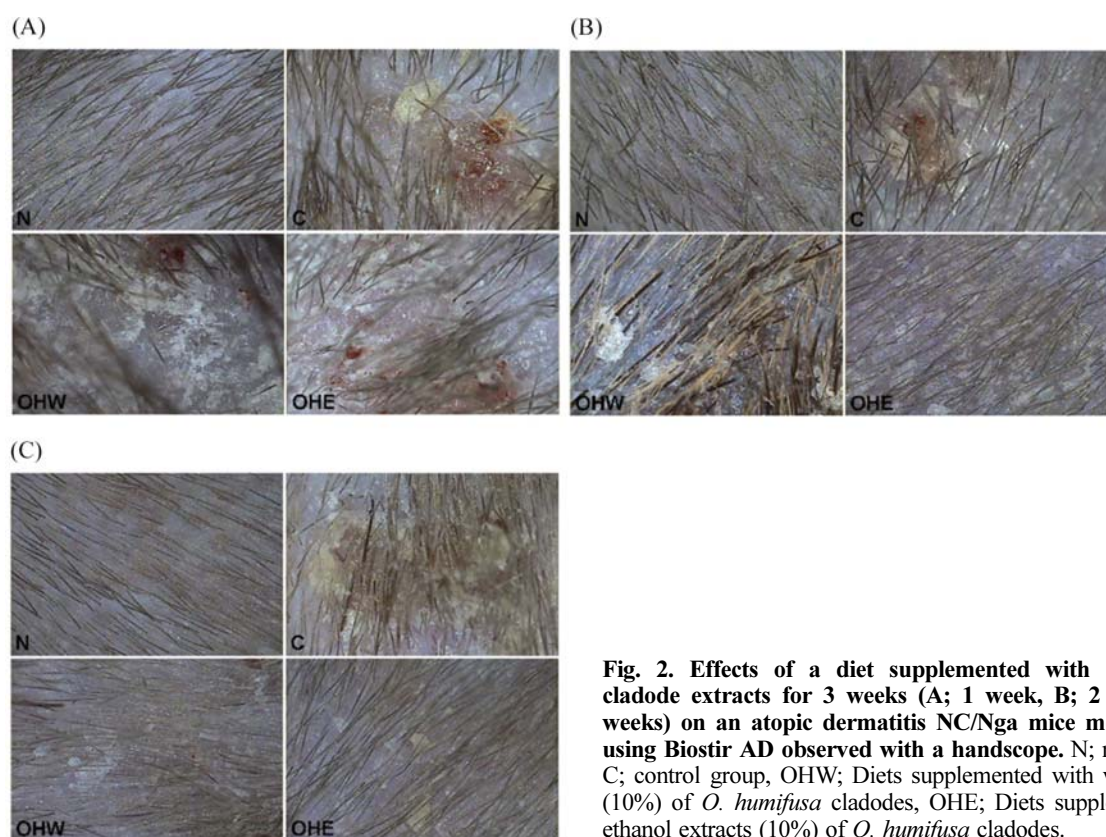


Fig. 2. Effects of a diet supplemented with *O. humifusa* cladode extracts for 3 weeks (A; 1 week, B; 2 weeks, C; 3 weeks) on an atopic dermatitis NC/Nga mice model induced using Biostir AD observed with a handscope. N; normal group, C; control group, OHW; Diets supplemented with water extracts (10%) of *O. humifusa* cladodes, OHE; Diets supplemented with ethanol extracts (10%) of *O. humifusa* cladodes.

an antigen bound to IgE with an FcεRI receptor, which is expressed by mast cells. After activation of the FcεRI receptor, mast cells accelerate inflammatory reactions by secreting substances, such as arachidonic acid metabolic products and inflammatory cytokines (21).

For investigation of whether the number of mast cells in the skin changed after treatment with extracts, mouse skin was stained with toluidin blue after mice had been fed *O. humifusa* cladode extracts for 3 weeks (Fig. 3). The number of mast cells in normal group mice was 14.4 ± 3.8 units, whereas the number in control group mice was 22.9 ± 5.2 . Thus, control group mice exhibited significantly ($p < 0.001$) more mast cells than normal group mice. Numbers of mast cells in OHW and OHE group mice were 17.1 ± 4.5 and 9.3 ± 2.7 , respectively, and were significantly ($p < 0.05$ and $p < 0.01$, respectively) decreased, compared with numbers in normal and control group mice. OHE group mice showed a larger decrease in the number of mast cells than OHW group mice. Moreover, fewer mast cells were observed in both the dermis and the subcutaneous skin tissue of OHE group mice than in control (C) and OHW group mice. In a study investigating the effects of *Lithospermum erythrorhizon* on atopic dermatitis by Kim *et al.* (22), granule rates of mast cells in normal and control groups were 6.10 ± 0.02 and $87.56 \pm 11.00\%$, respectively. In contrast, the granule rate of a group treated with *L. erythrorhizon* was $33.14 \pm 10.25\%$, consistent with this

