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(R)-(+)-pulegone suppresses allergic and inflammation responses on 2,4-dinitrochlorobenzene-induced atopic dermatitis in mice model

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ABSTRACT

Background: (R)-(+)-pulegone (PLG), a biotransformation of monoterpene ketones, is one of essential oils of Labiatae family. Although PLG was reported to have anti-inflammatory and anti-histamine effects, the therapeutic effects of PLG on atopic dermatitis (AD) have not been reported yet.

Objective: This study investigated the anti-AD effects and underlying mechanisms of PLG in AD-induced mice.

Methods: BALB/c male mice were challenged with 2, 4-dinitrochlorobenzene (DNCB, 1%) to induce AD. After 4 days of rest, PLG (0.1, 1 and 10 μ M) were topically applied to dorsal skin for 2 weeks with secondary elicitation using 0.5% DNCB. Histological changes were identified by H&E staining and mast cells were evaluated by toluidine blue staining. Pro-inflammatory cytokines and serum IgE levels were analyzed by ELISA. Inflammatory mediators were measured by western blotting assay.

Results: Topical treatment with PLG significantly suppressed skin thickness and scratching behavior compared with control group. Expression of nerve growth factor was also decreased by PLG treatment. PLG administration decreased serum IgE levels and the number of mast cells in mice model of DNCB-induced AD. The levels of IL-4, IFN- γ , IL-6, TNF- α and IL-1 β in dorsal skin of PLG-treated group were lower than those in the control group. PLG inhibited the phosphorylation of MAPKs, as well as I κ B α degradation and NF- κ B activation.

Conclusions: PLG attenuated the symptoms of AD by suppressing cytokines production, the phosphorylation of MAPKs and the activation of NF- κ B signaling. These data suggest that PLG may be an effective natural compound for the treatment of inflammatory skin diseases.

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1. Introduction

Atopic dermatitis (AD) is a type of inflammatory skin disease, which affects from infants and adolescents to adults in developed countries [1]. Although AD is not a life-threatening disease, it remains a serious health problem and the incidence rates of AD is still increasing worldwide [2]. AD patients exhibit the eczematous lesions, itching, destruction of epidermal barrier, and inflammatory cell infiltration of the dermis [3]. The pathology of AD has not been fully defined, it has been implicated to involve in complex interactions of genetic, immune and environmental factors [4].

Topical corticosteroids are commonly prescribed in the first-line treatment for AD [5]. Severe AD patients require additional treatment, such as systemic immunosuppressant drugs or

phototherapy (ultraviolet; UV light). In addition, several alternative therapies are also used to treat AD, such as anti-histamine drug to alleviate itching, emollients to reduce the evaporation of water [6–8]. However, long-term use of steroidal drugs often causes side effects, such as thinning, rashes of the skin and striae distensae, leading to cracking and bleeding [9]. Therefore, effectiveness-based anti-inflammatory agents with fewer side effects has attracted attention.

Pulegone, a transformation of monoterpene ketones, is present in the leaves and flowering tops of several members of the mint family. Pulegone exist as two enantiomeric forms of (+) and (–) in nature, and (+) form is rich in most essential oils [10–12]. In the previous report, pulegone displayed anti-histaminic activity in the guinea-pig ileum [13]. Recently, a combination containing (R)-(+)-pulegone exerted an immuno-modulatory effect by decreasing IL-13 levels from lymphocytes in the immediate-type hypersensitivity responses [14]. These findings suggest that (R)-(+)-pulegone is a potent immuno-modulatory agent, which might be a promising natural alternative for the treatment of AD. In addition, it has been reported that the extract of *Schizonepeta tenuifolia* var.

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japonica (Maxim.) Kitag, which contains pulegone, inhibits the development of AD in mice [15] and suppresses inflammatory reaction in mouse peritoneal macrophages [16]. However, the effects of (R)-(+)-pulegone on atopic dermatitis has not been clarified yet.

