



Topical application of *Pleurotus eryngii* extracts inhibits 2,4-dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice by the regulation of Th1/Th2 balance

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ABSTRACT

Pleurotus eryngii is a nutritional and medicinal food rich in polysaccharides that enhance the host immune system as a response to various diseases. The present study investigated the effects of *P. eryngii* extracts (PEE) on the progress of atopic dermatitis (AD)-like skin lesions in NC/Nga mice induced by 2,4-dinitrochlorobenzene (DNCB). We evaluated skin dermatitis severity, ear thickness, histopathological examination, and cytokines level in DNCB-applied mice treated with PEE. Continuous treatment of PEE inhibited the development of the AD-like skin lesions. PEE suppressed DNCB-induced dermatitis severity, serum level of IgE and thymus and activation-regulated chemokine (TARC), and mRNA expression of TNF- α , INF- γ , IL-4, IL-5, and IL-13 in mice. In addition, PEE reduced thickness of the dermis and dermal infiltration of inflammatory cells and mast cells in histopathological examination. These results indicate that PEE inhibits allergic contact dermatitis through the modulating of T helper (Th)1 and Th2 responses and diminishing the inflammatory cells and mast cells infiltration in the skin lesions in NC/Nga mice.

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1. Introduction

Atopic dermatitis (AD) is a chronic, relapsing and inflammatory skin disease in humans and animals, caused by a complex interrelationship among genetic, environmental, pharmacologic, psychological, immunologic and skin barrier dysfunction factors (Udompataikul and Limpa-o-vart, 2012). Various factors including the immunological abnormalities contribute to the pathogenesis and development of AD. The early onset of AD in infancy stands out as the most prevalent chronic skin disorder in childhood and can affect individuals throughout their lifetimes through its persistence or the onset of serious problems in adulthood (Choi et al., 2012). The itching is an important problem in AD patients because scratching worsens the dermatitis (Hashimoto et al., 2011). Reduction of itching-associated scratching is the most effective therapeutic strategy for improving the quality of life for AD patients. For a long time, therapeutic strategies of AD have been dominated by

the application of local or systemic corticosteroids (Blume-Peytavi and Wahn, 2011). These steroids are still the first treatment for AD because they are effective at reducing the inflammation by AD. However, these steroids are often observed the adverse effects in patients with AD (Hengge et al., 2006). Therefore, there is a great need for the development of new and effective therapies for AD.

The pathogenesis of AD is complex, involving genetic, environmental, and immunological factors, while interleukin (IL)-4, IL-5 and IL-13 produced by T-helper (Th) 2 cells may have especially key roles in its onset and development of AD (Jung et al., 2010). Although the etiology and pathology of AD are not fully understood, recent study reports that typical symptoms of AD involve increased levels of Th2-mediated cytokines and a deficiency in Th1-mediated cytokines (Sawada et al., 2012). Th2 cells are dominant during the acute phase of AD, Th1 cells are expressed and contribute to pathogenesis during the chronic phase (Vestergaard et al., 1999). Elevated immunoglobulin E (IgE) level is a hallmark of AD and the expression of IL-4 contributes to this elevation. IL-4 stimulates IgE production in B cells. IgE release from B cells bind to mast cells. Mast cells degranulate and release various biological mediators in IgE-mediated AD (Amin, 2012; Kishimoto and Hirano, 1988; Poulsen and Hummelshoj, 2007). AD is dependent on the secretion

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of the cytokines IL-4, IL-5, and IL-13 by Th2 cells that are generated from precursors. Most patients with AD have increased eosinophils and immunoglobulin E (IgE) due to elevated IL-4, IL-5, and IL-13 produced by Th2 cells (Yamanaka and Mizutani, 2011).

Nc/Nga mice, an inbred strain of Japanese origin, were the first mouse model of AD-like skin disease reported (Matsuda et al., 1997). AD-like skin disease is spontaneously developing eczematous skin lesions when maintained in conventional conditions but not in specific pathogen free conditions (Hiramoto et al., 2010). Along with the AD-like skin changes, Nc/Nga mice exhibit Th cells differentiation toward Th2 cells, accumulation of inflammatory cells and mast cells in the skin lesion, and increases of serum IgE level (Kohara et al., 2001; Vestergaard et al., 1999). The scratching behavior is the first sign of the skin changes, occurs at 6–8 weeks, and is followed by rapidly developing erythema, hemorrhage, scarring, dryness, hyperkeratosis, and hyperplasia in the skin lesions. Furthermore, Nc/Nga mice are linked to the increased IgE production as well as increased Th2 responses.

To reduce experimental variation and induce stable clinical AD-like skin diseases, many researchers have adopt chemical hapten, DNCB, 2,4-dinitrofluorobenzene (DNFB), 2,4,6-trinitro-1-chlorobenzene (TNCB). Chemical hapten, DNCB, DNFB, TNCB application produce AD-like skin disease in NC/Nga mice (Park et al., 2012; Kim et al., 2011). DNCB was used as a rational alternative in view of its structural similarity and the basis of evidence of reproducibility and repeatability in creating AD-like skin lesions in NC/Nga mice (Jung et al., 2010; Yang et al., 2011; Fujii et al., 2009). The pathogenesis of DNCB-induced contact hypersensitivity is predominantly the result of T cell-mediated immune responses (Zhang et al., 2009).

For thousands of years, mushrooms have been known to be a source of medicine and food. *Pleurotus eryngii* (*P. eryngii*), also known as the king oyster mushroom, is one of the most popular and edible mushrooms in Asia, Europe, and North America (Rodriguez Estrada and Royse, 2007). *P. eryngii* has been used for centuries in Korea as both a food and a traditional medicine. It contains little fat and digestible carbohydrates, but has higher protein content than most vegetables, and has been reported to have anti-microbial, anti-oxidant, and anti-atherosclerotic properties (Kalyoncu et al., 2010; Mori et al., 2008). Our previous studies demonstrated that *P. eryngii* extracts (PEE) suppresses the level of IL-4 and allergy-related signaling proteins by inhibiting of nuclear factor of activated T-cells (NF-AT)-, nuclear factor κ B (NF- κ B)-, and high affinity IgE receptor (Fc ϵ RI)-mediated signaling in antigen-stimulated mast cells (Han et al., 2011). Although PEE has been reported in various bioactivity assays, the effect of PEE as an anti-atopic agent for AD remains unclear. In this study, we examined the inhibitory effect of PEE on the development of AD in NC/Nga mice.

