

# Effect of pedunculagin investigated by non-invasive evaluation on atopic-like dermatitis in NC/Nga mice

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**Background/purpose:** Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder that is becoming increasingly prevalent. Experimental animal models have been an indispensable tool for studying its pathological mechanisms and for *in vivo* testing of novel therapeutic approaches. AD-like lesions can be induced experimentally in NC/Nga mice. Pedunculagin, an ellagitannin purified from the Manchurian alder, *Alnus hirsuta* var. *microphylla*, Betulaceae, is a novel immunomodulator. To evaluate the effect of pedunculagin for AD-like lesions in NC/Nga mice, using clinical and non-invasive methods.

**Methods:** AD-like lesions were induced in NC/Nga mice using 2,4,6-trinitrochlorobenzene (TNCB). A cream containing 0.1% or 0.5% pedunculagin was applied to the positive treatment group, and the base cream without pedunculagin was applied to the negative treatment group. The control group did not receive any kind of topical agents. We evaluated the therapeutic efficacy of pedunculagin for AD

by statistical evaluation of the clinical severity score using non-invasive biomedical engineering tools before treatment, and 1 day, 3 days, 1 week, 2 weeks and 4 weeks afterwards.

**Results:** An AD-like skin rash was successfully induced using TNCB in NC/Nga mice. The group receiving higher concentrations of pedunculagin showed faster and greater improvement.

**Conclusion:** Our results suggest that remedies made from natural materials like pedunculagin are now showing promise for medical applications, and many new studies are expected to explore this potential.

**Key words:** atopic dermatitis – pedunculagin – stereo-image optical topometer – 2,4,6-trinitrochlorobenzene – NC/Nga

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ATOPIC DERMATITIS (AD) is a chronic, relapsing, inflammatory skin disease that is representative of the allergic diseases, which include asthma and allergic coryza. Recently, the incidence of AD has rapidly increased, especially in industrialized countries, suggesting that environmental factors play a role. Research on the cause of AD is progressing and new treatments are being developed, but the main issue for AD is finding methods of safe, long-term control and cure. Medications to control skin moisture and inflammatory reactions are currently the dominant treatment, including non-steroidal immunosuppressive agents, cyclosporin, interferon- $\gamma$ , thymopentin, mycophenolate mofetil and intravenous immunoglobulin. However, new medicines that can be applied safely, for long periods, are needed to replace these medicines, because

they relieve symptoms only temporarily or cause side effects after an overdose or long-term use.

The diagnosis of most diseases is based on morbid histology, requiring invasive methods such as tissue biopsy (1). However, non-invasive bioengineering methods allow scientific analysis of the molecular interrelation of tissue and cells, *in vivo*, in real time. To measure AD quantitatively, medical bioengineering methods, such as the Evaporimeter<sup>®</sup>, Corneometer<sup>®</sup> and pH meters, are widely used. Stereoimage optical topometer (SOT), a method of research for the three-dimensional (3D) investigation of the skin surface, has also been used for comparative analysis of skin before and after the application of natural material (2, 3).

We induced an AD-like disease in a mouse model (4, 5), and analyzed the symptoms quanti-

tatively using a clinical severity score (6–8). In addition, non-invasive methods and methods for 3D analysis of the skin surface, such as SOT, were used to directly and quantitatively analyze AD. We comparatively and statistically analyzed the results to determine the effects of an AD medicine based on a natural substance.

## Materials and Methods

### *Experimental animals*

Forty NC/Nga (male, 6 weeks old) mice were obtained from Central Laboratories, Animal Inc. (Korea). They were used for experiments after being held under specific pathogen-free (SPF) conditions for 7 days (9). Temperature was maintained at  $23 \pm 3^\circ\text{C}$ , humidity at  $55 \pm 15\%$  and lighting from 07:00 to 19:00 hours. Unlimited food and water were provided for all mice.