In the present study, the therapeutic effects and underlying mechanisms of (R)-(+)-pulegone on DNCB-induced AD in mice models were elucidated. The clinical efficacy (R)-(+)-pulegone such as the histopathological features and scratching behavior for AD was evaluated. Several molecular biomarkers were analyzed in dorsal skin tissues and serum. Furthermore, nuclear factor (NF)- κ B and mitogen-activated protein kinase (MAPKs) in the skin lesion were evaluated.

2. Materials and methods

2.1. Animals

Male BALB/c mice (Six-weeks-old) were purchased from RaonBio Inc. (Yongin, Republic of Korea). The animals were housed in the room maintained at a temperature of $23 \pm 3^\circ\text{C}$ with a relative humidity of $55 \pm 10\%$ throughout the experiment. All mice were allowed access to water and food *ad libitum*. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The experiment was approved by Committee on Care and Use of Laboratory Animals of Kyung Hee University, Seoul, Korea (KHUASP (SE)-16-159).

2.2. Atopic dermatitis induction and sample treatments

After 7 days of adaptation, the mice randomly divided into 6 group as follow (n = 5): NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μM and DNCB; positive group), and PLG0.1, PLG1, PLG10 (treated with PLG 0.1, 1, 10 μM and DNCB, respectively). (R)-(+)-pulegone (PLG), 2,4-dinitrochlorobenzene (DNCB) and dexamethasone (DEX) were purchased from Sigma-Aldrich Chemical Co. (MO, USA). To develop atopic dermatitis-like skin lesion, the shaved dorsal skin of mice was treated with 200 μM of 1% DNCB solution (dissolved in acetone: olive = 4:1) once a day for 3 days,

expect NOR group. After four days of rest, 200 μl of dexamethasone 10 μM and (R)-(+)-pulegone (0.1, 1 and 10 μM , respectively) with 0.5% DNCB solution were applied on the dorsal skin for 11 days. Before sample treatment, 100 μl 4% SDS solution was applied to enhance skin barrier perturbation. The NOR group was treated with vehicle. DEX, PLG, SDS were dissolved in distilled water. Mice were sacrificed on day 19 of the experiment (Fig. 1A). After sacrifice, blood samples were collected by orbital puncture and dorsal skin were collected for histological analysis, immunohistological and molecular analyses. The chemical structure of the PLG used in this experiment is shown in Fig. 1B.

2.3. Histological analysis for histopathological features

For histopathological observation, the dorsal skins were fixed in 10% buffered neutral formalin for 24 h and embedded in paraffin wax. Paraffin-embedded blocks were cut to a thickness of 4 μm and the slides stained with hematoxylin and eosin for the skin thicknesses. For measurement of mast cell infiltration, the slides were stained with toluidine blue (Sigma-Aldrich, St. Louis, MO, USA). The slides of each group were evaluated by randomly selecting 5 photographs and were monitored using microscopy (LAS; Leica Microsystems, Buffalo Grove, IL, USA). The magnification was 100.

2.4. Measurement of scratching behavior

After 1 h from DNCB challenge, the number of scratching behaviors was evaluated (on 18 days; the day before sacrifice). The video camera (Model NEX-C3; Sony, Japan) was recorded their behavior for 20 min while fixed on the top of the mouse cage. Scratching movements by the hind paw were defined as a scratching in which a mouse lifted its hind paw until the paw was returned to the floor.

2.5. Measurement of total serum immunoglobulin E levels

The serum was obtained by centrifugation (10,000 $\times$ g, 10 min, RT) and then stored at -70°C until quantitative analysis. The serum concentrations of immunoglobulin E (IgE) was measured using a mouse IgE ELISA kit (BD Biosciences, New Jersey, USA) according to the manufacturer's instructions. After the reaction was stopped,

