



Therapeutic effects of *Artemisia scoparia* Waldst. et Kitaib in a murine model of atopic dermatitis

K. J. Ryu,¹ M. S. Yoou,¹ Y. Seo,² K. W. Yoon,³ H. M. Kim¹ and H. J. Jeong⁴

¹Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul, Korea; ²Division of Marine Environment and Bioscience, Korea Maritime University, Busan, Korea; and ³Departments of Biotechnology and ⁴Food Science and Technology, Hoseo University, Asan, Chungnam, Korea

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Summary

Background. *Artemisia scoparia* Waldst. et Kitaib (AS) (Oriental wormwood, known as Bissuk in Korea) is a plant used in cosmetic and pharmaceutical treatments. However, the effect of AS on atopic dermatitis (AD) has not been described.

Aim. To examine the inhibitory effect of AS on AD using a murine model.

Methods. We applied either AS, the butanol-extracted fraction of AS (Bu-OH) or 3,5-dicaffeoyl-epi-quinic acid (DEQA, a major component of Bu-OH) topically for 3 weeks to 2,4-dinitrofluorobenzene (DNFB)-induced skin lesions in BALB/c mice.

Results. AS, Bu-OH and DEQA suppressed the clinical symptoms of DNFB-induced skin lesions and the associated scratching behaviour. Numbers of inflammatory cells infiltrating skin lesions were significantly reduced by AS or Bu-OH application but not by DEQA. In addition, AS significantly suppressed serum levels of histamine and IgE, while Bu-OH significantly suppressed serum levels of histamine, IgE, thymic stromal lymphopoietin (TSLP), interleukin (IL)-4 and IL-6, and DEQA significantly suppressed serum levels of histamine, IgE, TSLP and IL-4 in DNFB-induced AD mice. In skin lesions, AS and Bu-OH significantly reduced inflammatory cytokines, whereas DEQA did not. AS, Bu-OH and DEQA all significantly suppressed caspase-1 activities.

Conclusions. These results demonstrate the anti-AD effects of AS, Bu-OH and DEQA, and suggest that all three have therapeutic potential.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder related to cutaneous hyper-reactivity to environmental triggers.¹ T cells, B cells, dendritic cells, natural killer cells and monocytes/macrophages influenced by mast cells promote inflammation, tissue injury and tissue remodelling during allergic reactions.² Mast cells play a critical role in both innate

and acquired immunity, and are increased in immunological skin diseases, including AD and contact dermatitis.³ Mast cells produce potential inflammatory mediators such as thymic stromal lymphopoietin (TSLP), histamine, cytokines, chemotactic factors and proteases.⁴ During the pathogenesis of AD, TSLP is an initiator of inflammatory cascades, and its expression is markedly increased in the skin lesions of patients with acute or chronic AD.^{5,6} TSLP is expressed in activated mast cells and generates allergic reactions,⁷ and is produced by the receptor interacting protein-2/caspase-1 signal pathway in mast cells.⁸ Caspase-1 is a principal inflammatory caspase, and plays an important role in several inflammatory diseases.^{8,9} It is activated by proinflammatory stimuli, and when activated, it increases the production of various proinflammatory cytokines, such as interleukin (IL)-1 β ,

Correspondence: Dr Hyun-Ja Jeong, Department of Food Science and Technology, Hoseo University, Asan, Chungnam, 31499, Korea
E-mail: hjeong@hoseo.edu

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KJR and MSY contributed equally to this work and should be considered joint first authors.

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IL-6, tumour necrosis factor (TNF)- α and TSLP in mast cells.⁸ Therefore, TSLP and caspase-1 are viewed as target molecules for the treatment of AD.^{5,8}

Artemisia scoparia Waldst. et Kitaib (AS) (Oriental wormwood, known as Bissuk in Korea) is a plant belonging to the Compositae family, and is distributed widely in Eurasia. AS possesses anti-inflammatory, antipyretic and antinociceptive activities,^{10,11} and its leaves and stems are used in the cosmetic and pharmaceutical industries.¹² Recently, we screened various AS fractions (butanol, methanol, hexane, ethyl acetate and water) *in vitro* for anti-allergic inflammatory effects, and found that this was greatest in the butanol fraction of AS (Bu-OH). However, the effects of AS, Bu-OH and 3,5-dicaffeoyl-epi-quinic acid (DEQA; a major component of Bu-OH) in an animal model of AD has not been investigated. Therefore, we examined the effects of these three agents in a 2,4-dinitrofluorobenzene (DNFB)-induced murine model of AD, which displays AD-like skin symptoms similar to those observed in human patients with AD.¹³

Methods

All animal protocols were approved by the institutional animal care committee of Kyung Hee University, and carried out in accordance with standard laboratory animal care.