### *Sensitization and repeated application with 2,4,6-trinitrochlorobenzene*

To induce AD artificially, 1% 2,4,6-trinitrochlorobenzene (TNCB) (acetone:ethanol = 1:4) was prepared and used to sensitize the mice (100  $\mu\text{L}$ /mouse). The backs of the mice were scratched using the tape-stripping method, and 7 days later, 100  $\mu\text{L}$  of 1% TNCB was applied for 36 days at 2-day intervals to induce AD (5). Applications were on the backs and ears of 20 mice, and on the backs of the others.

### *Therapeutic experiment*

After inducing AD, 3  $\mu\text{L}$  of cream was applied to the backs of the mice once a day. A cream containing 0.1% ( $n = 10$ ) or 0.5% ( $n = 10$ ) pedunculagin was applied to the positive treatment group, and the base cream with no pedunculagin was applied to the negative treatment group ( $n = 10$ ). The control group ( $n = 10$ ) received no cream. For ear thickness measurements, we applied cream to the ears of two groups: one that

received 0.5% pedunculagin and one that received base cream. Applications stopped 12 h before the efficacy test, which was conducted after stabilization at a room temperature of  $22^\circ\text{C}$  and a relative humidity of 60% for 30 min. The severity of AD was measured with a visual scoring and using bioengineering methods before treatment, and 1 day, 3 days, 1 week, 2 weeks and 4 weeks afterwards.

The three kinds of cream, 0.1% pedunculagin, 0.5% pedunculagin and base cream, were provided by Prof. Minwon Lee and his team at the College of Pharmacy at Chungang University (see Fig. 1).

### *Clinical severity score*

The clinical skin severity score was measured following the method of Leung et al. (10). We graded the levels of five symptoms, itching, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness, as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). The scored opinions of three dermatologists were aggregated and calculated for the final score.

### *Ear thickness*

Changes in ear thickness among mice that received base cream and 0.5% pedunculagin cream were measured using a traditional method. Three measurements with a dial thickness gauge (Ozaki Mfg, Osaka, Japan), with a precision of 1  $\mu\text{m}$ , were averaged (11–14).

### *Transepidermal water loss (TEWL)*

When a probe 12 mm in diameter can penetrate vertically into skin, a water vapor gradient exists between the skin surface and the air. Two hygrometers in an Evaporimeter<sup>®</sup> (Dermalab, Cortex Technology, Hadsund, Denmark) probe were used to measure the humidity gap between two areas, and TEWL was calculated from the mea-

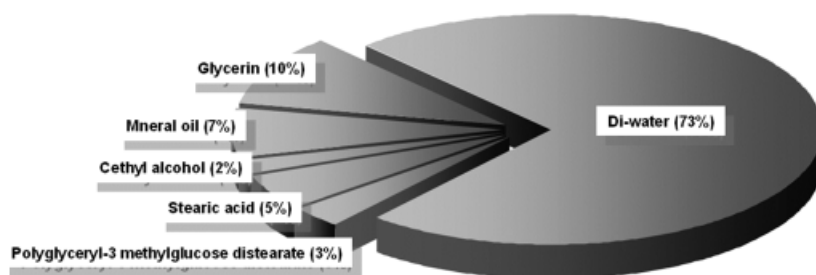


Fig. 1. Components of the vehicle.

sured values. Each measurement was conducted three times at the same area, and averaged (15–18). Measurements were performed at 24 °C and 46% relative humidity (19).

#### *Roughness of skin contours*

Using two charge-coupled device cameras, the disparity was calculated and 3D information was extracted from a pair of images, and then, parameters were selected for the 3D surface of the skin. A visualized image of the disparity of all digital image pixels is called a disparity map, and contains indirect 3D coordinates (20). The process of SOT was as follows: a digital stereo image was chosen with a stereo microscope (Fig. 2a), a disparity map was made with the image based on a matching algorithm and 3D coordinates, by pixel, were extracted from the map (2, 3). A pair of skin furrow stereo images extracted from normal skin by SOT is as follows (Fig. 2b) (2, 3).

We quantitatively analyzed skin roughness before and after treatment using four parameters. The selected parameters, arithmetic mean deviation of the surface ( $S_a$ ), 10-point height of the surface ( $S_z$ ), 3D length ( $SL$ ) and 3D area ( $SA$ ) of the SOT, were measured for length, area and volume

size in 3D, and quantitatively analyzed to determine values for the roughness of the skin (2, 3).