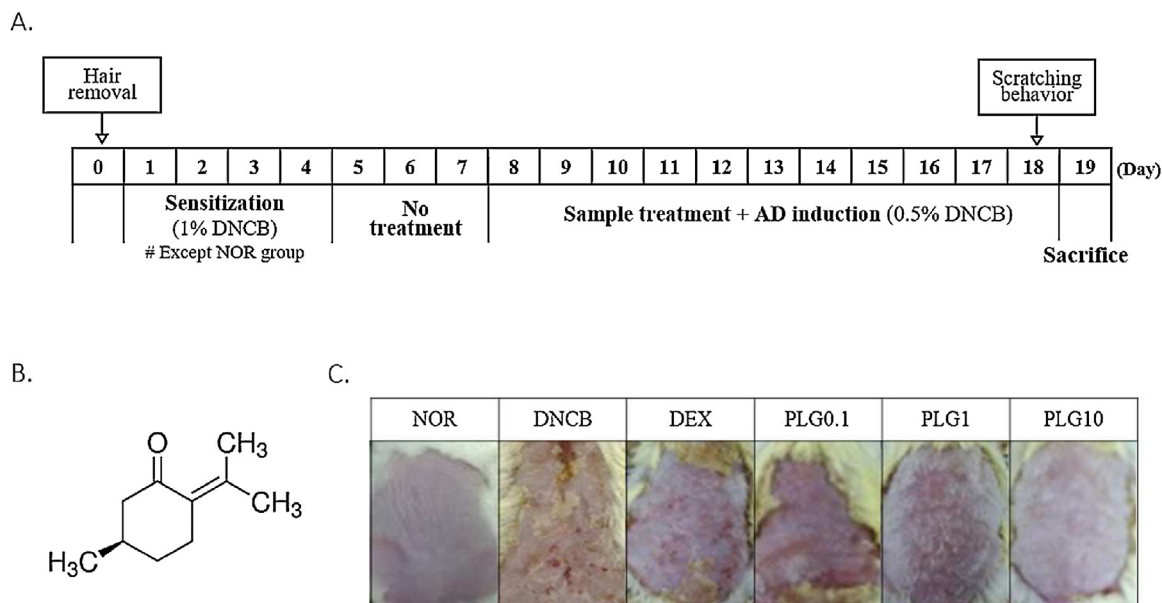


Fig. 1. The effects of PLG on DNCB-induced atopic dermatitis. (A) Experimental design (B) Chemical structure of PLG. (C) Photographs for the clinical features.