2. Materials and methods

2.1. Chemicals

DNCB (2,4-dinitrochlorobenzene) was obtained from Sigma–Aldrich (Milwaukee, WI, USA). Enzyme-linked immunosorbent assay (ELISA) kits for IgE and TARC were obtained from BD Biosciences (San Diego, CA, USA) and R&D Systems (Minneapolis, MN, USA). Polymerase chain reaction (PCR) oligonucleotide primers were custom synthesized by Bioneer Co. (Daejeon, Korea). All chemicals and solvents were of the highest grade commercially available.

2.2. Preparation of *P. eryngii* extracts (PEE)

Air-dried *P. eryngii* (2.5 kg) was extracted with ethanol; the yield of *P. eryngii* extracts (PEE) corresponded to 0.23%. The analysis of *P. eryngii* ethanol extracts were contained 86.1% water, 1.72% crude protein, 0.31% crude lipid, 0.63% ashes, 6.14% crude sugar, and 5.3% crude fiber. The minerals analysis was performed by the use of a PerkinElmer® ICP model Optima™ 4300 DV. The amount of minerals in *P. eryngii* ethanol extracts is 98.9 mg/kg K, 4.5 mg/kg Ca, 15.8 mg/kg Mg, 23.9 mg/kg Na, 1.8 mg/kg Mn, 2.9 mg/kg Fe, 0.6 mg/kg Zn, 0.5 mg/kg Cu, 0.4 mg/kg Al, and 75.5 mg/kg P. The fungal sterol and ergosterol peroxide were also determined by high-pressure liquid chromatography (HPLC) system and analyzed to contain 5.5% and 15.4%, respectively. PEE was mixed with distilled water and emollient cream at 100 or 200 mg/kg concentration.

2.3. Animals and treatment

Specific pathogen free male 6-week-old NC/Nga mice were obtained from SLC, Inc., (Shizuoka, Japan). Animals were acclimatized to the temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) of controlled rooms with a 12 h light/dark cycle for at least two weeks prior to experiments. Animals were allowed free access to Purina rodent chow (Seoul, Korea) and tap water. All experimental protocols for animal care were performed according to the rules and regulations of the Animal Ethics Committee, Chungnam National University. The mice were divided into four groups ($n = 5$ per group). To induce AD-like immunologic and skin lesions, DNCB was applied onto the dorsal skin and ears. After complete removal of dorsal hairs within an area approximately 8 cm^2 , 200 μL of 1% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied for three consecutive days for sensitization. Four days after sensitization, the dorsal skin and ears were challenged with 200 μL of 0.2% DNCB solution three times per week for eight weeks. After inducing AD, emulsion containing PEE was topically applied to the dorsal skin and ears of the mice six times per week for four weeks. PEE-treated mice were topically applied 1 h before DNCB applying each time. Control and DNCB-treated mice were topically applied to the emulsion without PEE on dorsal skin and ears. Animals were sacrificed 64 days after the first application of DNCB (Fig. 1). Blood was collected from the vena cava, and the right ear was removed and used for histopathological examination.

2.4. Measurement of ear thickness

Ear thickness was measured with a micrometer (Mitutoyo, Kawasaki, Japan) on the day of sacrifice. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges, and the thickness was recorded in micrometers. To minimize technique variations, a single investigator performed the measurements throughout each experiment.

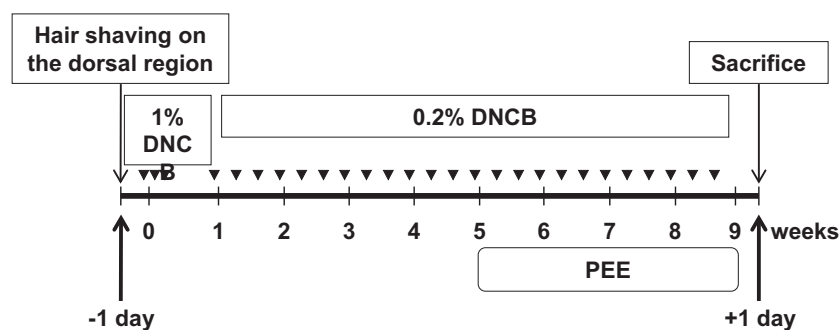


Fig. 1. Schematic diagram of the experimental protocol in mice. Mice were divided into four groups ($n = 5$ per group). To induce AD-like immunologic and skin lesions, DNCB was applied to the dorsal skin and ears. After complete removal of dorsal hairs within an area approximately 8 cm^2 , 200 μL of 1% DNCB was applied on the dorsal skin and ears for three consecutive days for sensitization. Four days after sensitization, the dorsal skin and ears were challenged with 200 μL of 0.2% DNCB solution three times per week for eight weeks. After inducing AD, emulsion containing PEE (100 or 200 mg/kg) was applied to the dorsal skin and ears of the mice six times per week for four weeks. Control and DNCB-treated mice were applied to the emulsion without PEE on dorsal skin and ears. Animals were sacrificed 64 days after the first application of DNCB.

Table 1

Primer sequences and the reaction conditions for semi-quantitative RT-PCR.

Gene	Primer sequences (5'→3')	Annealing temperature (°C)	Gene bank no.
TNF- α	(F) CCT GTA GCC CAC GTC GTA GC (R) TTG ACC TCA GCG CTG AGT TG	56	NM_013693.2
INF- γ	(F) CTC AAG TGG CAT AGA TGT (R) GAG ATA ATC TGG CTC TGC AGG ATT	60	NM_008337.3
IL-4	(F) GAA TGT ACC AGG AGC CAT ATC (R) CTC AGT ACT ACG AGT AAT CCA	58	NM_021283.2
IL-5	(F) TCA CCG AGC TCT GTT GAC AA (R) CCA CAC TTC TCT TTT TGG CG	62	NM_010558.1
IL-13	(F) GAC CCA GAG GAT ATT GCA TG (R) CCA GCA AAG TCT GAT GTG AG	62	NM_008355.3
GAPDH	(F) CAT CTT CCA GGA GCG AGA CC (R) TCC ACC ACC CTG TTG CTG TA	56	NM_008084.2

