

Inhibitory effects of *Solanum tuberosum* L. var. vitelotte extract on 2,4-dinitrochlorobenzene-induced atopic dermatitis in mice

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Keywords

atopic dermatitis; IgE; NC/Nga mice; *Solanum tuberosum* L. var. Vitelotte; Th2/Th1 ratio

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Abstract

Objectives We aimed to investigate the inhibitory efficacy of *Solanum tuberosum* L. var. Vitelotte (SV) extract on atopic dermatitis (AD)-like skin lesions induced by the topical application of 2,4-dinitrochlorobenzene in NC/Nga mice.

Methods SV extract was administered orally to NC/Nga mice at the dose of 75, 150 or 300 mg/kg for 4 weeks. The effectiveness of SV extract in NC/Nga mice was evaluated by measuring symptom severity, ear thickness, scratching behaviour, serum levels of IgE, IgG1 and IgG2a, T helper 1 (Th1; interferon- γ and IL-12) and Th2 cytokines (IL-4 and IL-13) in spleen, messenger RNA (mRNA) expression of inflammatory cytokines and chemokines in tissue and infiltration of inflammatory cells in tissue.

Key findings Oral administration of SV extract to NC/Nga mice resulted in the inhibition of the development of AD-like skin lesions. SV extract was attenuated AD-like skin lesion, ear thickening and scratching behaviour. SV extract also alleviated infiltrated inflammatory cells in tissue. Production of Th1 and Th2 cytokines was inhibited in splenocyte cultures. Additionally, reduced levels of IgE and IgG1/IgG2a ratio in serum and expression of AD-related mRNAs in lesional skins were observed in SV-treated mice compared with control group.

Conclusions SV extract alleviated the exacerbation of AD-like skin lesions in NC/Nga mice by suppressing total serum level of IgE and correcting the Th1/Th2 balance.

Introduction

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease, provoked by Th1/Th2 immune responses. AD was characterized by pruritic and eczematous skin lesions, along with elevated IgE levels relying on the cutaneous hyper-reactivity to environmental triggers.^[1] IgE activates mast cells to release histamine by binding to high-affinity IgE receptors (Fc ϵ RI) in the mast cell membrane.^[2] The incidence of AD is increasingly the common pruritic inflammatory skin disorder that affects approximately 20% of children and 1–3% of adults. AD is a biphasic inflammatory skin disease that can be considered to have two distinct phases. In the acute phase, Th2 cells secrete IL-4 and IL-13, which are known to induce isotype switching to IgE synthesis, whereas in the chronic phase, Th1 cells secrete interferon- γ (IFN- γ).^[3–5] Also, AD is a chronic inflammatory skin disease that is closely related to dysregulation of the

Th1/Th2 balance.^[6] In the recent research, it has been shown that Th17 subset also plays an important role in the pathogenesis of AD by secreting pro-inflammatory cytokines, such as IL-17A and IL-22.^[6–8]

NC/Nga mice, an inbred mouse strain, were the first mouse model of AD reported by Matsuda *et al.*^[9] NC/Nga mice remain normal when kept under specific pathogen-free (SPF) conditions, but spontaneously develop AD-like eczematous skin lesions when placed in conventional surroundings.^[1] However, when these mice are maintained under SPF conditions, chemical hapten (2,4-dinitrochlorobenzene (DNCB) or 2,4-dinitrofluorobenzene) application produces 100% reproducible AD-like lesions in NC/Nga mice.^[10,11] In AD skin, frequent characteristics include erythema, dryness, erosion, scratching behaviour, excessive infiltration of inflammatory cells such as lymphocytes,

eosinophils, neutrophils, macrophages and granulated mast cells into the skin lesions, eosinophilia in peripheral blood and a high level of serum IgE.^[12] Topical steroids have been used as the therapy for AD to reduce AD symptoms mentioned above. However, it cannot be used for long periods because of adverse side effects.^[4] Therefore, a variety of natural products without side effects are needed.

Solanum tuberosum L. var. Vitelotte (SV) is a dark purple-fleshed potato. SV has an extensive amount of polyphenols. SV is enriched with anthocyanins. Anthocyanins that are phenolic compounds cause the intense colour of many fruits and vegetables such as red grapes, berries, red cabbages and purple potatoes.^[13] The pigment in SV contains various anthocyanin derivatives.^[14] Previous researches have reported that anthocyanins have remarkable antioxidant, anti-inflammatory activity and anticancer activity.^[13,15] Although SV has been reported that it has lots of activity in various assays, the effects of SV as an anti-atopic agent for AD remain unknown. In this study, we examined the effects of SV on the cure of AD *in vivo*.

Materials and Methods

Preparation of SV extract

SV was purchased from Hamyang-gun Agricultural Development and Technology Center. *S. tuberosum* L. var. Vitelotte. (Solanaceae) is authenticated by Prof. Young Pyo Jang, Department of Pharmacology (Kyung Hee University, Seoul, Korea). The SV was washed with tap water and cut in slice. Then, these slices were soaked fivefold in a solution of 80% aqueous edible ethanol in a lightproof container and were incubated overnight at room temperature. These procedures were repeated once more with the addition new extraction solvent. After filtration, the extract was evaporated under vacuum and then freeze dried. The extract powder was stored in -70°C deep freezer until use.

Determination of the total polyphenols contents

The contents of total polyphenols in the extract of SV was determined using the Folin–Denis colorimetric method.^[16] Tannin acid was used as the standard, and the results are expressed as milligrams of tannin acid equivalent per gram of extract. The SV extract had the contents of total polyphenols (26.7 ± 0.7 mg/g).

Sample preparation for HPLC

Thirty milligrams of lyophilized powder was dissolved at room temperature with 1 ml of 50% (v/v) methanol adjusted to pH 2.0 by formic acid. The solution was filtered through a $0.45\ \mu\text{m}$ syringe filter (Millipore, Bedford, MA, USA) before being injected to HPLC.

HPLC-electrospray ionization-tandem mass spectrometry analysis

HPLC instrument was composed of Agilent 1200 series (Agilent Technologies, Santa Clara, CA, USA) and UV detector (Agilent Technologies). UV detector was set at 280 nm running Chemstation software (Waters, Milford, MA, USA). The UV spectra (UVD340U DAD detector; Dionex, San Diego, CA, USA) were recorded between 210 and 600 nm. The Atlantis T3 C18 column (150×2.1 mm, $3\ \mu\text{m}$) was selected for the HPLC study (Waters). The mobile phase was comprised of acidified acetonitrile with formic acid (0.1%, solvent A) and acidified water with formic acid (0.1%, solvent B). Flow rate was 0.2 ml/min and the injection volume was 4 μl .

The gradient program of SV was 0–20 min, 8% of solvent A; 20–25 min 12% of solvent A; 25–90 min, 18% of solvent A; 90–100 min 100% of solvent A; and 100–120 min 100% of solvent A.

Quadrupole time-of-flight mass spectrometry (MS) was performed on a Quattro micro API instrument with electrospray ionization (ESI) ion source (Waters). MS and tandem mass spectrometry (MS/MS) spectra obtained in the positive ion mode were more informative than those obtained from negative ions. Therefore, acquisition was performed in the positive ion modes. The parameters were as follows: capillary voltage at 3.5 kV, cone voltage at 35 eV and collision energy at 20 eV; argon gas was used as a collision source, ion source temperature at 120°C , cone gas N_2 100 l/h and desolvation gas N_2 850 l/h. The instrument was set to scan from 50 to 1500 Da. The MassLynx software version 4.1 (Waters) was used to control MS instruments.^[17]

Animals

Four-week-old male NC/Nga mice were purchased from SLC (Shizuoka, Japan) and were housed in individually ventilated cages at $23 \pm 3^{\circ}\text{C}$ in $55 \pm 5\%$ humidity with 12 h/12 h light/dark cycle under SPF conditions in experimental period. Animals were maintained under constant environmental conditions and fed a standard laboratory diet (Central Lab Animal, Seoul, Korea) with water *ad libitum*.