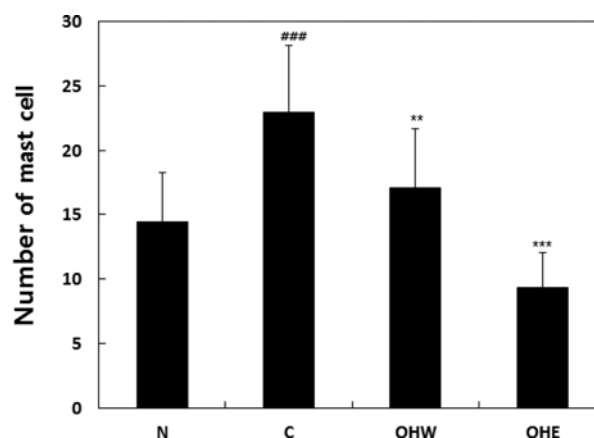


Fig. 3. Numerical changes of skin mast cells after feeding of experimental diets supplemented with extracts of *O. humifusa* cladodes for 3 weeks in an atopic dermatitis NC/Nga mice model induced using Biostir AD. Each data point represents a mean ± SD ($n = 5$). Values are significantly different from normal group (####) and control group (*); ### $p < 0.001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

study in that treatment with a natural compound resulted in a decrease in mast cell numbers and/or function.

Immunohistochemical analysis of mast cell tryptase and myeloperoxidase staining in skin tissue After feeding mice *O. humifusa* cladode extracts for 3 weeks, immunohistochemical staining was performed to determine

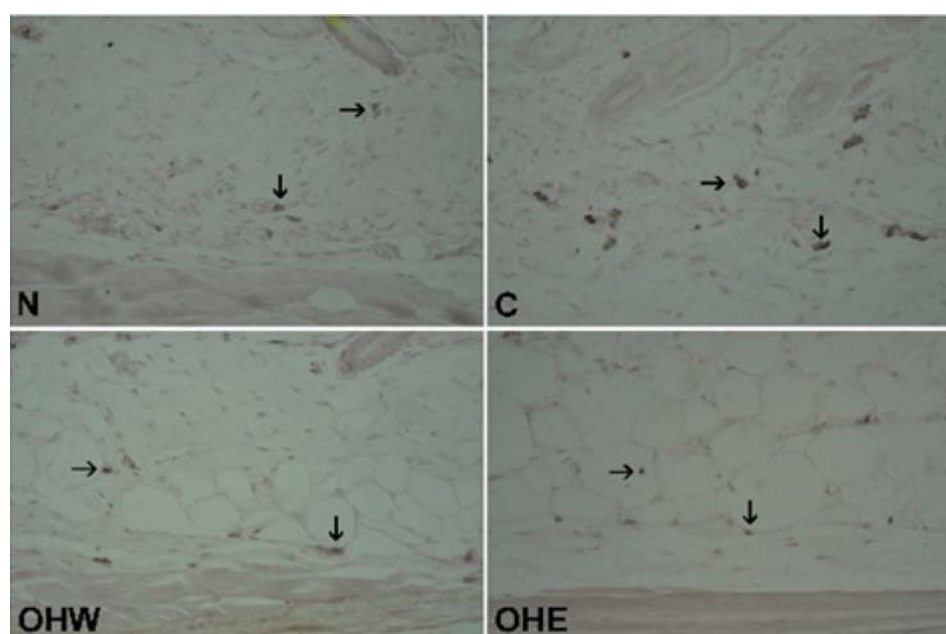


Fig. 4. Mast cell tryptase immunohistochemical staining of back skin after feeding of experimental diets supplemented with extracts of *O. humifusa* cladodes for 3 weeks in an atopic dermatitis NC/Nga mice model induced using Biostir AD (mast cell tryptase immunohistochemical stain ↓, ×200).

immune reactions occurring in mast cells of the skin. Immunohistochemical staining revealed that OHW and OHE group mice showed lower levels of mast cell tryptase staining, compared with control group mice. Moreover, the number of stained mast cells was also decreased (Fig. 4). Binding of allergens to the IgE receptor, which is present on the surface of mast cells, stimulates release of tryptase, which is an inflammatory mediator (23). OHW and OHE treatments in this *in vivo* model probably inhibited secretion of tryptase.