#### *Statistical analysis*

The results of data comparison were statistically reviewed using NCSS 2007<sup>®</sup> (NCSS Inc., Kaysville, UT, USA). To evaluate the improvement of AD at the points of pre-treatment, and after 1 day, 3 days, 1 week, 2 weeks and 4 weeks, we conducted Duncan multiple-range tests and Student's *t*-tests. *P*-values of <0.05 were considered statistically significant.

## Results

#### *Induction of AD*

Based on the continual increase in the clinical severity score after sensitizing TNCB, we found that an AD-like rash over the moderation appeared after 3 weeks. Mice in the test group with induced AD and mice in the non-induced groups showed statistically significant differences as measured by SOT and Evaporimeter<sup>®</sup>.

#### *Clinical severity score*

Analysis of the accumulated values of the clinical severity scores demonstrated that the rates of increase in groups receiving only the base cream and those in the no treatment group were similar to each other in each testing period. But the much faster improvement in the 0.5% pedunculagin group was comparatively lower than that in the 0.1% pedunculagin group. Groups that received pedunculagin showed greater improvement than groups that did not (Fig. 3).

#### *Ear thickness*

We compared mice that received base cream applications on their ears with mice that received applications of 0.5% pedunculagin cream in each testing period, and found a significant change 3 days after application. In addition, the 0.5% pedunculagin-treated mice showed a quantitatively significant difference in the period before application and 4 weeks after application (Fig. 4).

#### *TEWL*

Analysis using an Evaporimeter<sup>®</sup> to compare groups receiving 0.1% or 0.5% pedunculagin with the no treatment groups showed a statisti-

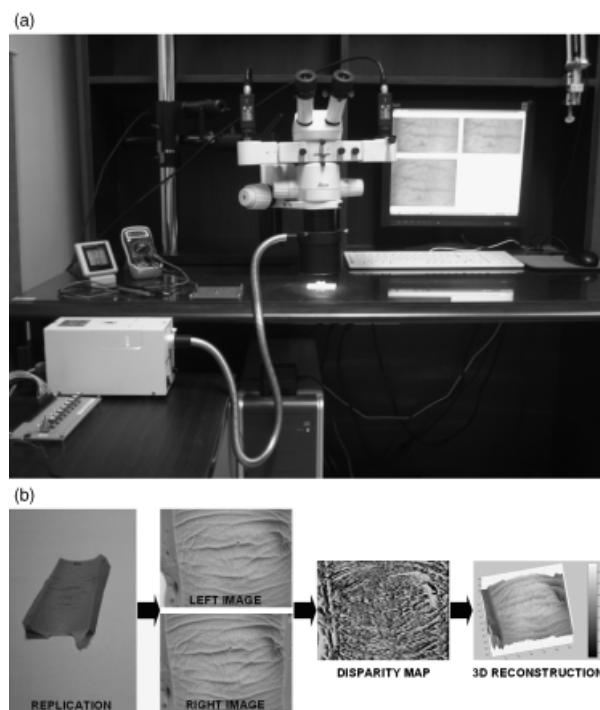


Fig. 2. (a) Stereomicroscope optical topometer. (b) Disparity map calculated by stereo matching based on left and right images. The 3D data view was reconstructed from the disparity map.

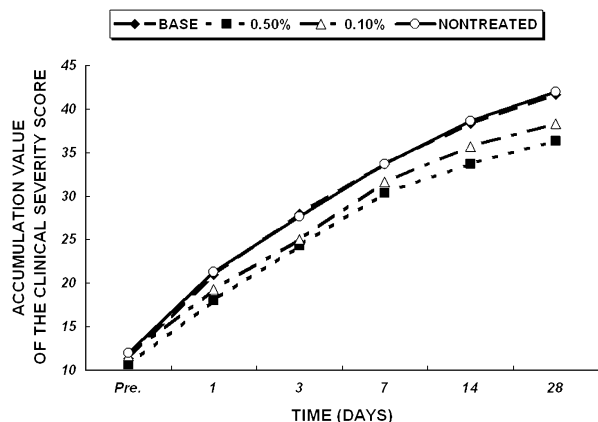


Fig. 3. Distribution of accumulation values for the clinical severity scoring test.