Animals

Eight-week old female BALB/c mice were obtained from the Dae-Han Experimental Animal Center (Eum-sung, Chungbuk, Korea) and maintained under standard conditions.

Sensitization with 2,4-dinitrofluorobenzene

For active sensitization, 100 μ L of 0.15% DNFB (Sigma Chemical Co; St. Louis, MO, USA) dissolved in acetone was topically challenged to the shaved abdominal skins of BALB/c mice, which were divided into seven groups,

with an eighth DNFB-unchallenged group ($n = 5$) acting as the negative vehicle control). A week later, the shaved dorsal skins of the experimental BALB/c mice were challenged with 50 μ L 0.15% DNFB. At that time, the mice were then treated with AS 20 mg/site, Bu-OH 20 mg/site or DEQA (2, 20 or 200 μ g/site; all generously provided by Professor Seo, Korea Maritime University). These compounds had been prepared by dissolving in distilled water, *n*-butanol or methanol, respectively, and then diluted with distilled water, and were topically applied to the skin as a cream formulation. In addition, the same volume of acetone was applied to the shaved dorsal skin as a vehicle group, while dexamethasone (DEXA; Sigma Chemical Co.) 5 mg/kg was orally administered to DNFB-challenged mice as a positive control (Fig. S1). Scratching behaviour was measured as the duration of scratching that occurred during a 10-min period 4 h after the final DNFB challenge. After the scratching test, the animals were euthanized by withdrawing blood from the heart. Serum was prepared by centrifugation at 400 **g** for 5 min at 4 °C. Clinical symptoms (erythema and haemorrhage) were observed twice a week for 4 weeks.

Histological analysis

Skin samples were fixed in formaldehyde, then embedded in paraffin wax and sectioned at 4 μ m. After dewaxing and dehydration, sections were stained with alcian blue and safranin O (A&S) (for mast cells) and haematoxylin and eosin (H&E) (for eosinophils), and numbers of inflammatory cells were counted at various sites in the dorsal skin lesions.

Histamine assay

Serum histamine is a known trigger factor of AD. Serum histamine contents were measured using a histamine assay kit (Oxford Biomedical Research, Oxford, MI, USA). Fluorescence intensities were measured at 440 nm (excitation at 360 nm) using a

Table 1 Primers used for reverse transcription PCR.

Name	Sequence 5'→3'	
TSLP	TGCAAGTACTAGTACGGATGGGGC	GGACTTCTGTGCCATTTCCTGAG
IL-4	ACGGAGATGGATGTGCCAAA	CGAGTAATCCATTTCATGA
IL-6	CGGGATCCATGTTCCCTACTTCACAA	CCCAAGCTTCTACGTTTGCC
TNF- α	TACAGGCTTGTCACCTCGAAT	ATGAGCACAGAAAGCATGAT
GAPDH	GGCATGGACTGTGGTCATGA	TTCAACCACCATGGAGAAGGC

GADPH, glyceraldehyde-3-phosphate; IL, interleukin; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.