Immunohistochemical staining for myeloperoxidase revealed that a large number of myeloperoxidase-immunoreactive cells were present in the dermic and hypodermic layers of control group mice. In contrast, the numbers of myeloperoxidase-immunoreactive cells were decreased in OHW and OHE group mice (Fig. 5). Myeloperoxidase is not expressed in lymph cells. Therefore, an absence of myeloperoxidase is used to diagnose marrow disease, such as leukemia (24). OHW and OHE treatments probably inhibited generation of myeloperoxidase.

Changes in serum levels of IgE IgE antibodies are directed against external protein antigens and levels of serum IgE and eosinophils are elevated in approximately 80% of all patients with atopic dermatitis (25). IgE antibodies are known to play an important role in the pathogenesis of atopic dermatitis and to cause pruritus via induction of the release of inflammatory mediators after stimulation of mast cells (26).

Serum IgE concentrations were monitored in this *in vivo*

model of artificially-induced atopic dermatitis (Fig. 6). The level of IgE in normal group mice was 379.9 ± 4.6 ng/mL. In contrast, the level in control group mice was $1,277.2 \pm 13.3$ ng/mL, which was significantly ($p < 0.001$) increased, compared with normal group mice. The IgE level in OHW group mice was $1,446.6 \pm 12.9$ ng/mL, which was significantly ($p < 0.001$) elevated compared with control group mice. In contrast, the IgE concentration in OHE group mice was $1,115.0 \pm 35.1$ ng/mL, which was significantly ($p < 0.01$) decreased, compared with control group mice. Thus, *O. humifusa* cladode extracts prevented elevation of serum IgE levels and OHE treatment was significantly ($p < 0.05$) effective in this respect, compared with controls. In a study by Ahn *et al.* (27) that focused on treatment of atopic dermatitis with water extracts of *Rumex japonicus*, experimental groups showed lower IgE concentrations than a control group. IgE concentrations in normal, control, low concentration (12.5 mg/kg), and high concentration (25.0 mg/kg) groups were 286.33 ± 45.61 , $1,531 \pm 61.22$, $1,180 \pm 250.59$, and $1,169.33 \pm 120.55$ ng/mL, respectively. Thus, the *O. humifusa* ethanol extracts used in this study appeared to be more effective for lowering the serum IgE concentration than water extracts of *R. japonicus*.

Changes in serum histamine levels Histamine is derived from mast cells and basophils and increases secretion of gastric acid, in addition to causing allergies via shrinkage of smooth muscles (28). Histamine is also an important mediator of immediate hypersensitive reactions and pruritus, and is an active agent in the allergic diseases of

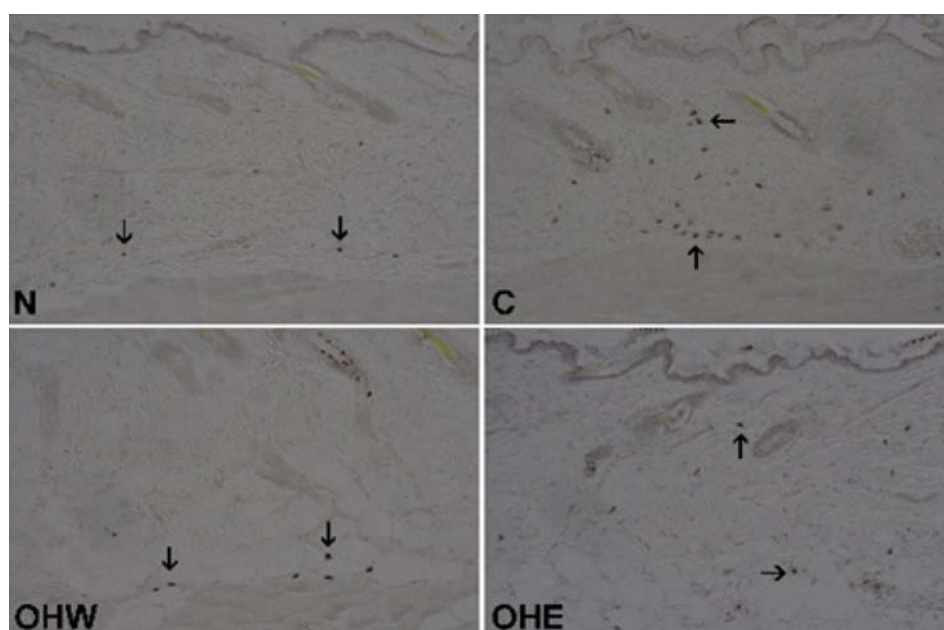


Fig. 5. Myeloperoxidase immunohistochemical staining of back skin after feeding of experimental diets supplemented with extracts of *O. humifusa* cladodes for 3 weeks in an atopic dermatitis NC/Nga mice model induced using Biostir AD (myeloperoxidase immunohistochemical stain ↓, ×100).