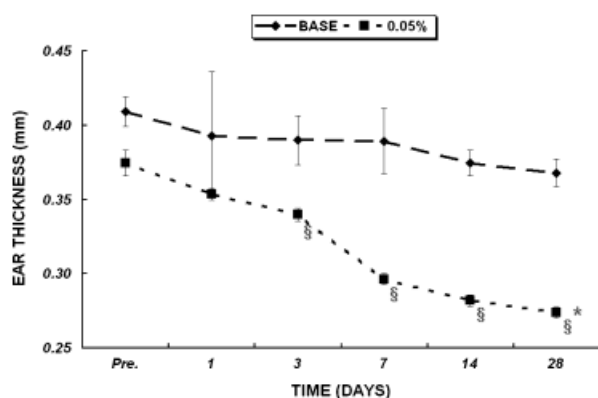


Fig. 4. Analysis of the response to treatment measured by changes in ear thickness. Data are mean  $\pm$  SD,  $n = 20$ . \* $P < 0.05$  for all groups by the Duncan multiple-range test.  $^{\S}P < 0.05$  for the base cream group vs. the 0.5% pedunculagin-treated group by the Student's  $t$ -test.

cally significant difference at 4 weeks after application (Fig. 5).

#### Roughness of skin contours

The SOT results for four parameters,  $S_a$ ,  $S_z$ ,  $SL$  and  $SA$ , are shown in Fig. 6. Statistically significant differences were found between the non-treated group and the 0.5% pedunculagin group for all parameters at all points, 3 days after application. Comparing the non-treated group with the 0.1% pedunculagin group,  $S_a$  and  $S_z$  showed significant differences 4 weeks after application, as did  $SL$  at all points after 3 days, and  $SA$  at 3 days, and at 2 and 4 weeks. We observed no significant differences in  $S_z$  and  $SL$  at any points when comparing the base cream group with the 0.5% pedunculagin group, but  $S_a$  showed differences 4 weeks after treatment, as did  $SA$  at all points 1 week after treatment. The

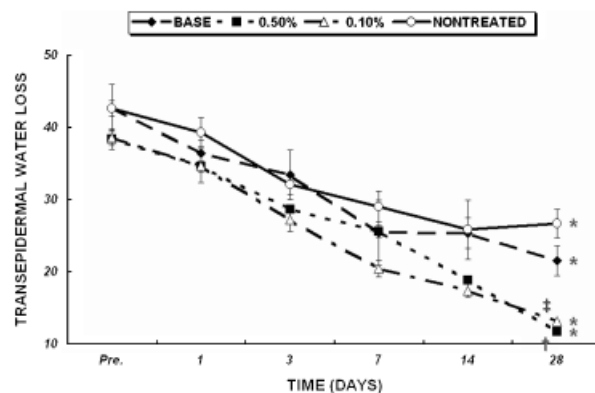


Fig. 5. Results for measurement of transepidermal water loss by an Evaporimeter<sup>®</sup>. Data are mean  $\pm$  SD,  $n = 40$ . \* $P < 0.05$  for all groups by the Duncan multiple-range test.  $^{\dagger}P < 0.05$  for the non-treated group vs. the 0.5% pedunculagin-treated group by the Student  $t$ -test.  $^{\ddagger}P < 0.05$  for the non-treated group vs. the 0.1% pedunculagin-treated group by the Student  $t$ -test.

base group and the 0.1% pedunculagin group showed no significant differences, except for  $SA$ , which showed significant differences after 4 weeks. A Duncan multiple-range test showed that the values of  $S_a$ ,  $S_z$  and  $SA$  were significantly different in all groups, except for the non-treated group, and differences in  $SL$  were found to be intermediate between the 0.1% and the 0.5% groups. Generally, the AD of the 0.5% group appeared to improve more quickly than the 0.1% group for  $S_a$ ,  $S_z$  and  $SA$ . Comparison of the base cream group with the 0.5% group showed significant differences only at  $S_a$  and  $SA$ , and with the 0.1% group, only at  $SA$ , confirming that the 0.5% group showed more improvement than the 0.1% and the base cream groups.