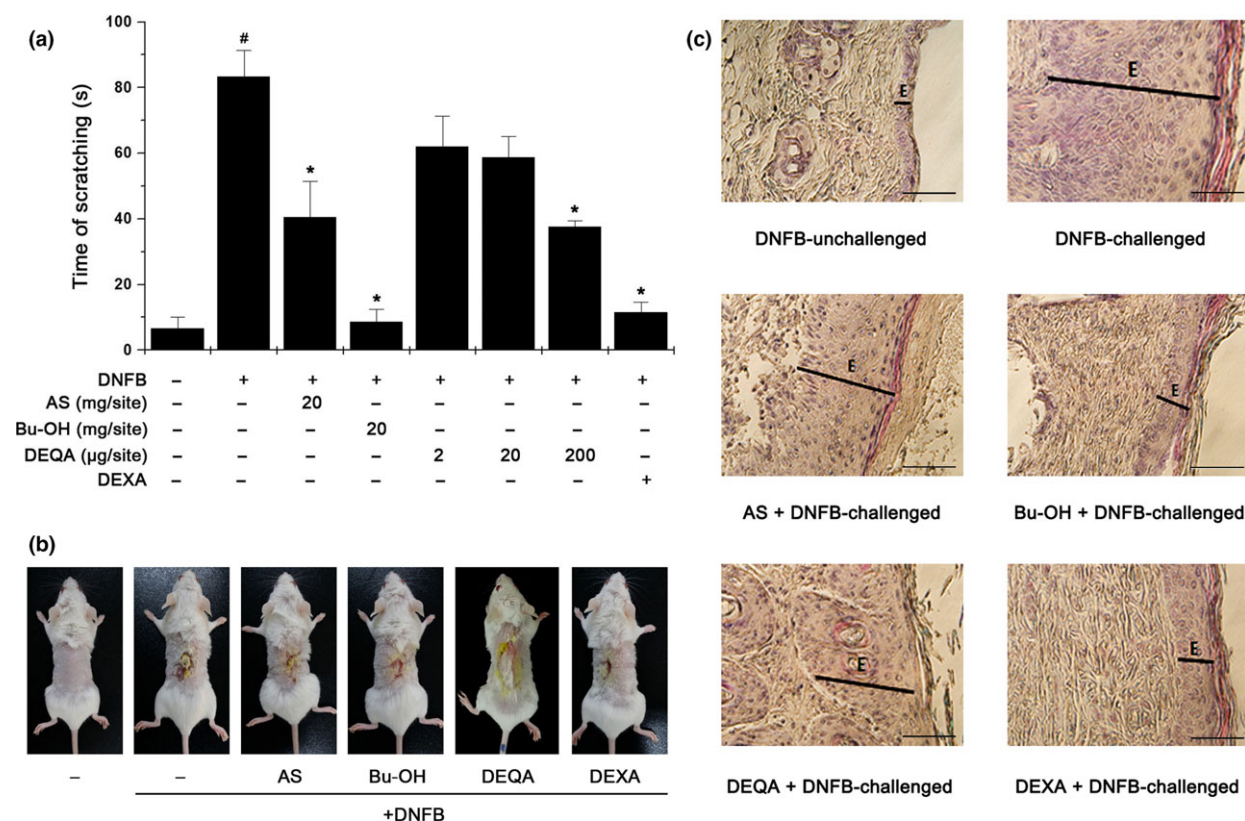


Figure 1 (a–c) Clinical and histological improvement of AD in a DNFB-challenged mouse model: AS 20 mg/site, Bu-OH 20 mg/site or DEQA 2, 20 or 200 μg/site were topically applied, whereas DEXA (5 mg/kg) was administered orally, and clinical features were observed 4 h after the final DNFB challenge. (a) Scratching test results and (b) AD-like skin lesions in BALB/c mice. (c) Histological analysis of skin lesions to assess epidermal thickness [haematoxylin and eosin, original magnification $\times 400$ (scale bar = 5 μm)]. Each data point represents mean $\pm$ SEM. [#] $P < 0.05$; significantly different from normal mice (independent *t*-test), ^{*} $P < 0.05$; significantly different from DNFB-treated mice (ANOVA with Tukey *post hoc* test), $n = 5$ /group. AD, atopic dermatitis; AS, *Artemisia scoparia* Waldst. et Kitaib; Bu-OH, the butanol fraction of AS; DEQA, 3,5-dicaffeoyl-epi-quinic acid; DEXA, dexamethasone; DNFB, 2,4-dinitrofluorobenzene; E, epidermis.

spectrofluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Cytokine assay

IgE and inflammatory cytokines (TSLP, IL-4 and IL-6) are known trigger factors of AD, thus their levels were measured by sandwich ELISA, according to the relevant manufacturer's instructions (TSLP antibody from R&D Systems, Minneapolis, MN, USA; IgE, IL-4 and IL-6 antibodies from BD Pharmingen, Torreyana Road, San Diego, CA, USA).

Reverse transcription-PCR analysis

Total RNA was isolated from lesioned skin samples (easy-BLUE™ RNA Extraction Kit; iNtRON Biotech, Korea). RT-PCR was performed using the primers shown in Table 1, and GAPDH was used as a reference

gene to confirm equal loadings. Annealing temperatures were 62 °C for TSLP and IL-4, 50 °C for IL-6, and 60 °C for TNF- α and GAPDH. Products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining. mRNA levels were normalized to GAPDH.

Caspase-1 assay

Caspase-1 plays an essential role in cytokine expression and production,⁸ and thus we investigated whether AS, Bu-OH, or DEQA could regulate caspase-1 activity in skin lesions. Caspase-1 activities were determined using a caspase-1 assay kit (R & D Systems).

Statistical analysis

Results were expressed as means $\pm$ SEM. Analyses were conducted using the independent *t*-test and ANOVA