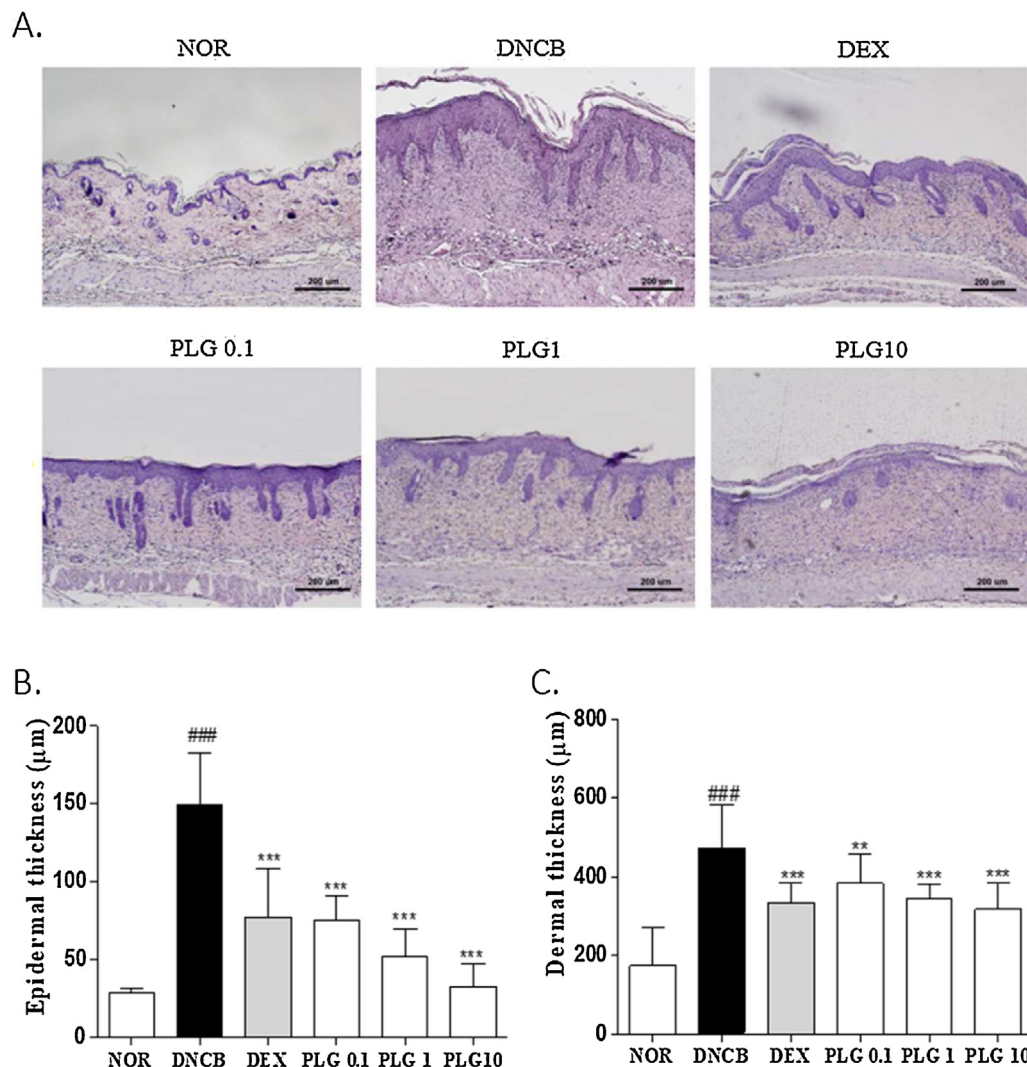


Fig. 2. The effects of PLG on histopathological features of mice with AD-like skin lesions. (A) The representative image of hematoxylin and eosin in dorsal skin. (B) Measurement of epidermis thickness and (C) dermis thickness. The magnification is 100 $\times$. ### $p < 0.001$ vs. NOR; *** $p < 0.001$ and ** $p < 0.01$ vs. DNCB. NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μ M and DNCB; positive group), and PLG0.1, PLG1 and PLG10 (treated with PLG 0.1, 1, 10 μ M and DNCB, respectively).

absorbance was measured at 450 nm using an ELISA reader (Molecular Devices, Downingtown, PA, USA).

2.6. Determination of Th2/ Th1 and pro-inflammatory cytokine expression in dorsal skin

100 mg of dorsal skin ($n = 5$) were homogenized in 1 ml tissue protein extraction (Pierce, Rockford, IL, USA) reagent with protease inhibitor (Roche, Indianapolis, IN, USA). The resulting homogenate was centrifuged at 12,000 $\times$ g for 30 min at 4 $^{\circ}$ C and the supernatant was carefully removed without disturbing the pellet. Bradford protein assay was used as a protein quantitative method. The concentrations of IL-4, -6, -1 β , IFN- γ and TNF- α in the supernatant were measured by an ELISA kit (BD Biosciences, New Jersey, USA), according to the manufacturer's instructions. After completion of the reaction, the optical density was read at 450 nm using an ELISA reader (Molecular Devices, Downingtown, PA, USA).