All of the experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees (approval no. KHP-2012-07-5).

Induction of AD-like skin lesions in NC/Nga mice and oral administration of SV extract

AD-like symptoms were induced in AD control, positive control and treatment groups of NC/Nga mice by the topical application of DNCB (Sigma-Aldrich, St Louis, MO,

USA) mixture as previously described.^[18] Briefly, dorsal hair of NC/Nga mice was removed repeatedly once in a week by using an electric shaver and hair-removing cream containing thioglycolic acid. At the next day of last hair removal, the dorsal skin and right ear of the NC/Nga mice were sensitized with 200 µl of 1% DNCB dissolved in acetone and ethanol (2 : 3 v/v) mixture. Four days later, the mice were treated once more by 200 µl of 1% DNCB mixture for second sensitization. A week after the first sensitization, the dorsal skin and right ear of NC/Nga mice were challenged with 150 µl of 0.4% DNCB dissolved in acetone and olive oil (3 : 1 v/v) mixture. Challenging with 0.4% DNCB mixture was repeated three times a week for 9 weeks.

The AD-induced mice were divided into six groups: normal, negative control, positive control, and SV extract groups 75, 150 and 300 mg/kg. SV extract was orally administered by gastric intubation using an animal-feeding needle daily for 4 weeks. Carboxymethyl cellulose (CMC; 0.5%) was administered instead of SV extract for the normal and negative control groups. Also, the positive control group was treated with prednisolone (3 mg/kg) dispersed into 0.5% CMC.

Measurement of skin severity and ear thickness

The severity of dermatitis was assessed macroscopically in a blinded fashion according to the scoring procedure described below.^[19] Briefly, the back and ear skin lesions were scored by the following criteria. A total clinical score of dermatitis severity was calculated as the sum of the individual scores graded as 0 (no symptoms), 1 (mild), 2 (moderate) or 3 (severe) for each of five signs and symptoms (dryness, lichenification, excoriation, erythema/oedema and erosion).^[20] Skin severity score was evaluated by a single experienced person 1 h before samples treatment. Ear thickness was measured weekly with a dial thickness gage (Mitutoyo Corporation, Tokyo, Japan) on the right ear of each mouse.

Evaluation of scratching behaviour

The scratching behaviour was observed three times a week as described previously with slight modification.^[21] Briefly, after oral administration of SV extract or prednisolone, mice of each group were placed into a new clear plastic cage for 1 h of habituation. After habituation, scratching behaviour was monitored macroscopically for 30 min.^[22] A series of scratching movements by the hind paw was regarded as one scratching episode. Each scratching episode within 30 min was scored from 0 to 4 : 0 (no scratching), 2 (scratching shorter than 1.5 s) and 4 (scratching longer than 1.5 s).^[23] The total scratching behaviour number was calculated as the sum of individual score within 30 min.

ELISA

Ig level of serum was determined using ELISA kits. The IgE level of serum was measured by mouse IgE ELISA kit (Shibayagi, Shibukawa, Japan), and level of IgG1 and IgG2a was measured by mouse IgG1 and IgG2a kits (Enzo Life Science, Exeter, UK) according to the manufacturer's instructions, respectively.

Analysis of cytokine production by splenocytes

After the last treatment, mice were anesthetized by diethyl ether, and spleens of each mouse were obtained. Splenocytes were seeded into 24-well plate at concentration of 1×10^6 cells/well in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 50 µg/ml streptomycin. Splenocytes were stimulated with 5 µg/ml of Con A (Sigma, St. Louis, MO, USA) and incubated in 5% CO₂ incubator for 72 h at 37°C. Following incubation, supernatants were collected by centrifugation and stored in -70°C until use. The levels of IL-4, IFN-γ, IL-12 and IL-13 in supernatant were measured by ELISA kits (Enzo Life Science/eBioscience, San Diego, CA, USA/Bioo Scientific, Austin, TX, USA/eBioscience) according to each manufacturer's instructions, and the results were reported for each protein.

Quantitative real-time PCR

The dorsal skin tissues of at least five mice from each group were collected at the end of treatment. Total RNA was isolated from tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After quantitative analysis of RNA was complete, reverse transcription was performed using M-MLV reverse transcriptase (Promega, Madison, WI, USA), 5× buffer, 10 mM deoxyribonucleotide triphosphates mix, Oligo dT and RNase inhibitor at 42°C for 1 h, 94°C for 5 min and 4°C for 1 h. Equal amounts of complementary DNA were mixed with SYBR Green supermix (TaKaRa, Shiga, Japan) mix and 5pM primer, and then were analysed using an ABI StepOnePlus Real-Time PCR machine (Applied Biosystems, Foster, CA, USA). Real-time PCR measured the amounts of gene expression by using primers for mouse IL-4, IL-5, IFN-γ, IL-12, CCL11, MCP-1, CCR3, CCR4, IL-17 and β-actin. The sequences of the primers are indicated in Table 1. The relative gene expression levels of target genes were normalized using housekeeping gene β-actin.^[4,24]

Histological analysis

Mice were sacrificed at the end of the experiment. Dorsal and ear skins were fixed in 10% formalin, embedded in

Table 1 Primer sequences and the reaction conditions for real-time PCR

Gene	Primer sequences (5' → 3')	Temperature (°C)
β-actin	(F) CCC AAC TTG ATG TAT GAA GG	55
	(R) TTG TGT AAG GTA AGG TGT GC	55
IL-4	(F) GTC TGC TGT GGC ATA TTC TG	57
	(R) GGC ATT TCT CAT TCA GAT TC	53
IL-5	(F) GGC TAC ACA GAG AAA CCC TGT	59
	(R) CAT GCA TAC ACA GGT AGT TCA	55
IFN-γ	(F) CTC TGA GAC AAT GAA CGC TAC ACA CT	61
	(R) TGG CAG TAA CAG CCA GAA ACA G	60
IL-12	(F) CAC CAG CAG CTT CTT CAT CAG A	60
	(R) CAA TGG CTT CAG CTG CAG GT	59
CCR3	(F) CCC GTA CAA CCT GGT TCT CC	61
	(R) AAA GAG CCG AAG GTG TTT CC	57
CCR4	(F) TCG CCT TGT TTC AGT CAG G	57
	(R) CTT GCC ATG GTC TTG GTT TT	55
Eotaxin-1/CCL11	(F) CAC CCT GAA AGC CAT AGT GT	57
	(R) TGT GTA CCT GGG AAA TTA G	53
MCP-1	(F) TTA AGG CAT CAC AGT CCG AG	57
	(R) TGA ATG TGA AGT TGA CCC GT	55
IL-17	(F) AAG GCA GCA GCG ATC ATC C	59
	(R) GGA ACG GTT GAG GTA GTC TGA G	61

paraffin wax and sectioned at 4 μm. The sections were stained with H&E and toluidine blue (TB).

Statistical analysis

The results were presented as mean ± standard error. Differences between groups were evaluated by statistical package for social sciences (SPSS; Chicago, IL, USA) using one-way analysis of variance. Multiple comparisons were performed with Tukey's test as described. A *P*-value < 0.05 was considered statistically significant.