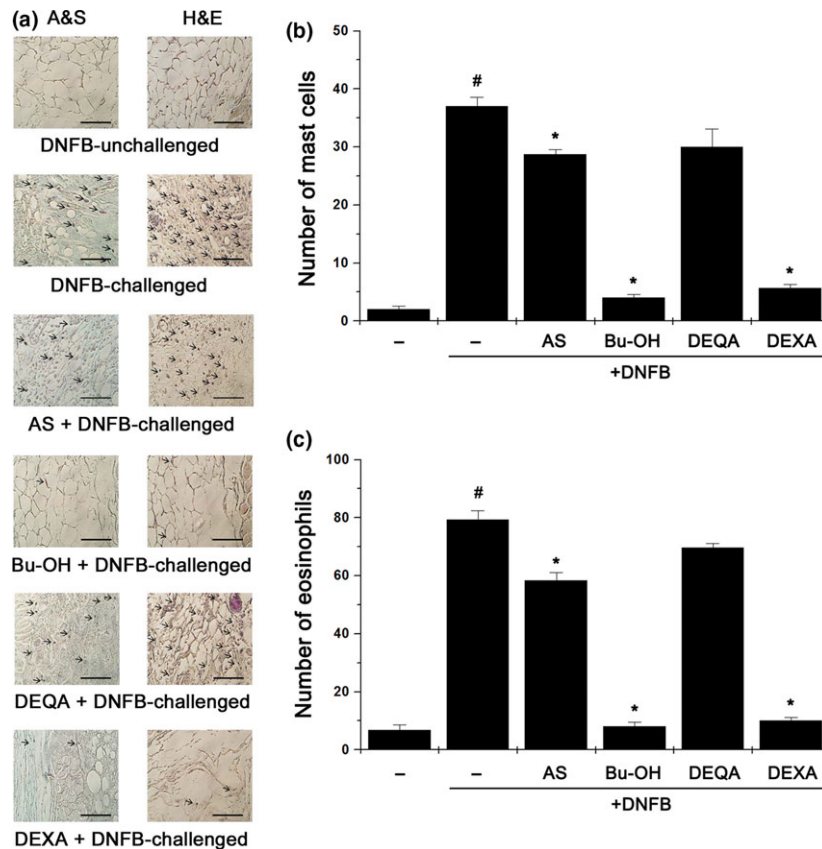


Figure 2 (a,b) Suppression of inflammatory cell infiltration in a DNFB-induced AD mouse model; AS 20 mg/site, Bu-OH 20 mg/site or DEQA 2, 20 or 200 µg/site were topically applied, whereas DEXA (5 mg/kg) was administered orally. (a) Histological analysis of skin lesions was conducted [A&S (mast cell stain; left) H&E (eosinophil stain; right); original magnification $\times 400$ (scale bar = 5 µm)]. (b,c) Numbers of (b) mast cells and (c) eosinophils were counted. Inflammatory cells are arrowed. Each data point represents mean $\pm$ SEM. [#] $P < 0.05$; significantly different from normal mice (independent *t*-test), ^{*} $P < 0.05$; significantly different from DNFB-treated mice (ANOVA with Tukey *post hoc* test), $n = 5$ /group. A&S; alcian blue and safranin O; AD, atopic dermatitis; AS, *Artemisia scoparia* Waldst. et Kitaib; Bu-OH, the butanol fraction of AS; DEQA, 3,5-dicaffeoyl-epi-quinic acid; DEXA, dexamethasone; DNFB, 2,4-dinitrofluorobenzene; H&E, haematoxylin and eosin.

with Tukey *post hoc* test in SPSS software (v22; IBM SPSS, Armonk, NY, USA). Statistical significance was accepted for $P < 0.05$.

Results

Topical application of AS, Bu-OH, or DEQA improved clinical symptoms in a DNFB-induced mouse model of atopic dermatitis

AS, Bu-OH, DEQA and DEXA all significantly suppressed scratching behaviour and improved clinical symptoms in DNFB-challenged BALB/c mice (Fig. 1a, b). DEQA 200 µg/site significantly ($P < 0.05$) suppressed scratching behaviour (Fig. 1a). Noticeable erythema, haemorrhage, dryness and erosion were

present in skin lesions of DNFB-induced AD mice, and topical application of AS, Bu-OH, or DEQA markedly improved these phenotypes (Fig. 1b). Epidermal thickness was reduced more in the skin lesions of mice treated with AS, Bu-OH or DEQA than in those treated with DNFB (Fig. 1c). Oral administration of DEXA significantly improved AD-like symptoms and reduced skin lesion thickness (Fig. 1b,c). In DNFB-unchallenged mice, neither AS, Bu-OH, DEQA nor DEXA produced AD symptoms or phenotypes (erythema, haemorrhage, dryness and erosion, data not shown). In addition, mast cell and eosinophil infiltration induced by DNFB were significantly ($P < 0.05$) suppressed by AS, Bu-OH and DEXA (Fig. 2). DEQA non-significantly reduced numbers of infiltrating inflammatory cells (Fig. 2).