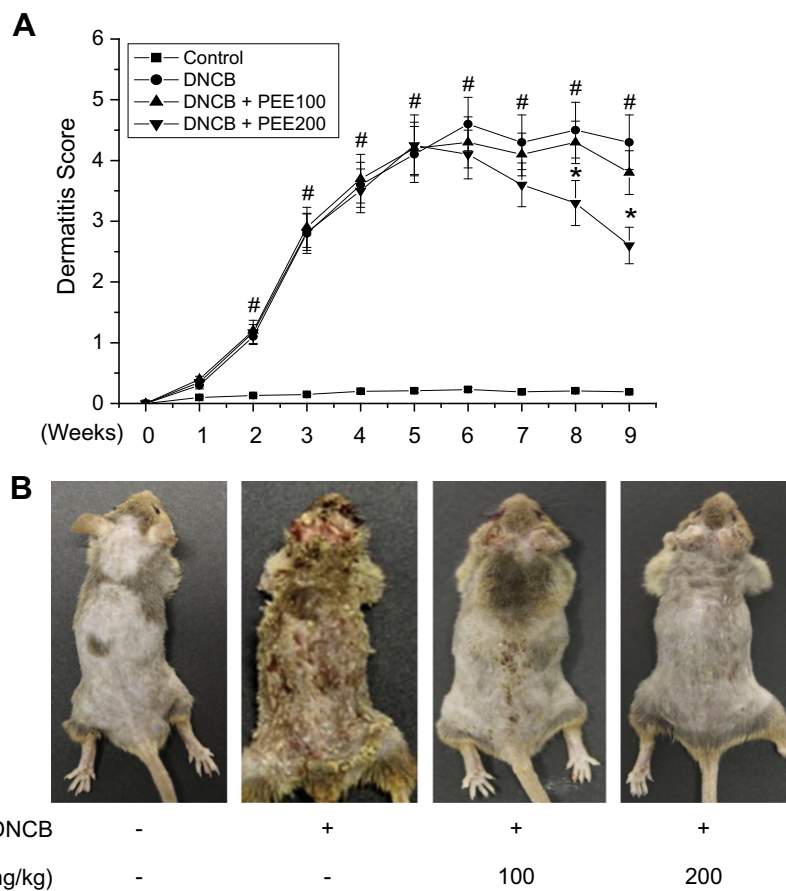


Fig. 2. Effects of PEE on DNCB-induced AD skin severity in NC/Nga mice. (A) The dermatitis score was defined as the sum of scores for seven clinical criteria: erythema, hemorrhage, edema, excoriation, erosion, scarring, and dryness. (B) Example of the clinical severity of AD-like skin lesions. The photographs were taken on day 64 after the sacrifice of the mice. Results shown are representatives of five observations. Results are presented as mean $\pm$ S.E.M. ($n = 5$). # $p < 0.05$, significantly different from the control group; * $p < 0.05$, significantly different from the DNCB-treated group.

2.5. Histopathological studies

The right ear lesions were sliced and tissue slices were fixed in 10% buffered-neutral formalin for 24 h. The fixed ear tissue slices were embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. Sections 5 μ m in thickness were subjected to hematoxylin and eosin (H&E) or toluidine blue for detecting various inflammatory cells and mast cells, respectively. Histopathological changes were examined by light microscopy. An arbitrary scope was given to each microscopic field viewed at a magnification of 100 $\times$.

2.6. Evaluation of skin dermatitis severity

The severity of dermatitis in the dorsal skin and ear lesions was evaluated twice a week. The development of (1) erythema/hemorrhage, (2) scarring/dryness, (3)

edema, and (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was defined as the dermatitis score (Matsuda et al., 1997).

2.7. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from left ear lesion using the RNAiso reagent (Takara, Kyoto, Japan), according to the manufacturer's protocol, and stored at -80°C until use. Then, 0.5 μ g RNA was used for reverse transcription and amplified by PCR using the access RT-PCR system Takara thermal cycler (Takara, Seoul, Korea). PCR was performed using primers for mouse TNF- α , INF- γ , IL-4, IL-5, IL-13, and GAPDH. The sequences of primers are indicated in Table 1. The PCR products were separated on a 2% agarose gel containing ethidium