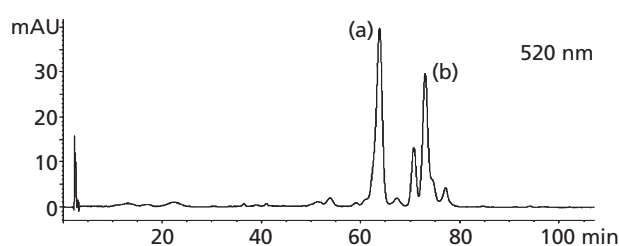
Results

Establishment of HPLC profile

To establish optimized chemical profile from the SV, HPLC gradient program was slightly modified for each. Optimized HPLC chromatogram recorded at 520 nm for the coloured potato is shown in Figure 1a and 1b. Further identification of major peaks (a and b) was performed by liquid chromatography (LC)-ESI-MS/MS study.

Identification of anthocyanins from SV by LC-ESI-MS/MS

An HPLC chromatogram of the SV extract recorded at 520 nm is shown in Figure 1. In the total ion chroma-

**Figure 1** HPLC chromatographic profile at 520 nm of *Solanum tuberosum* L. var. Vitelotte extract.

togram, the major peak at 64 min corresponds with the main peak from HPLC chromatogram. The major anthocyanin was identified by HPLC-ESI-MS and MS/MS using full spectral scan, precursor ion scan and product ion scan experiments. Figure 2a represents molecular and aglycone ions at *m/z* 933, 771, 479 and 317, respectively. The molecular ion and observed fragment ion coincide with previous study.^[25,26] Therefore, the compound (peak in Figure 1a) was proposed as petunidin3-(4'''-*p*-coumarylrutinoside)-5-glucoside.

Furthermore, Figure 2b represents molecular and aglycone ions at *m/z* 917, 755, 463 and 301 that coincide with peonidin3-(4'''-*p*-coumarylrutinoside)-5-glucoside (Figure 1b peak).

The chemical structures of anthocyanins are shown in Figure 3.

SV extract reduced DNCB-induced AD-like skin lesions and ear thickening in NC/Nga mice

To investigate the effect of SV extract on AD-like skin lesions and ear thickness, AD-induced NC/Nga mice were orally administrated with SV extract (75, 150 and 300 mg/kg per day) or prednisolone (3 mg/kg per day) on a daily basis for 4 weeks, and the progression of AD was observed. In the NC/Nga mice, AD developed by topical application of DNCB resulted in clinical signs and symptoms such as dryness, lichenification, excoriation, erythema/oedema and erosion on the ears and dorsal skin. In contrast, any skin lesions were not observed in normal controls (Figure 4). The total scores were calculated as the sum of the scores for five symptoms. As shown in Figure 5, dermatitis scores of DNCB-treated group significantly increased by repeated topical application of DNCB for 9 weeks compared with the normal group. After treatment of SV or prednisolone for 4 weeks, DNCB-induced AD severity was significantly decreased (Figure 6a).

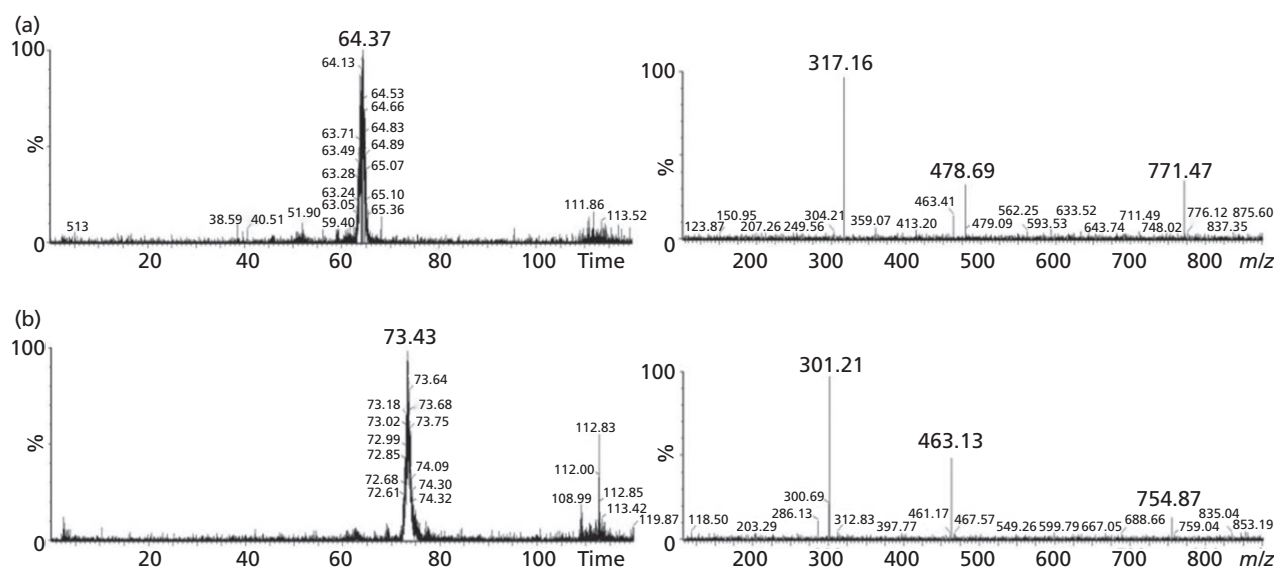


Figure 2 (a) Selected ion chromatogram of petunidin3-(4'''-p-coumaroylrutinoside)-5-glucoside and its fragmented spectrum. (b) Selected ion chromatogram of and peonidin3-(4'''-p-coumaroylrutinoside)-5-glucoside and its fragmented spectrum.

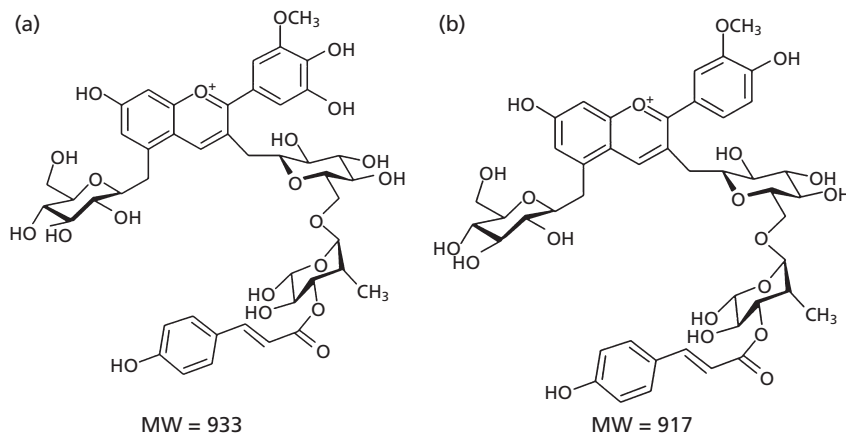


Figure 3 Chemical structures of anthocyanins. (a) petunidin3-(4'''-p-coumaroylrutinoside)-5-glucoside; (b) peonidin3-(4'''-p-coumaroylrutinoside)-5-O-β-glucoside.

The ear thickness was significantly increased after topical application of DNCB. However, the increase of ear thickness was significantly reduced in SV extract-treated group and positive control group. The decrease rates of ear thickness were 20, 30, 32 and 32%, respectively, at the fourth week in the groups treated with 75–300 mg/kg-body weight (bw) of SV extract and positive control group compared with nontreated AD control group (Figure 6b). The AD group that was not treated by SV extract did not show any effect on dermatitis score and ear thickness during the experimental periods.

These results indicated that SV extract decrease AD-like skin lesions and ear thickness in NC/Nga mice.