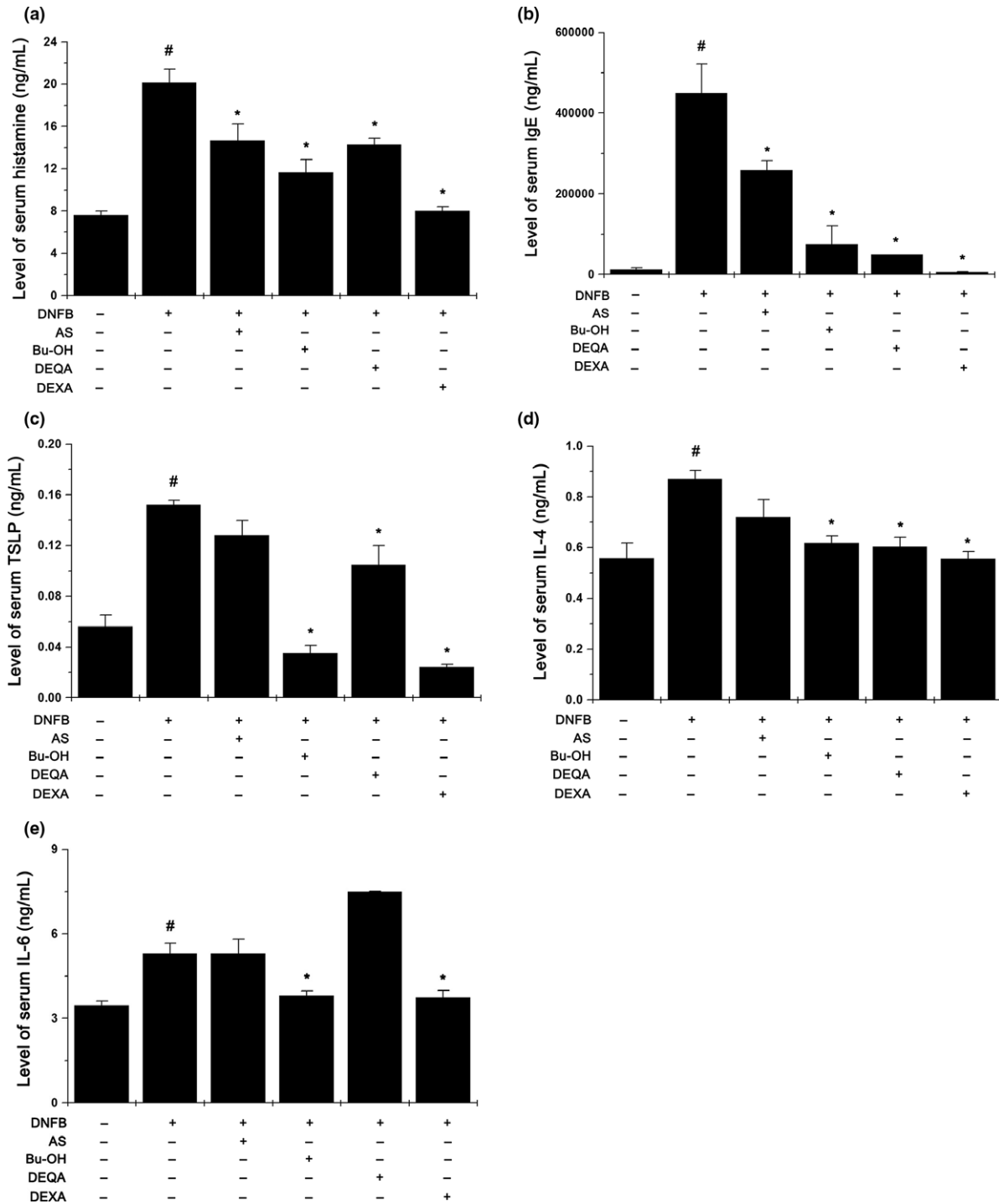


Figure 3 (a–e) Reduction in serum levels of histamine, IgE and inflammatory cytokines in a DNFB-induced AD mouse model; AS 20 mg/site, Bu-OH 20 mg/site or DEQA 2, 20 or 200 µg/site were topically applied, whereas DEXA (5 mg/kg) was administered orally. Serum levels of (a) histamine, (b) IgE, (c) TSLP, (d) IL-4 and (e) IL-6 were measured. Each data point represents mean ± SEM. [#] $P < 0.05$; significantly different from normal mice (independent t -test), $^*P < 0.05$; significantly different from DNFB-treated mice (ANOVA with Tukey *post hoc* test), $n = 5$ /group. AD, atopic dermatitis; AS, *Artemisia scoparia* Waldst. et Kitaib; Bu-OH, the butanol fraction of AS; DEQA, 3,5-dicaffeoyl-epi-quinic acid; DEXA, dexamethasone; DNFB, 2,4-dinitrofluorobenzene; IL, interleukin; TSLP, thymic stromal lymphopoietin.

Topical application of AS, Bu-OH, or DEQA suppressed serum levels of histamine, IgE and inflammatory cytokines in a DNFB-induced mouse model of atopic dermatitis

AS significantly ($P < 0.05$) suppressed serum histamine and IgE levels (Fig. 3b), while Bu-OH or DEXA significantly ($P < 0.05$) suppressed serum histamine, IgE, TSLP, IL-4 and IL-6 levels (Fig. 3), and DEQA significantly ($P < 0.05$) suppressed serum histamine, IgE, TSLP and IL-4 levels (Fig. 3).