2.7. Preparation of protein extraction in dorsal skin

Frozen dorsal skin tissues (100 mg) were homogenized in 1 ml cytoplasmic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM

EDTA, 0.1 mM EGTA, 1 mM DTT, 0.15% Nonidet P-40, 50 mM β -glycerophosphate, 10 mM NaF, and 5 mM Na_3VO_4) containing protease inhibitors. The homogenate was incubated on ice for 1 h after vortexing and centrifuged at 12,000 $\times$ g for 15 min at 4 $^{\circ}$ C. The cytoplasmic protein was carefully obtained from upper supernatants without disturbing the pellet for assay of activated I κ B α and phosphorylated I κ B α . To extract the nuclear proteins, nuclear buffer containing the protease inhibitor cocktail was added to the pellet. Nuclear proteins were obtained in the same manner as the cytoplasmic protein preparation to assess NF- κ B expression. The expression of NGF and MAPKs (ERK 1/2, p38, JNK) were confirmed by total protein extracts using RIPA assay buffer (50 mM Tris-HCl pH 7.6, 150 mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA) containing protease inhibitor cocktail. The total protein extraction method is the same as the above cytoplasmic and nuclear protein.

2.8. Detection of inflammatory protein expression in dorsal skin

Each protein (cytoplasmic, nuclear, and total proteins) from the dorsal skin was quantitated by the Bradford method. The protein lysates were denatured at 96 $^{\circ}$ C for 5 min after mixing with SDS

loading buffer. The denatured protein was applied on 10% SDS polyacrylamide gel for electrophoresis. Separated proteins were transferred onto a PVDF membranes and the membranes were blocked with 5% BSA for 2 h at RT. The membranes were incubated overnight with primary antibodies against β -actin, NF- κ B, phospho-I κ B α , I κ B α , NF- κ B, ERK1/2, phospho-ERK1/2, JNK, phospho-JNK, p38, phospho-p38 (1:1000, Cell Signaling Technology, Danvers, MA, USA). After washing with 0.1% PBST for 45 min, membranes were then incubated with secondary antibodies (1:2000, Santa Cruz Biotechnology, Inc., CA) for 1 h at RT. Band

signals were visualized using an enhanced chemiluminescence solution (Amersham Pharmacia, Piscataway, NJ, USA). The band density was quantified by an image J analyzer.

2.9. Statistical analysis

Data are presented as the means $\pm$ standard error of the mean (SEM). Statistical analysis was performed using an independent *t*-test and one-way ANOVA followed by Dunnett's test. For all experiments, *p* < 0.05 was considered to be significant.