Effect of SV extract on scratching behaviour

AD is characterized by severe itching and repeated scratching episodes. To examine the effect of SV extract on itching and scratching behaviour, the number of scratching episodes within 30 min was counted macroscopically 1 h after treatment, three times a week. As a result, the each sample-treated group showed decreased scratching behaviour as time passed. SV extract-treated group and positive control groups showed a significant reduction of scratching behaviour compared with AD control group. The decrease rates of scratching number were 35, 49, 55 and 55%, respectively, at fourth week in the groups treated with SV extract 75–300 mg/kg-bw and prednisolone (Figure 6c).

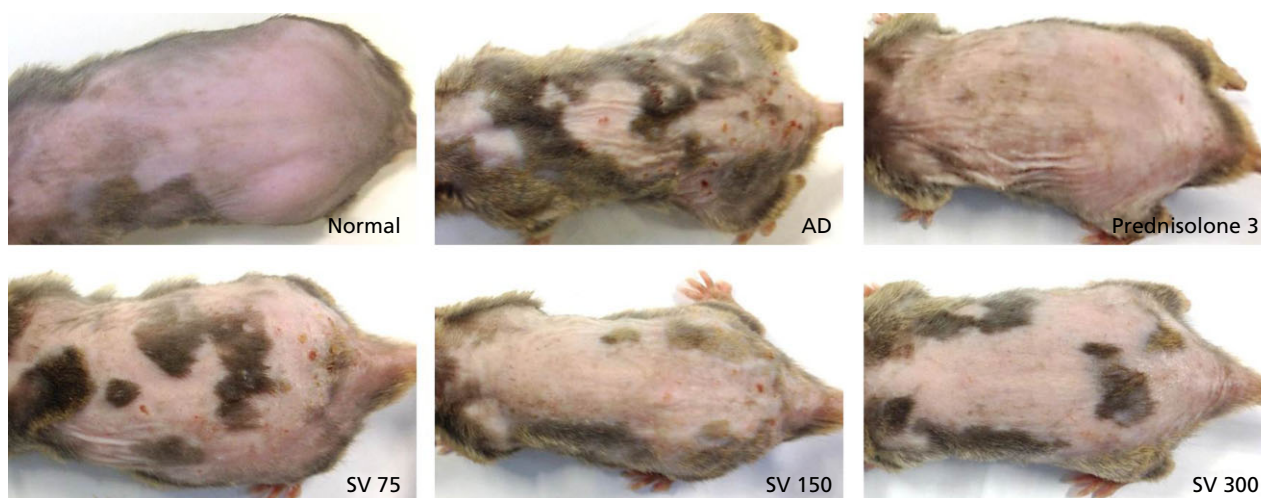


Figure 4 Representative clinical skin features in the normal group, atopic dermatitis group and treated groups with prednisolone (3 mg/kg-bw) and *Solanum tuberosum* L. var. Vitelotte extract (75, 150 and 300 mg/kg-bw).

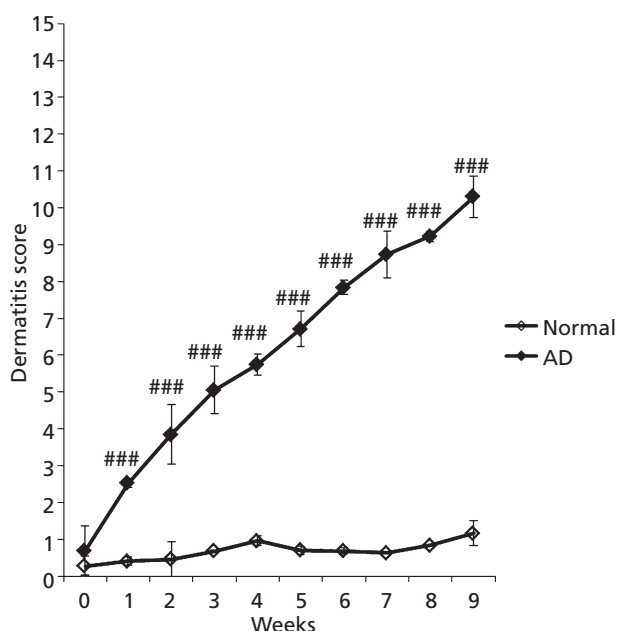


Figure 5 Evaluation of atopic dermatitis-like symptoms induced by treatment of 2,4-dinitrochlorobenzene. Dermatitis score was assessed once a week for 9 weeks following induction of atopic dermatitis. Severity of dermatitis was evaluated as sum of the score for five clinical symptoms. Dermatitis score was expressed as mean $\pm$ standard error (normal group: $n = 8$, atopic dermatitis group: $n = 40$). ### $P < 0.001$, significantly different from the normal group.

Effects of SV extract on DNCB-induced serum Ig level in NC/Nga mice

In addition to the visual clinical features imitating human AD, conventional NC/Nga mice also show elevated levels of

IgE in serum after the onset of dermatitis. Therefore, we tested whether SV extract could control the serum level of Th2-mediated IgE and IgG1 and Th1-mediated IgG2a. Sensitization with 0.4% DNCB highly increased the serum IgE level in NC/Nga mice, and the oral administration of the SV extract (75, 150 and 300 mg/kg) or prednisolone decreased the serum IgE levels with rates of 31, 36, 53 and 55% for 4 weeks, respectively (Figure 7a).

At the end of the experiment, the level of IgG1 and IgG2a in serum was measured. DNCB-sensitized mice exhibited significantly increased IgG1 level. However, increased IgG1 level was effectively reduced by the treatment of SV extract (Figure 7b). In contrast, IgG2a level was not decreased by the treatment of SV extract (Figure 7c). The induction of AD is known to be related to unbalance of Th1/Th2 ratio.^[27] Ratio of IgG1/IgG2a was decreased in SV extract 75, 150 and 300 groups, and positive control group compared with normal group, resulting in 54%, 57%, 69% and 62% reduction, respectively (Figure 7d). Thus, these data indicate that SV extract has immune regulative effects.

Inhibitory effects of SV extract on cytokine production in splenocytes

To investigate the effect of SV extract on the levels of Th1 type or Th2 type cytokines produced by splenocyte, spleens were obtained from NC/Nga mice treated with SV extract or prednisolone after sacrifice, and isolated splenocytes were stimulated by 5 μ g/ml of Con A and incubated for 72 h. The supernatant was collected after incubation, and the levels of IL-4, IL-13 (as a Th2 cytokines), IFN- γ and IL-12 (as a Th1 cytokines) in supernatant were measured by ELISA kits. The production of IL-4 was decreased by 42%, 20%, 31% and 37% in the prednisolone-treated group and