Topical application of AS, Bu-OH, or DEQA suppressed the mRNA levels of inflammatory cytokines in the skin lesions of a DNFB-induced mouse model of atopic dermatitis

Because topical applications of AS, Bu-OH and DEQA improved clinical symptoms, we studied the inhibitory effect of AS on the mRNA levels of inflammatory cytokines. The mRNA levels of TNF- α in skin lesions were significantly ($P < 0.05$) suppressed by topical AS application, while topical Bu-OH or oral DEXA significantly ($P < 0.05$) suppressed mRNA levels of TSLP, IL-4, IL-6 and TNF- α , and topical DEQA significantly ($P < 0.05$) suppressed mRNA levels of IL-6 and TNF- α (Fig 4a,b).

Topical application of AS, Bu-OH, or DEQA suppressed the activation of caspase-1 in skin lesions of a DNFB-induced mouse model of atopic dermatitis

AS, Bu-OH, DEQA and DEXA all significantly suppressed caspase-1 activities in skin lesions (Fig. 4c) ($P < 0.05$).

Discussion

AD is one of the most common and severe skin disorders and is characterized by eczematous skin lesion and high IgE levels.^{14,15} A wide array of drugs has been developed, but the prevalence of AD has considerably increased worldwide in recent years because of many factors known to worsen the disease, including climatic factors and chemical or physical irritants.^{5,15} Release of inflammatory cytokines in skin is an important component of the progress of AD. IgE synthesis triggered by allergens can induce release of IL-4, IL-5, IL-6 and IL-13 by basophils, eosinophils and mast cells.¹⁶ Recently, TSLP was found to act as a crucial switch in allergic reactions, based on its ability to differentiate Th2 cells and mast cells.^{5,17} TSLP-induced inflammation is T cell-

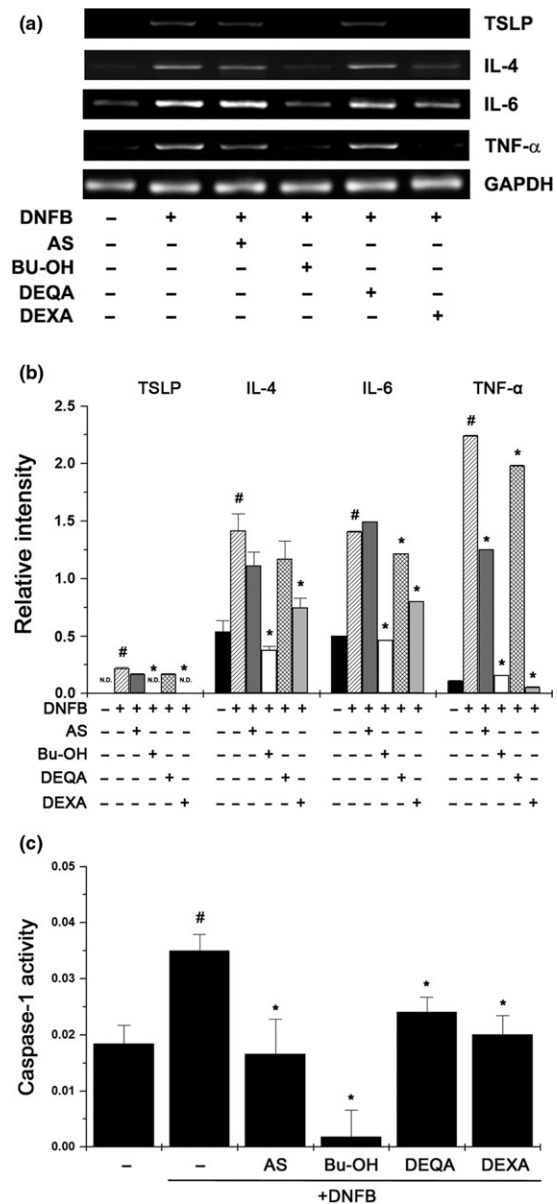


Figure 4 Reduction in mRNA levels in a DNFB-induced AD mouse model; AS 20 mg/site, Bu-OH 20 mg/site or DEQA 2, 20 or 200 μ g/site were topically applied, whereas DEXA (5 mg/kg) was administered orally. (a) mRNA expression of TSLP, IL-4, IL-6, TNF- α and GAPDH in skin lesions assessed by RT-PCR. (b) Relative intensities were quantified by densitometry means TSLP/GAPDH, IL-4/GAPDH, IL-6/GAPDH and TNF- α /GAPDH. (c) Skin lesions were prepared and analysed for caspase-1 as described in the Methods. # $P < 0.05$; significantly different from normal mice (independent t -test), * $P < 0.05$; significantly different from DNFB-treated mice (ANOVA with Tukey *post hoc* test), $n = 5$ /group. AS, *Artemisia scoparia* Waldst. et Kitaib; Bu-OH, the butanol fraction of AS; DEQA, 3,5-dicaffeoyl-epi-quinic acid; DEXA, dexamethasone; DNFB, 2,4-dinitrofluorobenzene; GAPDH, glyceraldehyde-3-phosphate; IL, interleukin; TSLP, thymic stromal lymphopoietin.