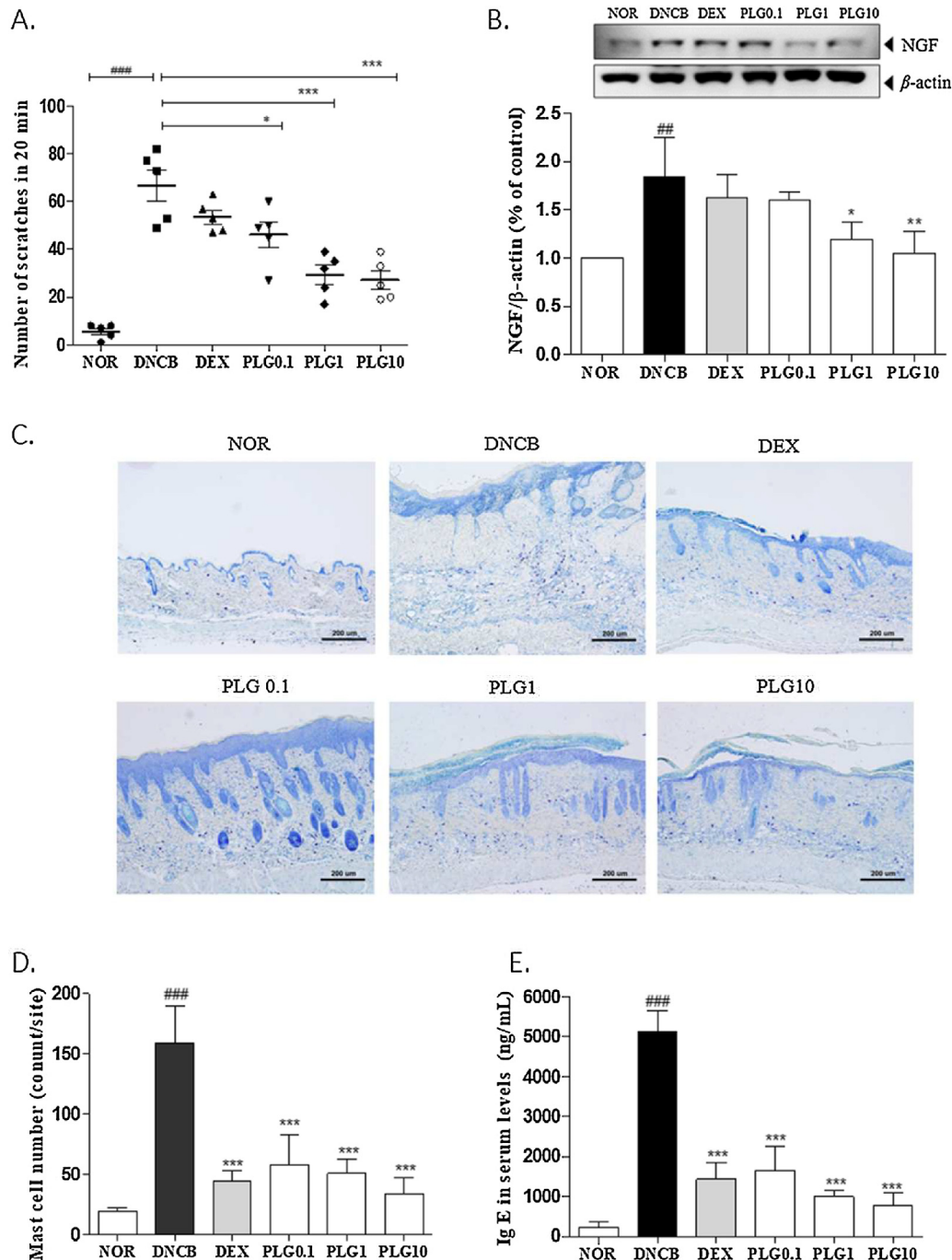


Fig. 3. The effects of PLG on allergic inflammation of mice with AD-like skin lesions. (A) The number of scratching behavior. (B) The NGF expression on dorsal skin. (C) Representative images of toluidine blue-stained mast cells in dorsal skin. The magnification is 100 $\times$. (D) The number of mast cells on dermis. (E) The levels of IgE in serum. ### *p* < 0.001 vs. NOR; *** *p* < 0.001, ** *p* < 0.01 and * *p* < 0.05 vs. DNCB. NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μ M and DNCB; positive group), and PLG0.1, PLG1 and PLG10 (treated with PLG 0.1, 1, 10 μ M and DNCB, respectively). Data from three independent experiments (*n* = 3).

3. Results

3.1. Effect of PLG on histopathological features

Clinical signs and symptoms of dermatitis rapidly was developed during DNCB application in all groups, except the NOR group. Topically application of PLG markedly suppressed the increased (Fig. 1C). The histopathological features of the dorsal skin lesions are shown in Fig. 2A. Following H&E staining, the DNCB group showed the thicker epidermal and dermal. The epidermal thickness in the DNCB group ($149 \pm 33.2 \mu\text{m}$) was approximately 5-folds higher than in NOR group ($29.2 \pm 2.3 \mu\text{m}$). PLG 0.1, 1 and 10 μM treatment clearly decreased the epidermis thickness to approximately 49.4%, 65.4% and 78.1% of DNCB group, respectively. The thickness of the epidermis in the DEX group was reduced by 48.2% as a positive control, compared to the DNCB group (Fig. 2B). The thickness of the dermis in the DNCB group was approximately $465.6 \pm 107.7 \mu\text{m}$, which was 2.6-fold thicker than the NOR group ($179.1 \pm 96.5 \mu\text{m}$), while PLG treatment reduced epidermal

thickness by approximately 19%, 26.1% and 32.6%, compared to the DNCB group (PLG0.1: $377.1 \pm 70.1 \mu\text{m}$, PLG1: $343.9 \pm 36.2 \mu\text{m}$, PLG10: $313.6 \pm 70.5 \mu\text{m}$). In addition, the dermis thickness of DEX group were $332.2 \pm 50.9 \mu\text{m}$, which was 20% lower than that of DNCB group (Fig. 2C).