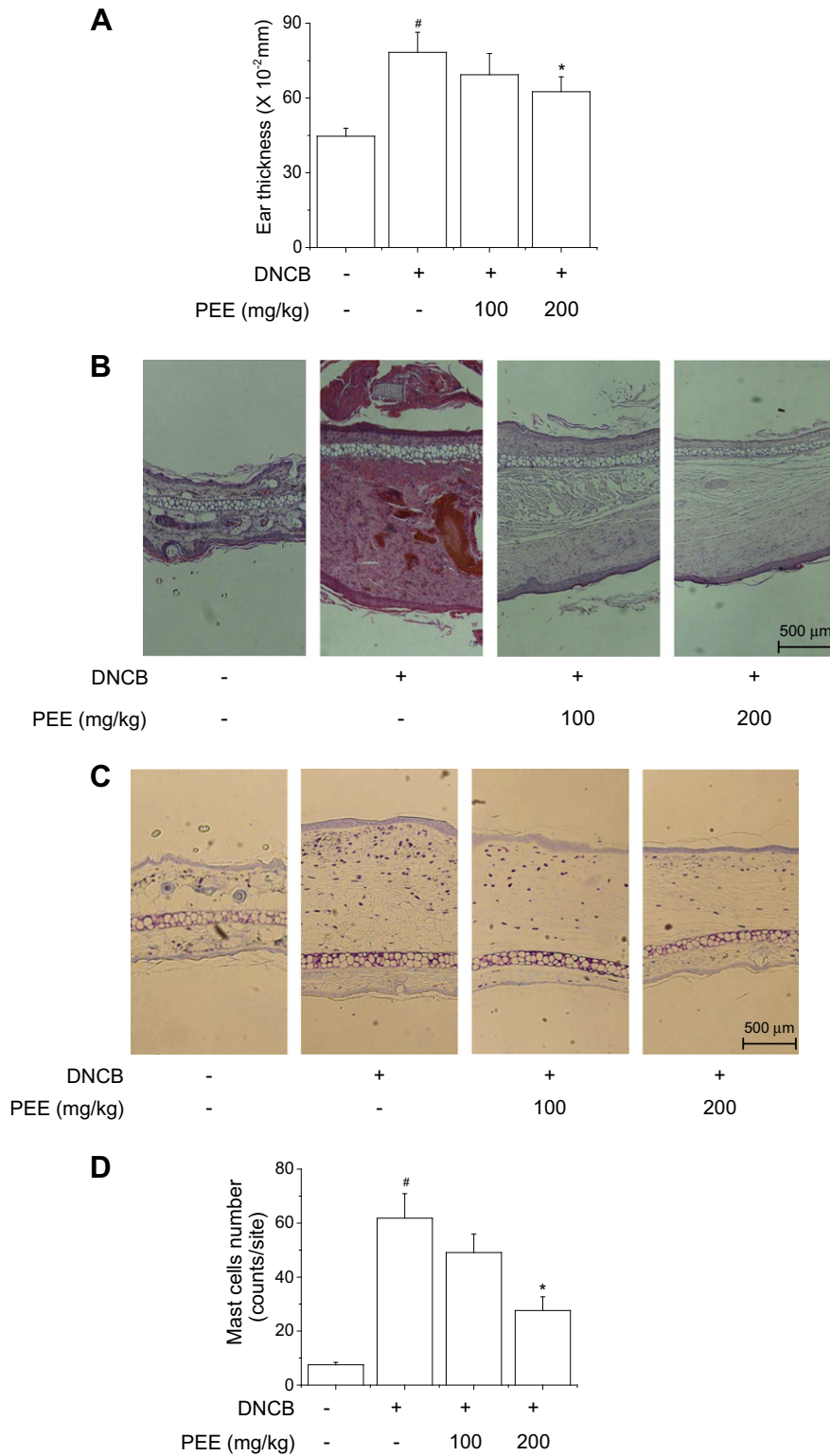


Fig. 3. Histopathological features of AD-like skin lesions treated with PEE in NC/Nga mice. (A) Ear thickness was measured with a micrometer. (B) Ear lesions were removed and fixed with 10% formaldehyde solution. Thin sections were cut and stained with hematoxylin and eosin. (C) Thin sections were cut and stained with toluidine blue. (D) The number of mast cells in five sites chosen at random was counted. Magnification, 100 $\times$. Results shown are representatives of five observations. Results are presented as mean $\pm$ S.E.M. ($n = 5$). [#] $p < 0.05$, significantly different from the control group; ^{*} $p < 0.05$, significantly different from the DNCB-treated group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bromide, visualized, and photographed using a gel documentation system (UVP, Cambridge, UK). Band intensity was quantified by densitometry using Image J software (Image Processing and Analysis in Java, NIH, USA). The relative expression levels of target genes were normalized using GAPDH as an internal control.

2.8. ELISA assay

Cytokines in serum level were measured by sandwich ELISA using the OptEIA Set mouse IgE kit from BD Biosciences and the DuoSet mouse CCL17/TARC kit from R&D Systems, according to the manufacturer's instructions. Cytokine

concentrations were calculated using a linear regression equation obtained from standard absorbance values.

2.9. Statistical analysis

All experiments were repeated at least three times. Results are reported as means $\pm$ standard error of the mean (S.E.M.). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test, using $p < 0.05$ as the level of significance.

3. Results

3.1. Effects of PEE on DNCB-induced AD-like skin severity in Nc/Nga mice

To evaluate the effectiveness of PEE against AD-like skin lesions, mice treated 100 or 200 mg/kg PEE per day after induction of AD-like skin lesions by DNCB application. The skin conditions were investigated twice a week for 64 days using the dermatitis severity. Repeated application of DNCB significantly increased dermatitis severity scores and symptoms of hemorrhage, edema, scarring, dryness, and erosion in NC/Nga mice. The DNCB-increased AD symptoms were suppressed by PEE treatment (Fig. 2).

3.2. Effect of PEE on DNCB-induced AD-like histopathological changes in Nc/Nga mice

On day 64 after the first application of DNCB, repeated application of DNCB significantly increased ear thickness in mice compared with control group (Fig. 3A). Furthermore, ear lesions showed hyperkeratosis and thickening of the dermis, as well as infiltration of inflammatory cells and mast cells in DNCB-treated group compared with the control group (Fig. 3B–D). These observations were all suppressed by PEE treatment. These results suggest that PEE decreases AD symptoms in NC/Nga mice.

3.3. Effect of PEE on DNCB-induced serum IgE and TARC levels in Nc/Nga mice

An important characteristic for AD is the secretion of IgE and TARC. Mast cells are known as key effector cells in IgE-mediated allergic disorders. Upon activation, mast cells undergo degranulation and release a variety of biologically active substances, which play an important role in the development of AD (Amin, 2012; Stone et al., 2010). Thymus and activation-regulated chemokine (TARC) is a functional ligand for CCR4, a chemokine receptor expressed by most skin-homing Th cells in the circulation by skin-infiltrating lymphocytes (Campbell et al., 1999). TARC is over-produced in the skin of patients with AD (Morita et al., 2010; Saeki and Tamaki, 2006). We examined the effect of PEE on the secretion of IgE and TARC by DNCB. Repeated application of DNCB significantly induced serum IgE and TARC levels in mice. PEE suppressed the serum levels of IgE and TARC by DNCB (Fig. 4).

3.4. Effect of PEE on DNCB-induced mRNA expression of TNF- α , INF- γ , IL-4, IL-5, and IL-13 in NC/Nga mice

Although Th2 cytokines are dominant in the acute phase, Th1 cytokines are highly expressed and contribute to the AD pathogenesis in the chronic phase (Leung and Bieber, 2003). We also investigated the inhibitory effect of PEE on the mRNA expression of Th1 and Th2 cytokines in the ear lesions. Repeated application of DNCB significantly increased mRNA expression of TNF- α , INF- γ , IL-4, IL-5, and IL-13. PEE treatment significantly inhibited these cytokines gene expression (Fig. 5). These results suggest that PEE suppresses DNCB-induced inflammatory cytokine expression, leading to the

inhibition of skin inflammation caused by the infiltration of inflammatory cells and mast cells.

4. Discussion

A number of biologically active compounds, including polysaccharides, vitamins, amino acids, and trace elements, has been identified in different mushroom species (Zaidman et al., 2005). Polysaccharides, mainly α - or β -glucans, protein-bound polysaccharides, or glycoproteins, demonstrated immunomodulatory activities through increased production of cytokines by dendritic cells and activation of natural killer cells (Borchers et al., 2008). Oyster mushrooms (PEE species) belong to the world consumed mushrooms, in addition to their nutritional value, demonstrate health-promoting (anti-oxidant, anti-atherosclerotic, and anti-tumor) effects, hypolipidemic effect, estrogen-like activity, and bone-protecting effect (Jayakumar et al., 2008; Mori et al., 2008; Jedinak and Sliva, 2008; Mizutani et al., 2010; Shimizu et al., 2006; Kim et al., 2006). Furthermore, acidic glycosphingolipids of mushroom (*Hypsizigus marmoreus* and *P. eryngii*) might contribute to the retention of immunohomeostasis through the natural killer T cell activation (Nozaki et al., 2008, 2010). Nevertheless, the effect of PEE on AD-like skin lesions remains unclear. Therefore, this study investigated the inhibitory effect of PEE on AD-like skin lesions induced by DNCB in NC/Nga mice.