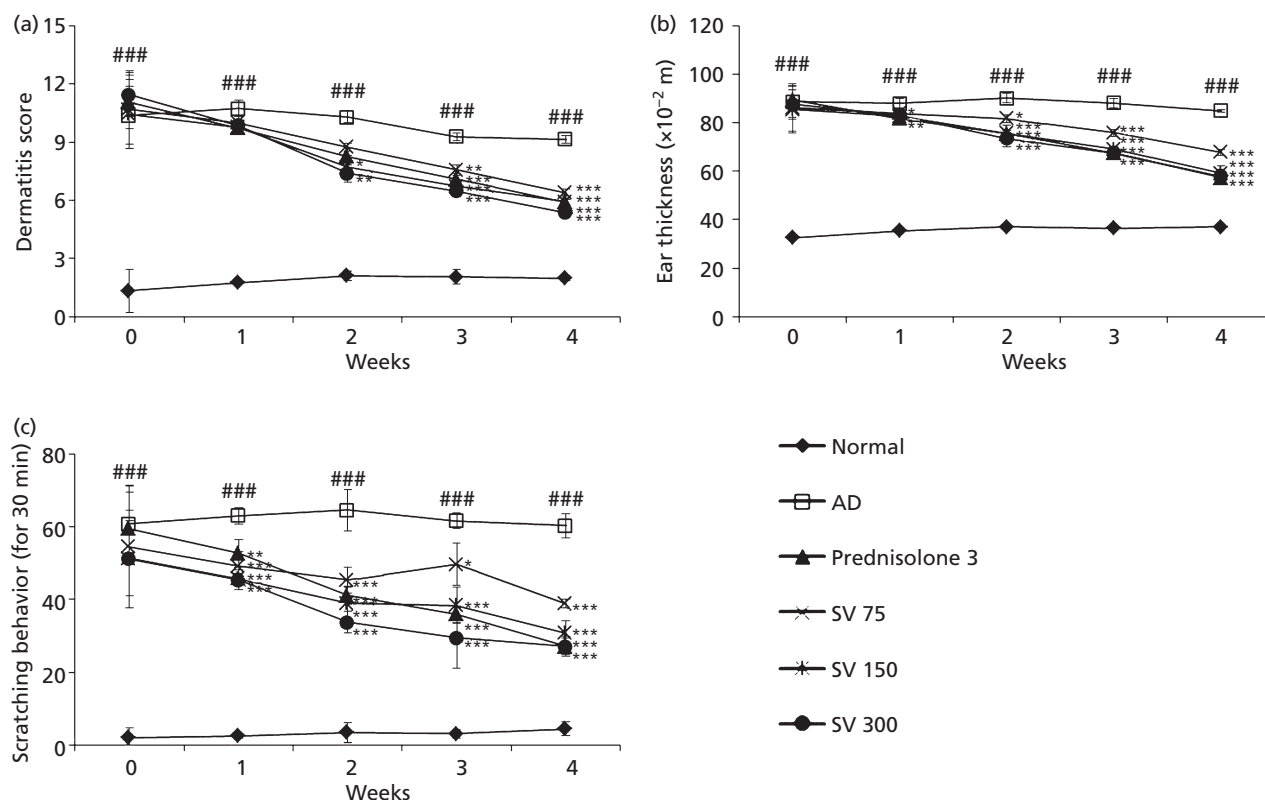


Figure 6 Effects of *Solanum tuberosum* L. var. Vitelotte extract on (a) dermatitis score, (b) ear thickness and (c) scratching behaviour. (a) Dermatitis score was evaluated as sum of the scores in five clinical symptoms. (b) Ear thickness was measured by dial thickness gauge. Each point represents the mean $\pm$ standard error of six mice per group. ### $P < 0.001$ when compared with normal group and * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with the atopic dermatitis group. (c) The number of scratching behaviour within 30 min was counted 1 h after each sample treatment, three times a week. Weekly data are presented as mean $\pm$ standard error of each scratching numbers of three days in a week. ### $P < 0.001$, significantly different from the normal group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from the atopic dermatitis group.

the SV extract-treated group (75–300 mg/kg), respectively. The production of IL-13 was decreased by 73%, 28%, 29% and 67% in the prednisolone-treated group and the SV extract-treated group (75–300 mg/kg), respectively. The production of IFN- γ was decreased by 82%, 3%, 39% and 77% in the prednisolone-treated group and the SV extract-treated group (75–300 mg/kg), respectively. IL-12 level was also significantly reduced by SV extract treatment (Figure 8). These results suggest that the treatment of the SV extract normalized both Th1 and Th2 cytokine production, and the suppressive effect of SV extract-treated group (300 mg/kg) is equivalent to that of prednisolone.

SV extract suppressed the expression of DNCB-induced cytokines and chemokines mRNAs in NC/Nga mice

We investigated the inhibitory effect of SV extract on the messenger RNA (mRNA) expression of Th1, Th2 and Th17 cytokines in the lesional skins. Th2 cytokines become

overexpression in the acute phase of AD. Th1 cytokines are contribute to the AD pathogenesis in the chronic phase.^[28] Repeated application of DNCB significantly increased mRNA expression of IL-4, IL-5, IFN- γ , IL-12, eotaxin-1, CCR3, MCP-1, CCR4 and IL-17 in the lesional skin, but SV extract significantly reduced the mRNA level of these genes (Figure 9). These results suggest that SV extract suppressed expression of IL-4, IL-5, IFN- γ , IL-12, eotaxin-1, CCR3, MCP-1, CCR4 and IL-17, and resulted in the inhibition of AD skin lesions caused by inflammatory cells.

SV extract reduced the infiltration of inflammatory cells in skin lesions and thickening of the epidermis

The infiltration of inflammatory cells in skin lesions is an important feature of AD. To investigate the effect of the SV extract on histopathological symptoms like AD in NC/Nga mice, dorsal and ear skins were sectioned, stained with H&E (Figure 10a) and TB (Figure 10d), and observed under