dependent but only partially dependent on IL-4.¹⁸ TSLP-deficient mice have significant reductions in the populations and maturation of mast cells.⁵ Previous studies have shown that levels of TSLP, IL-4, IL-6 and TNF- α in AD skin lesions are markedly elevated.^{19–21} In the present study, we observed that scratching duration, clinical symptoms and numbers of infiltrating mast cells and eosinophils were significantly suppressed by topically administered AS, Bu-OH or DEQA. In addition, these topical applications also significantly reduced serum levels of histamine, IgE, TSLP, IL-4 and IL-6 in AD-like mice, while topical Bu-OH significantly suppressed the mRNA levels of TSLP, IL-4, IL-6 and TNF- α in skin lesions.

Therefore, we suggest that the anti-AD effects of AS, Bu-OH and DEQA are due to their regulation of AD biomarkers. We found that whereas AS or DEQA (a major component of Bu-OH) partially inhibited biomarkers of AD, Bu-OH almost totally inhibited the levels of these biomarkers. These results suggest that Bu-OH contains active compounds in addition to DEQA, or that the dosage of DEQA administered was too low. Further investigation is required to resolve this situation.

Caspase-1 is a crucial regulator of inflammatory reactions, and its activation induces production of proinflammatory cytokines, which in turn, cause inflammation.^{22,23} In a previous study, transgenic mice overexpressing caspase-1 spontaneously developed chronic dermatitis and exhibited significant increase in serum levels of histamine and IgE.²³ In a previous study, we found that activation of caspase-1 significantly enhanced TSLP secretion by mast cells, and that pretreatment with a caspase-1 inhibitor markedly reduced this secretion.⁸ The present study showed that caspase-1 activities in AD-like skin lesions were significantly suppressed by topical AS, Bu-OH or DEQA. Therefore, we suggest that the inhibitory effects of AS, Bu-OH and DEQA on AD might be induced through the regulation of caspase-1 signalling pathways.

Conclusion

Natural substances tend to have fewer adverse effects and also effectively ameliorate the clinical symptoms of AD. In the present study, we investigated the anti-AD effects of AS, Bu-OH and DEQA on DNFB-induced AD mice. Collectively, our results suggest that AS, Bu-OH and DEQA ameliorate AD-like skin lesions and their symptoms in mice, and that these three agents offer a potential novel means of treating AD.

What's already known about this topic?

- AS possesses anti-inflammatory, antipyretic and anti-nociceptive activities.
- AS is used in the cosmetic and pharmaceutical industries.
- However, the effect of AS on AD has not been described.

What does this study add?

- We investigated the anti-AD effects of AS on DNFB-induced AD mice.
- We found that use of AS in an AD mouse model suppressed the clinical symptoms of DNFB-induced skin lesions and the associated scratching behaviour.
- AS also produced reductions in numbers of inflammatory cells, serum levels of histamine, IgE and inflammatory cytokines, and activities of caspase-1.
- AS offers a potential novel means of treating AD.

References

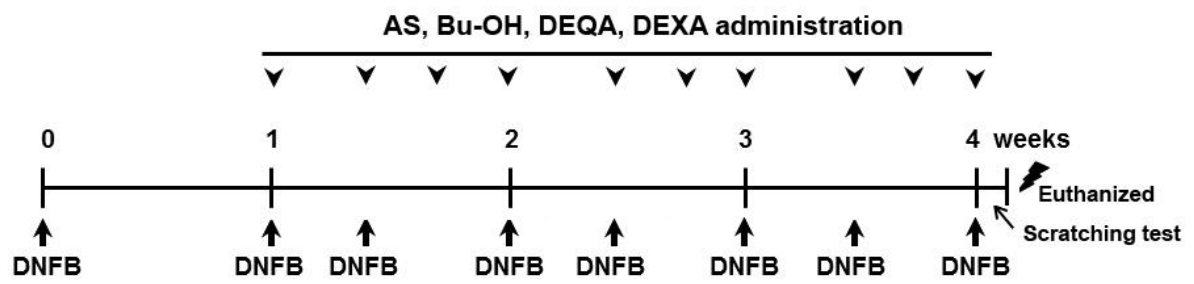
- 1 Bonness S, Bieber T. Molecular basis of atopic dermatitis. *Cur Opin Allergy Clin Immunol* 2007; **7**: 382–6.
- 2 Galli SJ, Grimaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat Rev Immunol* 2008; **8**: 478–86.
- 3 Navi D, Saegusa J, Liu FT. Mast cells and immunological skin diseases. *Clin Rev Allergy Immunol* 2007; **33**: 144–55.
- 4 Amin K. The role of mast cells in allergic inflammation. *Respir Med* 2012; **106**: 9–14.
- 5 Han NR, Oh HA, Nam SY *et al*. TSLP induces mast cell development and aggravates allergic reactions through the activation of MDM2 and STAT6. *J Invest Dermatol* 2014; **134**: 2521–30.
- 6 Zhu Z, Oh MH, Yu J *et al*. The role of TSLP in IL-13-induced atopic march. *Sci Rep* 2011; **1**: 23.
- 7 Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 2006; **203**: 269–73.
- 8 Moon PD, Kim HM. Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF- κ B pathway in mast cells. *Cytokine* 2011; **54**: 239–43.