## Discussion

Historically, people have looked to nature for solutions to medical problems. As technology has advanced to allow separation, structural analysis and examination of activity on very small scales, development of new medicines has become an active pursuit in the field of medical compounds.

The pedunculagin used in this study is an ellagitannin refined from alder trees (*Alnus hirsuta* var. *microphylla*, Betulaceas) (21). Ellagitannins have been reported to not only provide protection against inflammation, cancer, virus and bacteria but also to suppress lipid peroxidation, reduce blood urea nitrogen and improve mental condition (21–24). According to a study of

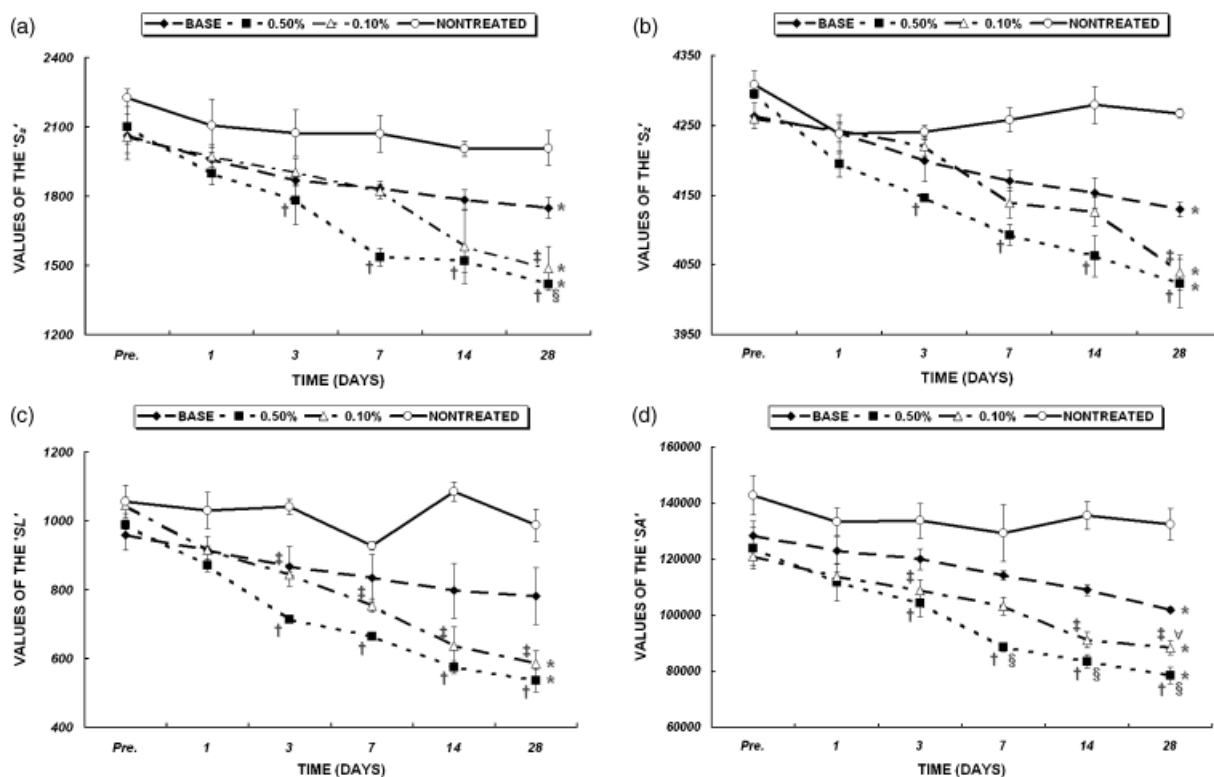


Fig. 6. Results of the skin roughness test. (a) Sa (arithmetic mean deviation of the surface), (b) Sz (10-point height of the surface), (c) SL (3D length) and (d) SA (3D area). Data are means  $\pm$  SD,  $n = 40$ . \* $P < 0.05$  for all groups by the Duncan multiple-range test. † $P < 0.05$  for the non-treated group vs. the 0.5% pedunculagin-treated group by the Student's  $t$ -test. ‡ $P < 0.05$  for the non-treated group vs. the 0.1% pedunculagin-treated group by the Student's  $t$ -test. § $P < 0.05$  for the base cream group vs. the 0.5% pedunculagin-treated group by the Student's  $t$ -test. ∇ $P < 0.05$  for the base cream group vs. the 0.1% pedunculagin-treated group by the Student's  $t$ -test.

the mechanisms underlying pedunculagin's alleged anti-inflammatory effects, pedunculagin increases the expression of IL-1 $\beta$  mRNA in Langerhans cells, and thereby increases the secretion of IL-1 $\beta$ . This increases the activation of macrophages, natural killer cells and lymphocytes, as well as the expression of TNF- $\alpha$  mRNA, and so the expression of TNF- $\alpha$  is also increased. Monocytes are also activated, which increases the secretion of other cytokines (IL-1 $\beta$ , IL-6 and GM-CSF), increasing immunity (23, 24). We used an NC/Nga mouse model for an AD-like disease to evaluate the efficacy of pedunculagin in this context.

NC/Nga mice are an excellent natural model for symptoms of AD that are similar to those observed in humans in terms of progress, histopathological and immunopathological findings, and heredity under conventional or non-specific pathogen-free (non-SPF) conditions. However, NC/Nga mice do not develop symptoms of skin diseases in an SPF environment (4). An NC/Nga mouse transferred from an SPF envi-