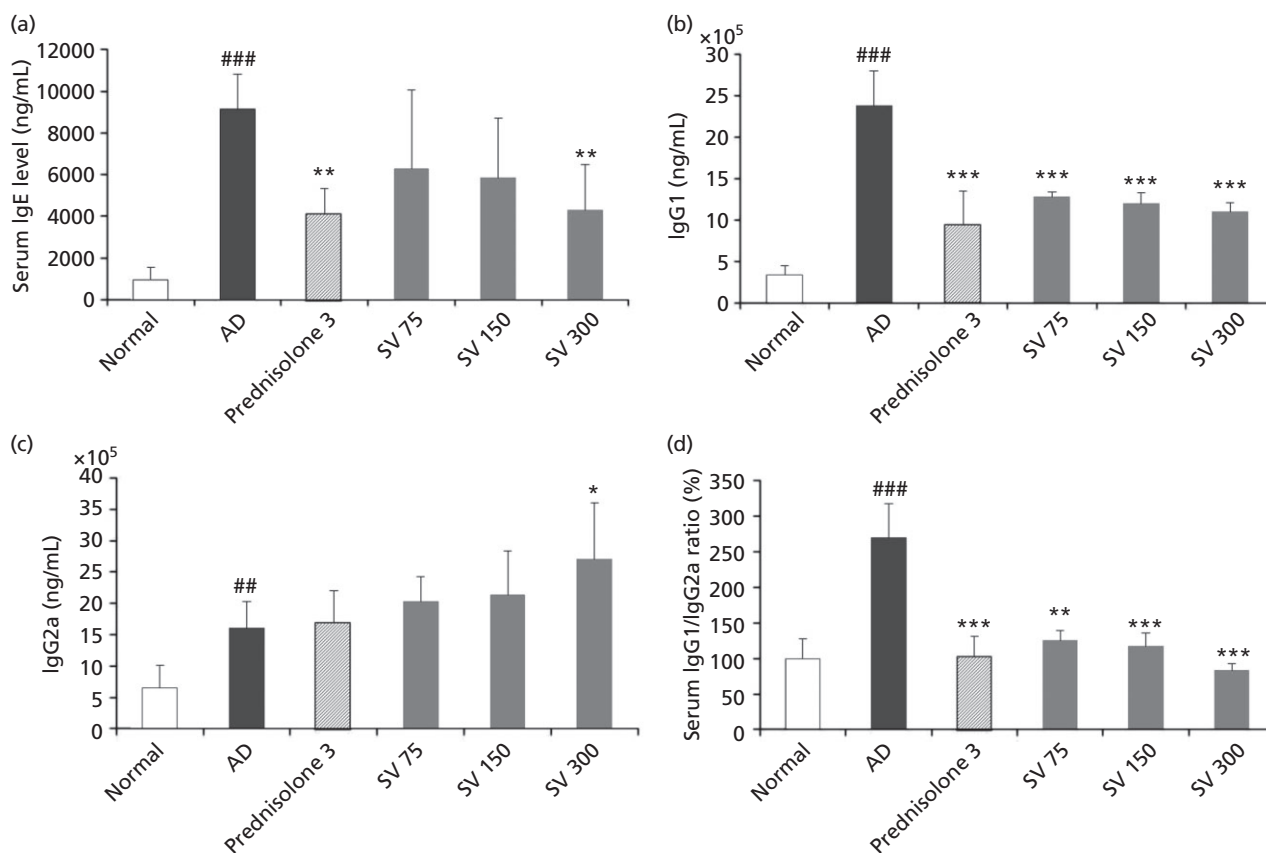


Figure 7 The effect of *Solanum tuberosum* L. var. Vitelotte extract on serum (a) IgE, (b) IgG1, (c) IgG2a and (d) IgG1/IgG2a ratio levels in the NC/Nga mice. Serum was collected 24 h after the last sample treatment. Serum levels of (a) IgE, (b) IgG1 and (c) IgG2a were measured using ELISA kit. The (d) IgG1/IgG2a ratio in serum was calculated as IgG1/IgG2a for each mouse. Data are presented as means $\pm$ standard error of six mice per group. ^{###} $P < 0.001$, significantly different from the normal group. ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$, significantly different from the atopic dermatitis group.

microscope at a magnification of $\times 400$. AD control group exhibited marked thickening of the epidermis and infiltration of inflammatory cells in particularly eosinophils and mast cells. However, the treatment with SV extract or prednisolone inhibited the thickening of the epidermis and the infiltration of inflammatory cells in the dermis. Oral administration of the SV extract 75, 150, 300 and prednisolone lessened the epidermal thickness with rates of 32%, 66%, 75% and 67%, respectively (Figure 10b). The infiltration of eosinophil (Figure 10c), mast cell (Figure 10e) and degranulated mast cell (Figure 10f) were macroscopically counted in randomly selected four fields (area of $\times 400$ view). The number of infiltrated eosinophil, mast cell and degranulated mast cell significantly decreased by the treatment of SV extract compared with AD control group. These results showed that oral intake of SV extract could effectively suppress the exacerbation of dermatitis in NC/Nga mice by reduction the infiltrations of inflammatory cells in the lesional skin.

Discussion

Oral administration of SV extract markedly improved DNCB-induced AD-like skin lesions. In addition, SV extract significantly normalized the Th2/Th1 ratio, infiltration of inflammatory cells and so on.

AD is a biphasic inflammatory skin disease provoked by an imbalance between Th1 and Th2 immune responses.^[29,30] The NC/Nga mice with AD lesions show clinical symptoms such as erythema/oedema, erosion, lichenification, dryness and excoriation. Also, itching is one of the major features of AD and scratching causes lesions to relapse.^[31]

There are side effects of the medicine from steroids that is used for curing AD.^[32] Many researchers need development of new treatment to replace steroids for AD therapy.^[33,34] Recently, several studies have reported that herbal extract therapy is effective in AD patients. Potato (*S. tuberosum* L.) is one of the most widely cultivated major crops in the world. Generally, the potato has not been

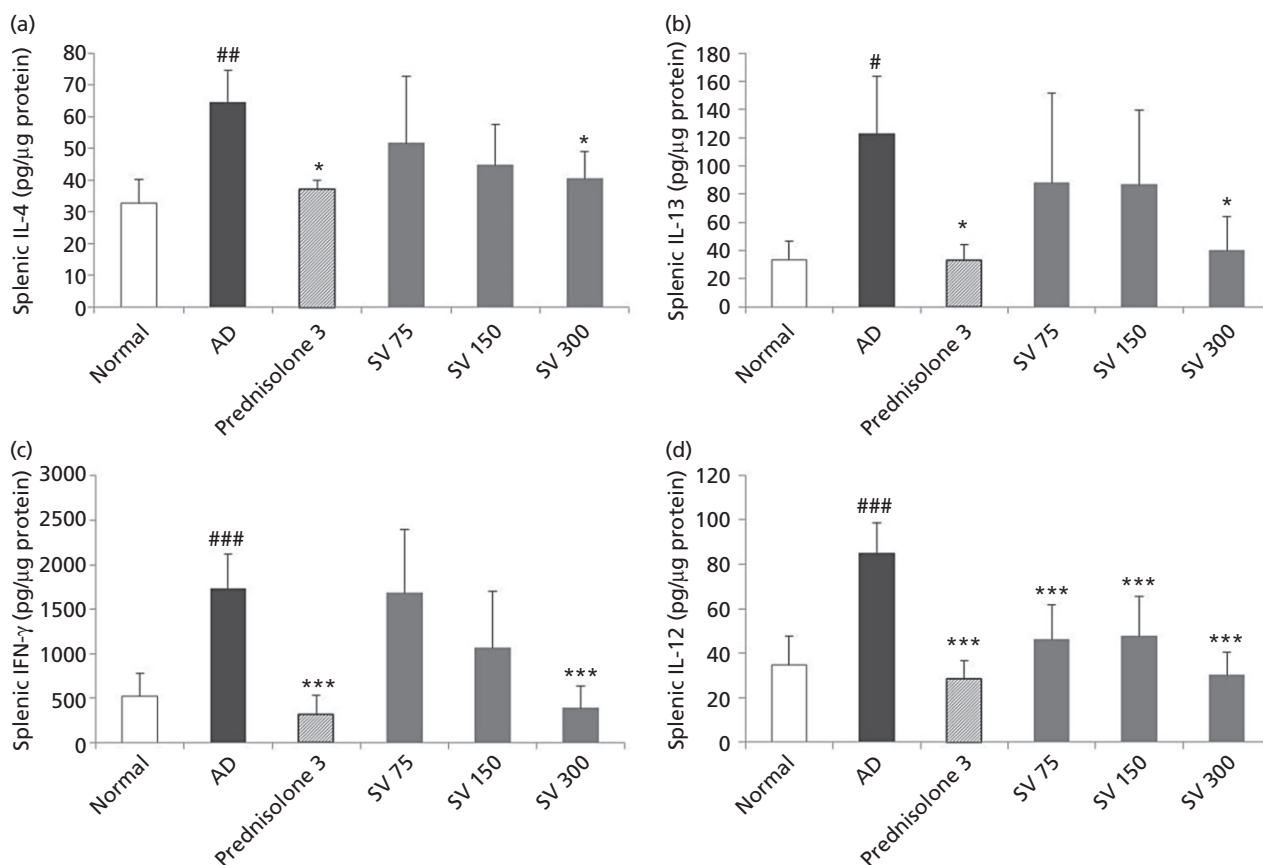


Figure 8 Effects of *Solanum tuberosum* L. var. Vitelotte extract on (a) IL-4, (b) IL-13, (c) IFN- γ and (d) IL-12 production from Con A-activated splenocytes supernatant. Spleen of each mouse was obtained 24 h after the last sample treatment. Isolated splenocytes were stimulated by Con A and incubated for 72 h. The levels of (a) IL-4, (b) IL-13, (c) IFN- γ and (d) IL-12 in supernatant were measured by ELISA kits. Data are presented as means $\pm$ standard error of six mice per group. $^{###}P < 0.001$, significantly different from the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the atopic dermatitis group.

regarded as a food rich in antioxidants. However, depending on the genetic variety, the SV has extensive amounts of polyphenols and anthocyanins.^[13,35,36] The major pigments in the SV were anthocyanins, especially petunidin3-(4'''-p-coumarylrutinoside)-5-glucoside and with peonidin3-(4'''-p-coumarylrutinoside)-5-glucoside. Petunidin3-(4'''-p-coumarylrutinoside)-5-glucoside has antioxidant activity.^[37] SV has potential health benefit to people because of its antioxidant capacity, mainly due to the presence of polyphenols including anthocyanins.^[13,38] Polyphenols and anthocyanins are known to be kinds of flavonoids. The flavonoids have been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antiviral and anticarcinogenic activity.^[39] Therefore, this study investigated the inhibitory effect of SV extract on AD-like skin lesions induced by DNCB in NC/Nga mice. In this study, we demonstrated that oral administration of SV extract decreased AD-like skin lesions and scratching behaviour in NC/Nga mice, and also reduced ear thickening.