AD is a chronic relapsing inflammatory skin disease in prevalence around the world. Many researches are needed to

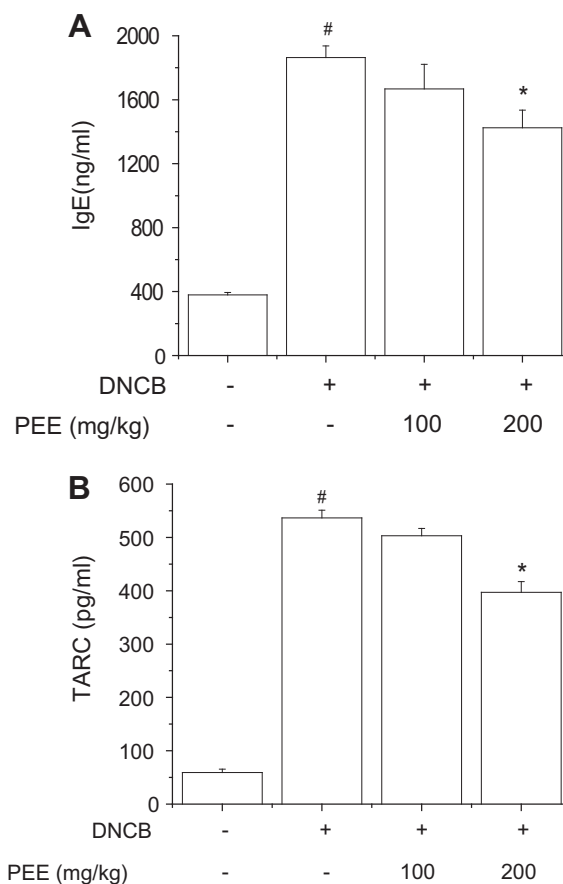


Fig. 4. Effects of PEE on DNCB-induced IgE and TARC level in NC/Nga mice. Serum was collected 24 h after the last PEE and/or DNCB treatment. Serum levels of (A) IgE and (B) TARC were analyzed by ELISA. Results are presented as mean $\pm$ S.E.M. ($n = 5$). [#] $p < 0.05$, significantly different from the control group; ^{*} $p < 0.05$, significantly different from the DNCB-treated group.

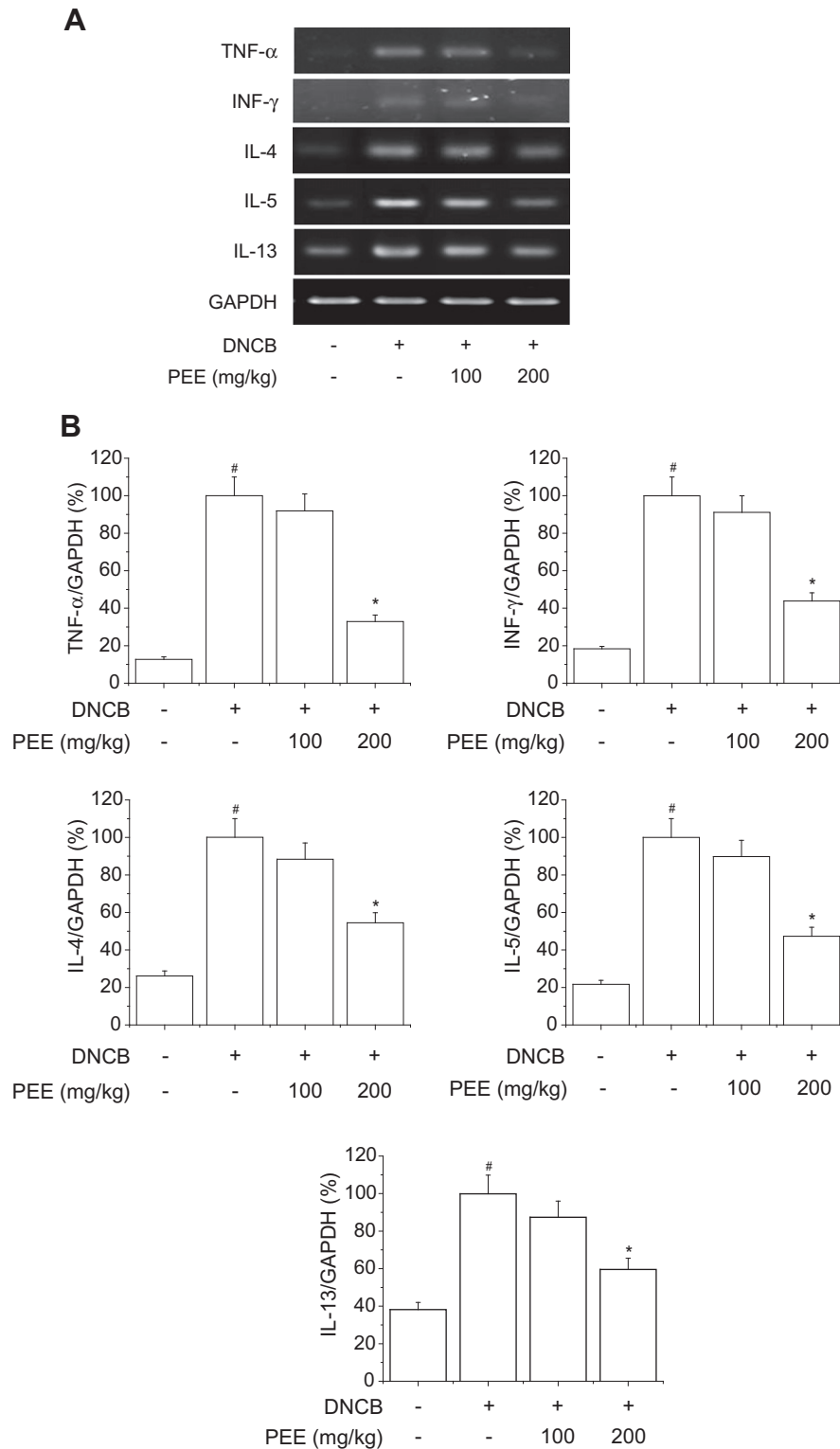


Fig. 5. Effects of PEE on DNCB-induced mRNA expression of Th1 and Th2 cytokines in NC/Nga mice. (A) Total RNA was extracted from ear tissue and mRNA levels of TNF- α , INF- γ , IL-4, IL-5, IL-13, and GAPDH were determined by semi-quantitative RT-PCR. Results shown are representatives of five observations. (B) The intensity of PCR bands was measured using the NIH Image J program. Results are presented as mean $\pm$ S.E.M. ($n = 5$). # $p < 0.05$, significantly different from the control group; * $p < 0.05$, significantly different from the DNCB-treated group.