ronment to a normal environment develops atopic-like dermatitis symptoms that are not as severe as those in mice raised in a normal environment from birth (4). The first symptoms that an NC/Nga mouse develops under conventional conditions begin at 6–8 weeks with scratching and erythematous erosion with edemas and hemorrhage, which rapidly appears on the face, ears and neck. Symptoms worsen by 17 weeks, and finally, may cause lichenification, blepharitis and ophthalmia in the worst cases (4, 25, 26). Like AD in humans, AD-like symptoms in mice show an improvement after 6 months in many cases (4).

A hapten-induced mouse model can easily be constructed by repetitive application of 2,4-dinitrochlorobenzene (DNCB) or TNCB. This model is valuable because it generates various symptoms of chronic inflammation, depending on the hapten used, is easily induced and can be evaluated quantitatively (27, 28).

Xeroderma and problems with skin barrier function are the main features of human AD, and NC/Nga mice also develop symptoms, such

as an increase in TEWL and abnormal skin conductance. Dysbolism of ceramide and complicated disorders of several genes, cytokines, chemokines and proteolytic enzymes have all been considered as factors contributing to the skin inflammation of NC/Nga mice (4).

The stratum corneum plays the role of a barrier in the skin, to maintain humidity. It consists of hydrophilic components like keratin and hydrophobic components, and therefore contains water, prevents water loss and protects the skin from foreign substances. Some researchers assert that disorders of skin barrier function caused by the abnormal proliferation of the corneum from eczema or psoriasis are directly related to the symptoms of those diseases.

Various bioengineering methods for quantitative analysis of skin water distribution are currently in use, including the Corneometer<sup>®</sup>, which gauges the capacitance of skin and allows the study of the relationship between the moisture status of the corneum and damaged skin and the Dermaflex<sup>®</sup>, which measures the elasticity of skin (29). Viscoelasticity skin analyzer and shear wave propagation (SWP) have also been used to elucidate the mechanism of skin's barrier function, as well as the effects of optional, active moisturization and dehydration, but have not yielded clear results (1, 30). Ultrasonic waves have also been used to measure the moisture of the corneum in many studies. However, new methods using other technologies, such as radiation, magnetic resonance and photonics, are being developed to replace this method, because of the error caused by the process of placing water directly into the corneum.