3.2. Effect of PLG on scratching behavior and nerve growth factor expression

In itching test after last application of DNCB (on 18 day), DNCB group (66.8 ± 14.8) exhibited significant increases in scratching behavior compared with NOR group (5.6 ± 3.0). This increases of scratching behavior were significantly inhibited by topical application of DEX (53.6 ± 6.6). Significant decreases of scratching behaviors were detected at all concentrations of PLG in concentration-dependent manner, compared with DNCB group (46.2 ± 12.1 , 29.4 ± 8.8 and 27.2 ± 8.6 , respectively, Fig. 3A). The expression of nerve growth factor (NGF) in AD skin tissue was investigated by western blotting assay. DNCB challenge up-

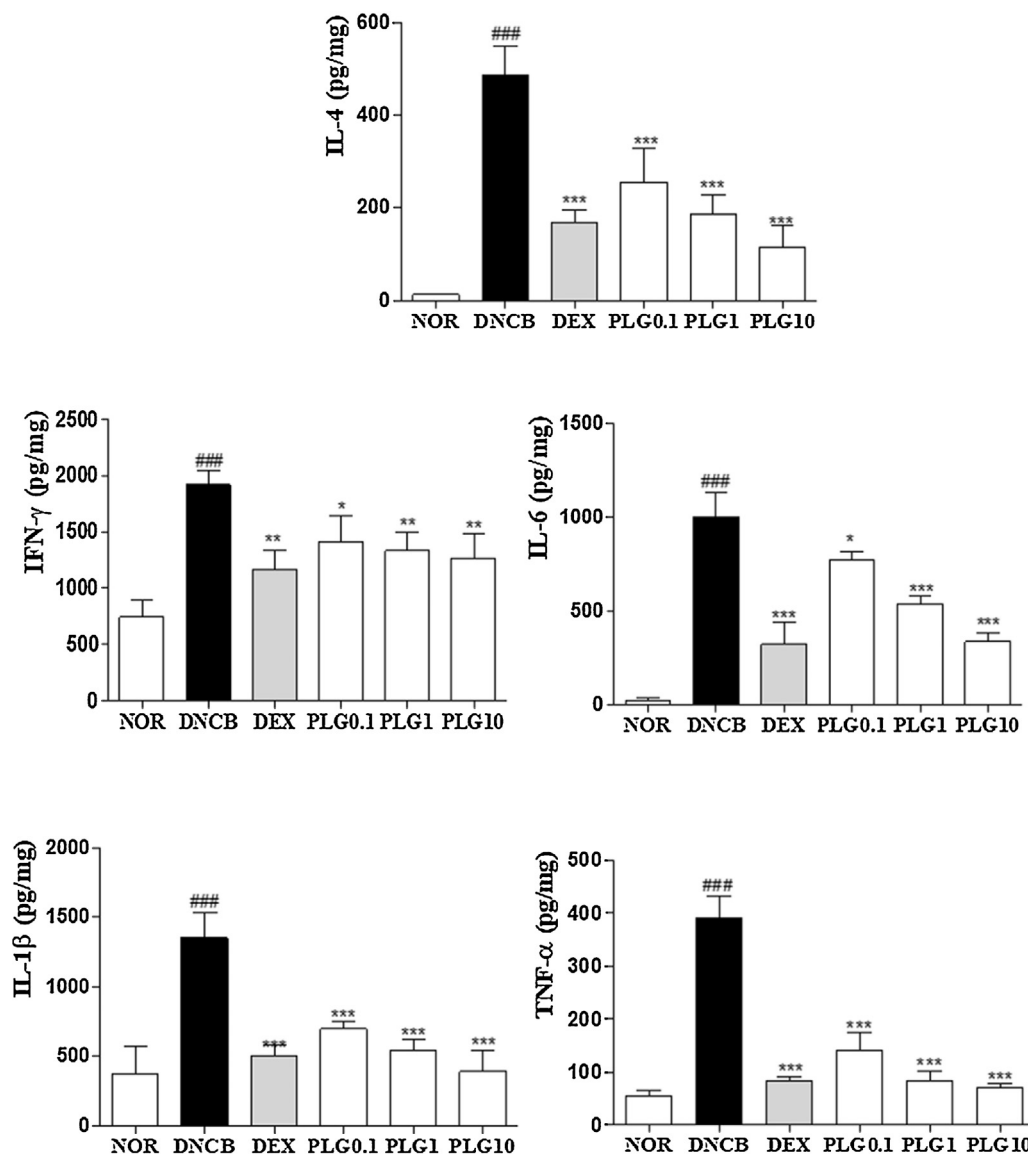


Fig. 4. The effects of PLG on the expression of Th2/Th1 and pro-inflammatory cytokines of mice with AD-like skin lesions. The levels of IL-4, IFN-γ, IL-6, IL-1β and TNF-α were performed on the dorsal skin by ELISA. ### $p < 0.001$ vs. NOR, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. DNCB. NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μM and DNCB; positive group), and PLG0.1, PLG1 and PLG10 (treated with PLG 0.1, 1, 10 μM and DNCB, respectively). Data from three independent experiments ($n = 3$).

regulated the NGF expression (about 1.8 folds) compared to the NOR group, whereas treatment with 1 μ M and 10 μ M PLG attenuated the DNCB induced NGF expression (the inhibition rate: PLG1: 35.3%, PLG10: 42.9%). However, the effect of dexamethasone on NGF expression was not shown significantly different (Fig. 3B).

3.3. Effects of PLG on the levels of IgE in serum and the number of mast