Differentiation of CD4⁺ T cells leads to the generation of Th1 cells that secrete IFN- γ and IL-12, Th2 cells that secrete IL-4, IL-5 and IL-13, and Th17 cells that secrete IL-17 and IL-22.^[29,40] Cytokines released from Th2 cells and Th1 cells are involved in AD development.^[29] Th1 cells were defined on the basis of their production of IFN- γ , a potent macrophage-activating cytokine important in the clearance of certain intracellular pathogens and a switch factor for induction of IgG2a production by B cells. Th2 cells became defined as producers of IL-4 and IL-13 that are responsible for the promotion of IgG1 and the induction of IgE synthesis by B cells, and IL-5 that is important for the recruitment and survival of eosinophils.^[1,7] Th1 cytokines and Th2 cytokines play an important role in AD disease in DNCB-induced NC/Nga mice. Our results demonstrated that SV extract effectively reduced production of Th1 cytokines and Th2 cytokines similar to the normal group. Hence, we found that treatment with the SV extract has an effect on both acute and chronic AD lesions. The Th1/Th2 balance appears to be important in AD. Under normal conditions,

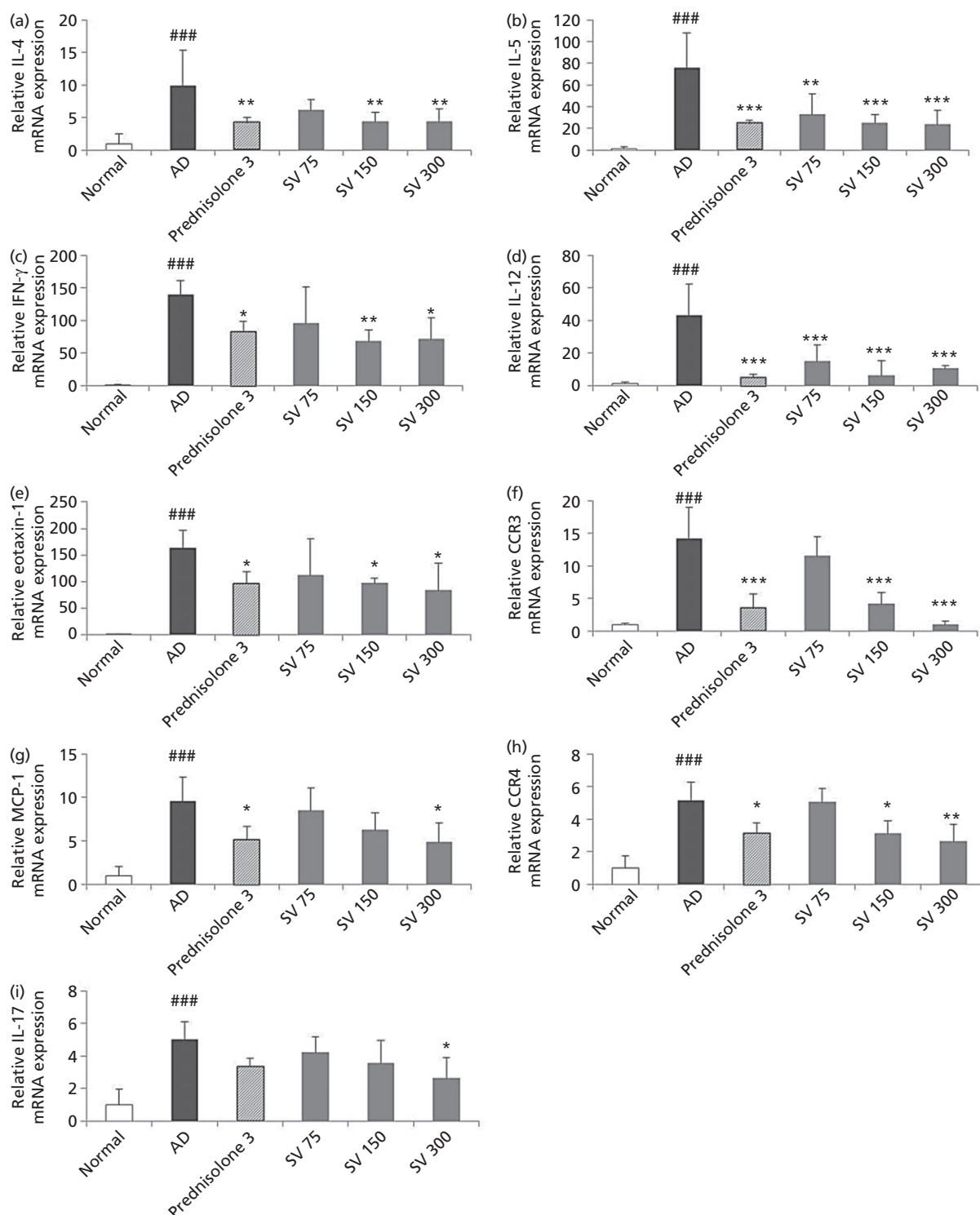


Figure 9 Effects of *Solanum tuberosum* L. var. Vitelotte extract on 2,4-dinitrochlorobenzene-induced IL-4, IL-5, IFN- γ , IL-12, eotaxin-1, CCR3, MCP-1, CCR4 and IL-17 mRNA expression in NC/Nga mice. Twenty-four hours after the last sample treatment, mice were sacrificed, and lesional skin tissues of each mouse were obtained. RNA was extracted from lesional skin. mRNA expression of (a) IL-4, (b) IL-5, (c) IFN- γ , (d) IL-12, (e) eotaxin-1, (f) CCR3, (g) MCP-1, (h) CCR4 and (i) IL-17 were measured by real-time PCR machine. Data are expressed as means $\pm$ standard error of six mice per group. ### P < 0.01, ### P < 0.001, significantly different from the normal group. * P < 0.05, ** P < 0.01, *** P < 0.001, significantly different from the atopic dermatitis group.