In this study, non-invasive methods using bioengineering devices, such as a dial thickness gauge and Evaporimeter<sup>®</sup>, were used to measure ear thickness and TEWL, to monitor improvement after the treatment of AD. The roughness of the skin was assessed using SOT and 3D analysis of the skin's surface was also performed. Statistically significant results and morphological improvements were seen in groups that received 0.5% pedunculagin cream 3 days after application, as measured by ear thickness using a dial thickness gauge. Comparing the SOT results of identical groups confirmed that a long treatment period is needed to improve the roughness of skin, because *Sa* and *SA* showed a statistically significant improvement only after 4 weeks and 1 week, respectively.

All groups showed significant differences in biomedical analyses by Evaporimeter<sup>®</sup>, using Duncan multiple-range tests, but when comparing all groups that received pedunculagin with the non-treatment group, we could observe a statistically significant improvement only after 4 weeks.

Experiments using bioengineering devices, such as a dial thickness gauge or Evaporimeter<sup>®</sup>, might lead to errors because of uncertainties in the depth of skin to be measured. This limits the physiological analysis of the ultrastructure of troubled skin. As a result, only information on the tendency of morphological changes in AD was obtained. Measurements using SOT provide 3D and morphological parameters, however, and so results could be verified in this way. The group receiving higher concentrations of pedunculagin showed faster and greater improvement, but additional studies on the proper amount of pedunculagin are needed to test its use as a treatment for AD.

The mechanisms of pedunculagin, including the mechanism of immunological activation, should be examined, and additional studies on its efficacy against other inflammatory diseases are also needed. Remedies made from natural materials like pedunculagin are now showing promise for medical applications, and many new studies are expected to explore this potential.

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*Conflict of interest:* The authors stated that they have no conflicts of interest.

## References

1. Seidenari S, Giusti F, Pellacani G. Non-invasive methods and assessment of skin disease. In: Serup J, Jemec BE, Grove GL, eds. Handbook of non-invasive methods and the skin, 2nd edn. Boca Raton: CRC/Taylor & Francis, 2006: 37–46.
2. Park S, Ha S-H, Yu D-S et al. Quantitative evaluation of severity in psoriatic lesions using three-dimensional morphometry. *Exp Dermatol* 2004; 13: 223–228.
3. Kim M-G, Park S-Y, Ha S-H et al. Objective interpretation of severity of SLS induced edema by stereoinaging. *J Dermatol Sci* 2004; 35: 125–131.