development new treatment for patients suffering from AD. The T cell according to cytokine and chemokine receptor expression has revealed a new Th cell classification, Th1, Th2, Th17, and regulatory T cell subsets that play an important role in allergic disorders (Werfel, 2009). AD skin contains an increased number of Langer-

hans cells and dendritic cells expressing the high-affinity receptor for IgE (Novak et al., 2001). These antigen-presenting cells (APCs) seem to have an important role in allergen to Th2 and Th1 cells (von Bubnoff et al., 2001). AD has been associated with Th2 responses is driven by the production of IL-4, IL-5, and IL-13. Acute

AD skin lesions exhibit Th2-dominant response characterized by dermal infiltration of CD4⁺ or CD8⁺ T cells, eosinophils, and increased skin expression of Th2 cytokines. Conversely, Th1 responses are characterized by the producing the effector cytokine TNF- α and IFN- γ . Chronic phase demonstrates a local Th1 response and tissue remodeling with increased deposition of collagen and dermal thickening (Niebuhr and Werfel, 2010; Yuan et al., 2010, 2011).

Experimental animal models of AD are used DNCB treatment to simulate the clinical features of human AD-like skin symptoms, such as erythema, hemorrhage, and edema (Pokharel et al., 2008). NC/Nga mouse is a valuable model for research on the pathogenesis of AD. Therefore, in this study, AD-like skin lesions were induced in NC/Nga mice by the topical application of DNCB. Topical application of PEE significantly inhibited the DNCB-induced AD-like skin symptoms in NC/Nga mice.

At present, the pathogenesis of DNCB-induced contact hypersensitivity is predominantly the result of T cell-mediated immune responses. After subsequent exposures to DNCB, macrophages accumulate at the site of DNCB exposure, contributing to the development of AD (Tuckermann et al., 2007). Many studies reported that the severity of contact hypersensitivity was significantly lessened in mice lacking either CD4⁺ or CD8⁺ T cells, and these reports suggested that the involvement of T cells in the development of contact hypersensitivity (Wang et al., 2000; Ulker et al., 2011; von Bonin et al., 2011). DNCB has also reported that animals exposed to chemical allergens, such as 2,4-dinitrofluorobenzene, picryl chloride, and oxazolone, had an increased T cell population in their draining lymph nodes (Larsen et al., 2007). Similarly, exposure of animals to contact allergens also led to an increased percentage of antigen-presenting cells (APCs) in the draining lymph nodes (Watanabe et al., 2007; Askenase, 2001; Campos et al., 2006).

AD is one of the most common and severe skin diseases, which is characterized by eczematous skin lesion and IgE hypersecretion (Lee and Yu, 2011). Furthermore, AD results from dermal inflammation, a hallmark feature of which is a breakdown in the immunologic balance between Th1 and Th2 cells (Oyoshi et al., 2009). Consistent with these studies, we found that serum IgE level was significantly increased by repeated DNCB application in NC/Nga mice, as well as TNF- α , INF- γ , IL-4, IL-5 and IL-13 mRNA expression by activated Th1 and Th2 cells. These cytokines were associated with severe skin lesions. Topical application of PEE significantly attenuated DNCB-induced AD-like skin severity, IgE level, and TNF- α , INF- γ , IL-4, IL-5, and IL-13 mRNA expression in a dose dependent manner.

Most patients with AD are pathologically characterized by infiltration of inflammatory cells and mast cells (Fujii et al., 2009; Plagier et al., 2009). The infiltration of mast cells into the dermis is a necessary factor to define an appropriate animal model for AD (Matsuoka et al., 2003). In this study, we showed that repeated topical application of DNCB resulted in inflammatory cells and mast cells infiltration into the dermis in the ears of NC/Nga mice. Also, ear lesions illustrated that ears area of DNCB-applied group was thickened because of dermal hyperplasia and consequent infiltration of inflammatory cells and mast cells. Topical application of PEE significantly inhibited the DNCB-induced infiltration of inflammatory cells and mast cells and thickness of dermis in a dose dependent manner.

TARC is a member of the CC chemokine group that is constitutively expressed in the thymus and is produced by dendritic cells, langerhans cells, and keratinocytes (Imai et al., 1996; Sallusto et al., 1998; Vestergaard et al., 1999; Saeki and Tamaki, 2006). TARC is a selective chemoattractant that assist in the recruitment and migration of Th2 cells. TARC plays important roles in the development of skin diseases. In this study, we found that serum TARC level was

significantly increased by repeated DNCB application in NC/Nga mice. Topical application of PEE significantly attenuated DNCB-induced TARC level in a dose dependent manner.

In conclusion, this study suggested that topical application of PEE attenuated the development of AD-like skin lesions in NC/Nga mice. Attenuation of the Th1 and Th2 responses and inflammatory cells and mast cells infiltration may be the major mechanisms for the therapeutic effect. These results suggest that PEE treatment could provide an effective alternative therapy for the management of AD. Further studies are needed to elucidate the molecular mechanism of therapeutic potentials of PEE in the prevention of AD.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