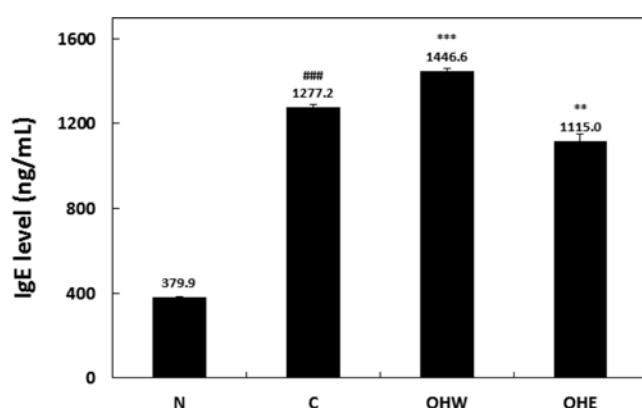


Fig. 6. Effects on the serum IgE level after feeding of experimental diets supplemented with extracts of *O. humifusa* cladodes for 3 weeks in an atopic dermatitis NC/Nga mice model induced using Biostir AD. Each data point represents a mean±SD ($n=5$). Values are significantly different from normal group (###) and control group (*); ### $p<0.001$; *** $p<0.001$; ** $p<0.01$.

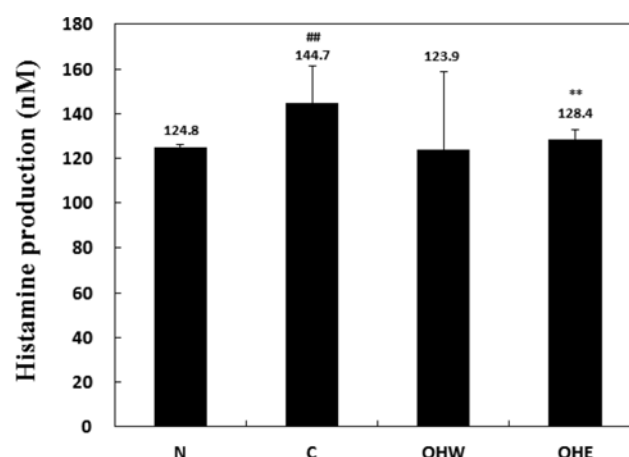


Fig. 7. Effects on the serum histamine level after feeding of experimental diets supplemented with extracts of *O. humifusa* cladodes for 3 weeks in an atopic dermatitis NC/Nga mice model induced using Biostir AD. Each data point represents a mean±SD ($n=5$). Values are significantly different from normal group (##) and control group (**); ## $p<0.01$, ** $p<0.01$.

hives, asthma, and allergic rhinitis. Patients with these reactions have been shown to exhibit increased serum or tissue concentrations of histamine (29).

Serum histamine concentrations were also assessed in the *in vivo* model in this study of artificially-induced atopic dermatitis (Fig. 7). Histamine concentrations of normal and control group mice were 124.8 ± 1.5 and 144.7 ± 16.4 nM, respectively. Thus, the level of histamine in control group mice was significantly ($p<0.01$) increased, compared with normal group mice. Histamine concentrations in OHW and OHE group mice, which received *O. humifusa* extracts,

were 123.9 ± 34.9 and 128.4 ± 4.3 nM, respectively. These mice also showed significantly ($p<0.01$) increased levels of histamine, compared with control group mice. No significant ($p>0.05$) differences were observed between OHW and OHE treatment group mice. Kim *et al.* (30) reported that application of water extracts of *Rabdosia japonicus* to mice (100 and 1,000 mg/kg) resulted in significantly reduced levels of histamine; however, the *in vivo* model and methods used differed from this study.

In conclusion, *O. humifusa* cladode extracts can exert

protective effects against atopic dermatitis in an NC/Nga mouse model. Moreover, ethanol extracts generally provided greater protection than water extracts. Thus, *O. humifusa* cladode extracts appear to have potential for future use as a functional food material.

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Disclosure The authors declare no conflict of interest.

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