4. Gutermuth J, Ollert M, Ring J, Behrendt H, Jakob T. Mouse models of atopic eczema critically evaluated. *Int Arch Allergy Immunol* 2004; 135: 262–276.
5. Matsumoto K, Mizukoshi K, Oyobikawa M, Ohshima H, Tagami H. Establishment of an atopic dermatitis-like skin model in a hairless mouse by repeated elicitation of contact hypersensitivity that enables to conduct functional analyses of the stratum corneum with various non-invasive biophysical instruments. *Skin Res Technol* 2004; 10: 122–129.
6. Matsuda H, Watanabe N, Geba GP et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997; 9: 461–466.
7. Sugarman J, Fluhr J, Fowler A, Bruckner T, Diepgen T, Williams M. The objective severity assessment of atopic dermatitis score: an objective measure using permeability barrier function and stratum corneum hydration with computer-assisted estimates for extent of disease. *Arch Dermatol* 2003; 139: 1417–1422.
8. Angelova-Fischer I, Bauer A, Hippler UC et al. The objective severity assessment of atopic dermatitis (OSAAD) score: validity, reliability and sensitivity in adult patients with atopic dermatitis. *Br J Dermatol* 2005; 153: 767–773.
9. Yamashita H, Michibata Y, Mizukami H, Ogihara Y, Morita A, Nose M. Dermal mast cells play a central role in the incidence of scratching behavior in mice induced by multiple application of the hapten, 2,4,6-trinitrochlorobenzene. *Exp Dermatol* 2005; 14: 438–444.
10. Leung DY, Hirsch RL, Schneider L et al. Thymopentin therapy reduces the clinical severity of atopic dermatitis. *J Allergy Clin Immunol* 1990; 85: 927–933.
11. Takano H, Yanagisawa R, Inoue K-I, Ichinose T, Sadakane K, Yoshikawa T. Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. *Environ Health Perspect* 2006; 114: 1266–1269.
12. Gao XK, Fuseda K, Shibata T, Tanaka H, Inagaki N, Nagai H. Kampo medicines for mite antigen-induced allergic dermatitis in NC/Nga mice. *Evid Based Complement Alternat Med* 2005; 2: 191–199.
13. Kang J, Lee K, Han S-B et al. Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of *Dermaphagoides pteronyssinus* in NC/Nga mice. *Int Immunopharmacol* 2006; 6: 1616–1622.
14. Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T. Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. *J Invest Dermatol* 1995; 105: 749–755.
15. Rodrigues L, Pinto P, Pereira L. Quantitative description of human skin water dynamics by a disposition–decomposition analysis (DDA) of trans-epidermal water loss and epidermal capacitance. *Skin Res Technol* 2003; 9: 24–30.
16. Bornkessel A, Flach M, Arens-Corell M, Elsner P, Fluhr JW. Functional assessment of a washing emulsion for sensitive skin: mild impairment of stratum corneum hydration, pH, barrier function, lipid content, integrity and cohesion in a controlled washing test. *Skin Res Technol* 2005; 11: 53–60.
17. Yoshizawa Y, Kitamura K, Kawana S, Maibach H. Water, salts and skin barrier of normal skin. *Skin Res Technol* 2003; 9: 31–33.
18. Nicander I, Rantanen I, Rozell B, Sderling E, Ollmar S. The ability of betaine to reduce the irritating effects of detergents assessed visually, histologically and by bioengineering methods. *Skin Res Technol* 2003; 9: 50–58.
19. Aioi A, Tonogaito H, Suto H et al. Impairment of skin barrier function in NC/Nga Tnd mice as a possible model for atopic dermatitis. *Br J Dermatol* 2001; 144: 12–18.
20. Hartley RI. In defense of the 8-point algorithm. *Proceedings of the 5th international conference on computer vision*. IEEE Computer Society Press, MA, USA, 1995: 1064–1070.
21. Lee DI, Kim HK, Lee MW et al. Enhancement of NK cytotoxicity and antitumor effect on melanoma by pedunculagin. *Yakhak Hoeji* 2000; 44: 169–174.
22. Lee DI, Kim HK, Lee MW et al. Augmentation of macrophage cytotoxicity and NO production by pedunculagin. *Yakhak Hoeji* 2000; 44: 175–181.
23. Joo SS, Kwon HS, Kang HC et al. Effect of pedunculagin on IL-1 $\beta$  mRNA expression in Langerhans cells. *Yakhak Hoeji* 2002; 46: 472–476.
24. Joo SS, Oh WS, Park JH et al. Effect of pedunculagin in production of TNF- $\alpha$  of Langerhans cells. *Yakhak Hoeji* 2002; 46: 477–481.
25. Gao X, Nakamura N, Fuseda K, Tanaka H, Inagaki N, Nagai H. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. *Biol Pharm Bull* 2004; 27: 1376–1381.
26. Sasakawa T, Higashi Y, Sakuma S et al. Atopic dermatitis-like skin lesions induced by topical application of mite antigens in NC/Nga mice. *Int Arch Allergy Immunol* 2001; 126: 239–247.
27. Shiohara T, Hayakawa J, Mizukawa Y. Animal models for atopic dermatitis: are they relevant to human disease? *J Dermatol Sci* 2004; 36: 1–9.
28. Lee SH, Baek SJ, Kim HA et al. 2,4-Dinitrochlorobenzene-induced atopic dermatitis like immune alteration in mice. *J Toxicol Pub Health* 2006; 22: 357–364.
29. Blichmann CW, Serup J. Assessment of skin moisture. Measurement of electrical conductance, capacitance and transepidermal water loss. *Acta Derm Venereol* 1988; 68: 284–290.
30. Mridha M, Odman S, Oberg PA. Mechanical pulse wave propagation in gel, normal and oedematous tissues. *J Biomech* 1992; 25: 1213–1218.

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