- Amin, K., 2012. The role of mast cells in allergic inflammation. *Respir. Med.* 106, 9–14.
- Askenase, P.W., 2001. Yes T cells, but three different T cells (alphabeta, gammadelta and NK T cells), and also B-1 cells mediate contact sensitivity. *Clin. Exp. Immunol.* 125, 345–350.
- Blume-Peytavi, U., Wahn, U., 2011. Optimizing the treatment of atopic dermatitis in children: a review of the benefit/risk ratio of methylprednisolone aceponate. *J. Eur. Acad. Dermatol. Venereol.* 25, 508–515.
- Borchers, A.T., Krishnamurthy, A., Keen, C.L., Meyers, F.J., Gershwin, M.E., 2008. The immunobiology of mushrooms. *Exp. Biol. Med.* 233, 259–276.
- Campbell, J.J., Haraldsen, G., Pan, J., Rottman, J., Qin, S., Ponath, P., Andrew, D.P., Warnke, R., Ruffing, N., Kassam, N., Wu, L., Butcher, E.C., 1999. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 400, 776–780.
- Campos, R.A., Szczepanik, M., Itakura, A., Lisbonne, M., Dey, N., Leite-de-Moraes, M.C., Askenase, P.W., 2006. Interleukin-4-dependent innate collaboration between iNKT cells and B-1 B cells controls adaptative contact sensitivity. *Immunology* 117, 536–547.
- Choi, J.H., Han, E.H., Park, B.H., Kim, H.G., Hwang, Y.P., Chung, Y.C., Lee, Y.C., Jeong, H.G., 2012. Platycodi radix suppresses development of atopic dermatitis-like skin lesions. *Environ. Toxicol. Pharmacol.* 33, 446–452.
- Fujii, Y., Takeuchi, H., Sakuma, S., Sengoku, T., Takakura, S., 2009. Characterization of a 2,4-dinitrochlorobenzene-induced chronic dermatitis model in rats. *Skin Pharmacol. Physiol.* 22, 240–247.
- Han, E.H., Hwang, Y.P., Kim, H.G., Choi, J.H., Im, J.H., Yang, J.H., Lee, H.U., Chun, S.S., Chung, Y.C., Jeong, H.G., 2011. Inhibitory effect of *Pleurotus eryngii* extracts on the activities of allergic mediators in antigen-stimulated mast cells. *Food Chem. Toxicol.* 49, 1416–1425.
- Hashimoto, Y., Takaoka, A., Sugimoto, M., Honma, Y., Sakurai, T., Futaki, N., Arai, I., 2011. Itch-associated scratching contributes to the development of dermatitis and hyperimmunoglobulinemia E in NC/Nga mice. *Exp. Dermatol.* 20, 820–825.
- Hengge, U.R., Ruzicka, T., Schwartz, R.A., Cork, M.J., 2006. Adverse effects of topical glucocorticosteroids. *J. Am. Acad. Dermatol.* 54, 1–15.
- Hiramoto, K., Kobayashi, H., Ishii, M., Sato, E., Inoue, M., 2010. Increased alpha-melanocyte-stimulating hormone (alpha-MSH) levels and melanocortin receptors expression associated with pigmentation in an NC/Nga mouse model of atopic dermatitis. *Exp. Dermatol.* 19, 132–136.
- Imai, T., Yoshida, T., Baba, M., Nishimura, M., Kakizaki, M., Yoshie, O., 1996. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J. Biol. Chem.* 271, 21514–21521.
- Jayakumar, T., Sakthivel, M., Thomas, P.A., Geraldine, P., 2008. *Pleurotus ostreatus*, an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain. *Chem. Biol. Interact.* 176, 108–120.
- Jednak, A., Sliva, D., 2008. *Pleurotus ostreatus* inhibits proliferation of human breast and colon cancer cells through p53-dependent as well as p53-independent pathway. *Int. J. Oncol.* 33, 1307–1313.
- Jung, B.G., Cho, S.J., Ko, J.H., Lee, B.J., 2010. Inhibitory effects of interleukin-10 plasmid DNA on the development of atopic dermatitis-like skin lesions in NC/Nga mice. *J. Vet. Sci.* 11, 213–220.
- Kalyoncu, F., Oskay, M., Sağlam, H., Erdoğan, T.F., Tamer, A.U., 2010. Antimicrobial and antioxidant activities of mycelia of 10 wild mushroom species. *J. Med. Food* 13, 415–419.