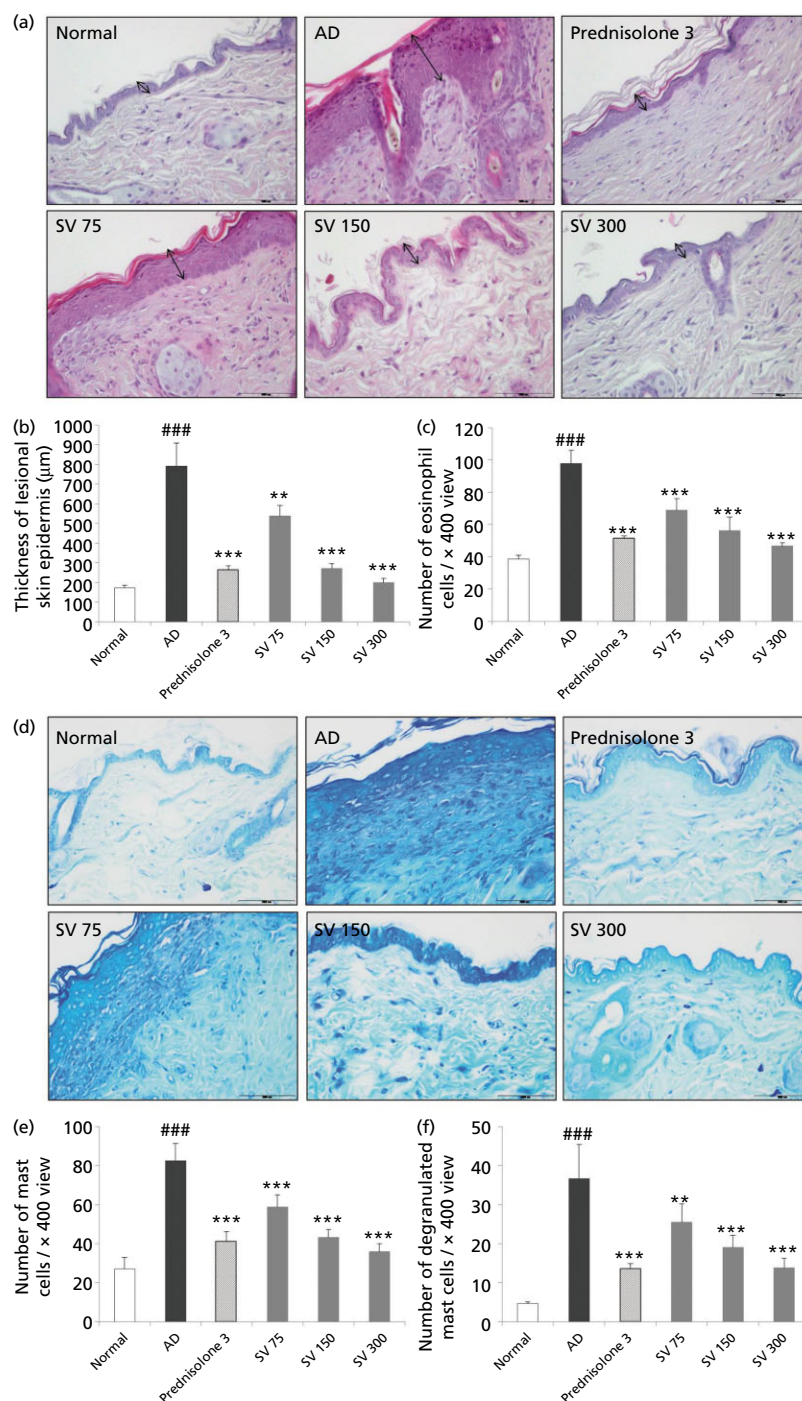


Figure 10 Histopathological features of atopic dermatitis-like skin lesions treated with *Solanum tuberosum* L. var. Vitelotte extract in NC/Nga mice. Dorsal lesions were removed and fixed with 10% formaldehyde solution. Thin sections were cut and stained with H&E or toluidine blue. H&E- or toluidine blue-stained sections were observed under microscope at a magnification of $\times 400$. (a) H&E-stained lesional skin of normal, atopic dermatitis, prednisolone (3 mg/kg-bw) and *Solanum tuberosum* L. var. Vitelotte extract (75, 150 and 300 mg/kg-bw) treated groups; (b) epidermal thickness and (c) infiltration of eosinophil was quantified as means in randomly selected four fields per mouse. (d) Toluidine blue-stained lesional skin of normal, atopic dermatitis, prednisolone (3 mg/kg-bw) and *Solanum tuberosum* L. var. Vitelotte extract (75, 150 and 300 mg/kg-bw) treated groups; (e) infiltration of mast cell and (f) infiltration of degranulated mast cell were quantified as means in randomly selected four fields per mouse. Scale bar = 1000 μm . Data are presented as means $\pm$ standard error of six mice per group. ^{###} $P < 0.001$, significantly different from the normal group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significantly different from the atopic dermatitis group.

the differentiation of naïve T cells to Th1 and Th2 lineages is regulated by cytokines that are secreted from various cells, and the Th1/Th2 balance is maintained.^[5,41] However, Th1/Th2 balance change into Th1/Th2 imbalance skewed to Th2 at AD-like skin lesion.^[42] We showed the inhibitory effect of Th1/Th2 balance through the IgG2a and IgG1 ratio as markers for Th1 and Th2 lymphocytes, respectively.^[43] Oral administration of SV extract decreased the level of IgG1 and increased the level of IgG2a. The calculated IgG1/IgG2a ratio for mice showed that SV extract significantly reduced Th2/Th1 ratio.

AD frequently exhibit elevated serum IgE levels by class switching from IgM to IgE through IL-4 overproduction.^[44] IgE binds to the high affinity receptor for IgE (FcεRI) on the surface of epidermal Langerhans cell, mast cells and basophils.^[1] After binding, mast cell becomes degranulation. A variety of mediators such as histamine releases from mast cells.^[45] SV extract inhibited production of IgE which plays a key role in the AD.

The dermal accumulation of inflammatory cells is an important histopathological feature of AD.^[46] AD is characterized by infiltrated with CD4⁺ T cells, eosinophils, mast cells and epidermal LCs into the dermis, and the number of infiltrated inflammatory cell is increased.^[47,48] In particular, activated mast cells release a variety of biologically active substances that play important roles in allergic reactions, such as AD.^[45,49] In this study, we showed that repeated topical application of DNCB resulted in infiltration of inflammatory cells and mast cells into the dermis of NC/Nga mice. Oral administration of SV extract significantly inhibited the infiltration of eosinophils, mast cells and degranulated mast cells. In addition, SV extract suppressed thickness of epidermis and dermis in a dose-dependent manner.

Chemokines can recruit both inflammatory and adaptive immune cells including dendritic cells, T cells and B cells. Eotaxin-1, also called CCL11, was initially discovered using a biological assay in guinea pigs.^[50,51] There is increased mRNA expression of the eosinophil chemotactic factor CCL11/eotaxin and of the CCL11/eotaxin receptor CCR3 in acute and chronic skin lesions of AD.^[52–54] MCP-1, a potent monocyte chemoattractant, and MCP-1 receptor CCR4 are also expressed in inflammatory tissue. MCP-1 directly

stimulates basophils to secrete histamine.^[55] Administration of SV extract decreased DNCB-induced CCL11/eotaxin, CCR3, MCP-1 and CCR4 mRNA expression in the tissue. Recently, the role of Th17 cells secreting IL-17 in AD has been investigated in several studies.^[40] In addition, a subset of IL-17-inducing Th cells (Th17) has been described as an essential factor in the pathophysiology of AD.^[44,56] Recently, Th1/Th2 paradigm in autoimmunity and allergic reactions has been expanded, following the discovery of a third subset of effector Th cells that produce IL-17 (Th17) and exhibit effector functions distinct from Th1 and Th2 cells.^[57] IL-17 is also highly expressed in acute AD.^[58,59] However, the role of differentiated Th17 cells *in vivo* is as yet unclear. In this study, ingeminated application of DNCB increased mRNA expression of Th1, Th2 and Th17 related genes. The mRNA expression of IL-17 was significantly increased in the chronic AD-like skin lesions. However, administration of SV extract decreased DNCB-induced IL-17 mRNA expression in the tissue. Ultimately, elevated mRNA expression of Th1-, Th2- and Th17-related genes reduced in SV extract-treated groups.

Conclusions

SV extract ameliorated AD symptoms as effectively as prednisolone. SV extract reduced the DNCB-induced AD-like skin lesions in NC/Nga mice by suppressing total serum IgE levels and Th1 and Th2 cytokines production in activated CD4⁺ T cells. Also, SV extract has an emollient effect on AD via the modulation of Th1/Th2 balance and reduction of IL-17 expression. Therefore, SV extract has the great potential as a safe and effective reagent for the treatment of AD.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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