- 9 Song YH, Nam SY, Choi Y *et al.* Socioeconomic impact of traditional Korean medicine, Pyeongwee-San (KMP6) as an anti-allergic inflammatory drug. *TANG* 2012; **2**: e29.
- 10 Habib M, Waheed I. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of *Artemisia scoparia* hydromethanolic extract. *J Ethnopharmacol* 2013; **145**: 18–24.
- 11 Yahagi T, Yakura N, Matsuzaki K, Kitanaka S. Inhibitory effect of chemical constituents from *Artemisia scoparia* Waldst. et Kit. on triglyceride accumulation in 3T3-L1 cells and nitric oxide production in RAW 264.7 cells. *J Nat Med* 2014; **68**: 414–20.
- 12 Jamwal KS, Sharma ML, Chandhoke N, Ghatak BJ. Pharmacological action of 6,7-dimethoxy coumarin (Scoparone) isolated from *Artemisia scoparia*, Waldst & Kit. *Indian J Med Res* 1972; **60**: 763–71.
- 13 Schuepbach-Mallepell S, Philippe V, Brügger MC *et al.* Antagonistic effect of the inflammasome on thymic stromal lymphopoietin expression in the skin. *J Allergy Clin Immunol* 2013; **132**: 1348–57.
- 14 Kang JS, Lee K, Han SB *et al.* Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of *Dermatophagoides pteronyssinus* in NC/Nga mice. *Int Immunopharmacol* 2006; **6**: 1616–22.
- 15 Wong CK, Ho CY, Ko FW *et al.* Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN- γ , IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin Exp Immunol* 2001; **125**: 177–83.
- 16 Hulse KE, Reefer AJ, Engelhard VH *et al.* Targeting allergen to Fc γ RI reveals a novel T(H)2 regulatory pathway linked to thymic stromal lymphopoietin receptor. *J Allergy Clin Immunol* 2010; **125**: 247–56.
- 17 Jessup HK, Brewer AW, Omori M *et al.* Intradermal administration of thymic stromal lymphopoietin induces a T cell and eosinophil-dependent systemic Th2 inflammatory response. *J Immunol* 2008; **181**: 4311–19.
- 18 Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF- α and their degranulation is associated with expression of ICAM-1 in the epidermis. *Arch Dermatol Res* 1998; **290**: 353–9.
- 19 Fedenko ES, Elisyutina OG, Filimonova TM *et al.* Cytokine gene expression in the skin and peripheral blood of atopic dermatitis patients and healthy individuals. *Self Nonself* 2011; **2**: 120–4.
- 20 Wang G, Savinko T, Wolff H *et al.* Repeated epicutaneous exposures to ovalbumin progressively induce atopic dermatitis-like skin lesions in mice. *Clin Exp Allergy* 2007; **37**: 151–61.
- 21 Furlan R, Martino G, Galbiati F *et al.* Caspase-1 regulates the inflammatory process leading to autoimmune demyelination. *J Immunol* 1999; **163**: 2403–9.
- 22 Han NR, Moon PD, Kim NR *et al.* Schisandra chinensis and its main constituent schizandrin attenuate allergic reactions by down-regulating caspase-1 in ovalbumin-sensitized mice. *Am J Chin Med* 2017; **45**: 159–72.
- 23 Murakami T, Yamanaka K, Tokime K *et al.* Topical suplastat tosilate (IPD) ameliorates Th2 cytokine-mediated dermatitis in caspase-1 transgenic mice by downregulating interleukin-4 and interleukin-5. *Br J Dermatol* 2006; **155**: 27–32.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The schedule diagram for DNFB-induced AD animal model.



Supplementary Fig. 1. The schedule diagram for DNFB-induced AD animal model.