- Kim, H.S., Kim, D.H., Kim, B.K., Yoon, S.K., Kim, M.H., Lee, J.Y., Kim, H.O., Park, Y.M., 2011. Effects of topically applied Korean red ginseng and its genuine constituents on atopic dermatitis-like skin lesions in NC/Nga mice. *Int. Immunopharmacol.* 11, 280–285.
- Kim, S.W., Kim, H.G., Lee, B.E., Hwang, H.H., Baek, D.H., Ko, S.Y., 2006. Effects of mushroom, *Pleurotus eryngii*, extracts on bone metabolism. *Clin. Nutr.* 25, 166–170.
- Kishimoto, T., Hirano, T., 1988. Molecular regulation of B lymphocyte response. *Annu. Rev. Immunol.* 6, 485–512.
- Kohara, Y., Tanabe, K., Matsuoka, K., Kanda, N., Matsuda, H., Karasuyama, H., Yonekawa, H., 2001. A major determinant quantitative-trait locus responsible for atopic dermatitis-like skin lesions in NC/Nga mice is located on chromosome 9. *Immunogenetics* 53, 15–21.
- Larsen, J.M., Geisler, C., Nielsen, M.W., Boding, L., Von Essen, M., Hansen, A.K., Skov, L., Bonefeld, C.M., 2007. Cellular dynamics in the draining lymph nodes during sensitization and elicitation phases of contact hypersensitivity. *Contact Dermatitis* 57, 300–308.
- Lee, C.H., Yu, H.S., 2011. Biomarkers for itch and disease severity in atopic dermatitis. *Curr. Probl. Dermatol.* 41, 136–148.
- Leung, D.Y., Bieber, T., 2003. Atopic dermatitis. *Lancet* 361, 151–160.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C., 1997. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int. Immunol.* 9, 461–466.
- Matsuoka, H., Maki, N., Yoshida, S., Arai, M., Wang, J., Oikawa, Y., Ikeda, T., Hirota, N., Nakagawa, H., Ishii, A., 2003. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* 58, 139–145.
- Mizutani, T., Inatomi, S., Inazu, A., Kawahara, E., 2010. Hypolipidemic effect of *Pleurotus eryngii* extract in fat-loaded mice. *J. Nutr. Sci. Vitaminol.* 56, 48–53.
- Mori, K., Kobayashi, C., Tomita, T., Inatomi, S., Ikeda, M., 2008. Antiatherosclerotic effect of the edible mushrooms *Pleurotus eryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizygus marmoreus* (Bunashimeji) in apolipoprotein E-deficient mice. *Nutr. Res.* 28, 335–342.
- Morita, E., Takahashi, H., Niihara, H., Dekio, I., Sumikawa, Y., Murakami, Y., Matsunaka, H., 2010. Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy* 65, 1166–1172.
- Niebuhr, M., Werfel, T., 2010. Innate immunity, allergy and atopic dermatitis. *Curr. Opin. Allergy Clin. Immunol.* 10, 463–468.
- Novak, N., Kraft, S., Bieber, T., 2001. IgE receptors. *Curr. Opin. Immunol.* 13, 721–726.
- Nozaki, H., Itonori, S., Sugita, M., Nakamura, K., Ohba, K., Suzuki, A., Kushi, Y., 2008. Mushroom acidic glycosphingolipid induction of cytokine secretion from murine T cells and proliferation of NK1.1 alpha/beta TCR-double positive cells in vitro. *Biochem. Biophys. Res. Commun.* 373, 435–439.
- Nozaki, H., Itonori, S., Sugita, M., Nakamura, K., Ohba, K., Ohnishi, M., Imai, K., Igarashi, Y., Suzuki, A., Kushi, Y., 2010. Invariant Valpha14 natural killer T cell activation by edible mushroom acidic glycosphingolipids. *Biol. Pharm. Bull.* 33, 580–584.
- Oyoshi, M.K., He, R., Kumar, L., Yoon, J., Geha, R.S., 2009. Cellular and molecular mechanisms in atopic dermatitis. *Adv. Immunol.* 102, 135–226.
- Park, S., Lee, J.B., Kang, S., 2012. Topical application of chrysanthemum indicum I. attenuates the development of atopic dermatitis-like skin lesions by suppressing serum IgE levels, IFN- γ , and IL-4 in Nc/Nga mice. *Evid. Based Complement. Alternat. Med.* 2012, 1–8.
- Plager, D.A., Henke, S.A., Matsuwaki, Y., Madaan, A., Squillace, D.L., Dierkhising, R.A., Kita, H., 2009. Pimecrolimus reduces eosinophil activation associated with calcium mobilization. *Int. Arch. Allergy Immunol.* 149, 119–126.
- Pokharel, Y.R., Lim, S.C., Kim, S.C., Heo, T.H., Choi, H.K., Kang, K.W., 2008. Sopungyangjae-Tang inhibits development of dermatitis in Nc/Nga mice. *Evid. Based Complement. Alternat. Med.* 5, 173–180.
- Poulsen, L.K., Hummelshoj, L., 2007. Triggers of IgE class switching and allergy development. *Ann. Med.* 39, 440–456.
- Rodriguez Estrada, A.E., Royse, D.J., 2007. Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. *Bioresour. Technol.* 98, 1898–1906.
- Saeki, H., Tamaki, K., 2006. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J. Dermatol. Sci.* 43, 75–84.
- Sallusto, F., Schaerli, P., Loetscher, P., Schaniel, C., Lenig, D., Mackay, C.R., Qin, S., Lanzavecchia, A., 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* 28, 2760–2769.
- Sawada, E., Yoshida, N., Sugiura, A., Imokawa, G., 2012. Th1 cytokines accentuate but Th2 cytokines attenuate ceramide production in the stratum corneum of human epidermal equivalents: an implication for the disrupted barrier mechanism in atopic dermatitis. *J. Dermatol. Sci.* 68, 25–35.
- Shimizu, K., Yamanaka, M., Gyokusen, M., Kaneko, S., Tsutsui, M., Sato, J., Sato, I., Sato, M., Kondo, R., 2006. Estrogen-like activity and prevention effect of bone loss in calcium deficient ovariectomized rats by the extract of *Pleurotus eryngii*. *Phytother. Res.* 20, 659–664.
- Stone, K.D., Prussin, C., Metcalfe, D.D., 2010. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* 125, S73–S80.
- Tuckermann, J.P., Kleiman, A., Moriggl, R., Spanbroek, R., Neumann, A., Illing, A., Clausen, B.E., Stride, B., Förster, I., Habenicht, A.J., Reichardt, H.M., Tronche, F., Schmid, W., Schütz, G., 2007. Macrophages and neutrophils are the targets for immune suppression by glucocorticoids in contact allergy. *J. Clin. Invest.* 117, 1381–1390.
- Udompatikul, M., Limpa-o-vart, D., 2012. Comparative trial of 5% dexpanthenol in water-in-oil formulation with 1% hydrocortisone ointment in the treatment of childhood atopic dermatitis: a pilot study. *J. Drugs Dermatol.* 11, 366–374.
- Ulker, O.C., Atak, A., Ates, I., Karakaya, A., 2011. Evaluation of auricular lymph node cell lymphocyte proliferation and cytokine production as non-radioactive endpoints during murine contact allergy. *J. Immunotoxicol.* 8, 131–139.
- Vestergaard, C., Yoneyama, H., Murai, M., Nakamura, K., Tamaki, K., Terashima, Y., Imai, T., Yoshie, O., Irimura, T., Mizutani, H., Matsushima, K., 1999. Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J. Clin. Invest.* 104, 1097–1105.
- von Bonin, A., Rausch, A., Mengel, A., Hitchcock, M., Krüger, M., von Ahsen, O., Merz, C., Röse, L., Stock, C., Martin, S.F., Leder, G., Döcke, W.D., Asadullah, K., Zügel, U., 2011. Inhibition of the IL-2-inducible tyrosine kinase (Itk) activity: a new concept for the therapy of inflammatory skin diseases. *Exp. Dermatol.* 20, 41–47.
- von Bubnoff, D., Geiger, E., Bieber, T., 2001. Antigen-presenting cells in allergy. *J. Allergy Clin. Immunol.* 108, 329–339.
- Wang, B., Fujisawa, H., Zhuang, L., Freed, I., Howell, B.G., Shahid, S., Shivji, G.M., Mak, T.W., Sauder, D.N., 2000. CD4+ Th1 and CD8+ type 1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. *J. Immunol.* 165, 6783–6790.
- Watanabe, R., Fujimoto, M., Ishiura, N., Kuwano, Y., Nakashima, H., Yazawa, N., Okochi, H., Sato, S., Tedder, T.F., Tamaki, K., 2007. CD19 expression in B cells is important for suppression of contact hypersensitivity. *Am. J. Pathol.* 171, 560–570.
- Werfel, 2009. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J. Invest. Dermatol.* 129, 1878–1891.
- Yamanaka, K., Mizutani, H., 2011. The role of cytokines/chemokines in the pathogenesis of atopic dermatitis. *Curr. Probl. Dermatol.* 41, 80–92.
- Yang, G., Lee, K., Lee, M.H., Kim, S.H., Ham, I.H., Choi, H.Y., 2011. Inhibitory effects of *Chelidonium majus* extract on atopic dermatitis-like skin lesions in NC/Nga mice. *J. Ethnopharmacol.* 138, 398–403.
- Yuan, X.Y., Liu, W., Zhang, P., Wang, R.Y., Guo, J.Y., 2010. Effects and mechanisms of aloperine on 2,4-dinitrofluorobenzene-induced allergic contact dermatitis in BALB/c mice. *Eur. J. Pharmacol.* 629, 147–152.
- Yuan, X.Y., Ma, H.M., Li, R.Z., Wang, R.Y., Liu, W., Guo, J.Y., 2011. Topical application of aloperine improves 2,4-dinitrofluorobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice. *Eur. J. Pharmacol.* 658, 263–269.
- Zaidman, B.Z., Yassin, M., Mahajna, J., Wasser, S.P., 2005. Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* 67, 453–468.
- Zhang, E.Y., Chen, A.Y., Zhu, B.T., 2009. Mechanism of dinitrochlorobenzene-induced dermatitis in mice. role of specific antibodies in pathogenesis. *PLoS. One* 4, 1–12.