To determine whether PLG reduces the number of mast cells, we performed toluidine blue-staining (Fig. 3C). The number of mast cells were higher in the DNCB group (165.4 ± 29.7) than in the NOR group (19.6 ± 2.5). PLG treatment markedly suppressed the increased number of mast cells (PLG0.1: 58.2 ± 27.2 , PLG1: 50.8 ± 12.7 and PLG10: 33.0 ± 15.1). DEX group also exhibited reduction in the number of mast cells (44.6 ± 9.2 , Fig. 3D). Repetitive topical application of DNCB significantly induced total IgE levels in serum compared with in NOR group (NOR: 228 ± 137.6 ng/ml, DNCB: 5117.3 ± 528.4 ng/ml). Daily topically treatment with 0.1, 1 or 10 mg/ml PLG markedly inhibited total

IgE production as compared with DNCB alone (PLG0.1: 1649.1 ± 620.8 ng/ml, PLG1: 1003.3 ± 159.0 ng/ml, PLG10: 774.3 ± 307.2 ng/ml). The treatment of dexamethasone reduced the increase of total serum IgE secretion (1433.6 ± 416.2 ng/ml, Fig. 3E).

3.4. Effects of PLG on the levels of Th2, Th1 and pro-inflammatory cytokines

To confirm the anti-inflammatory action of PLG in AD mice, the expression of Th2, Th1 and pro-inflammatory cytokines was performed on the dorsal skin by ELISA (Fig. 4). As shown in Fig. 4, the levels of IL-4, IFN- γ , IL-6, IL-1 β and TNF- α in the dorsal skin of DNCB-induced AD was significantly increased compared with the NOR group (about- 36.4 fold, -2.6 fold, -41.2 fold, -3.6 fold and -7.0 fold). The elevated expression of IL-4, IFN- γ , IL-6, IL-1 β and TNF- α levels on dorsal skin was attenuated in the PLG group. Specially, the levels of IL-4, IL-1 β and TNF- α in PLG10 group are significantly lower than that in the DEX group.

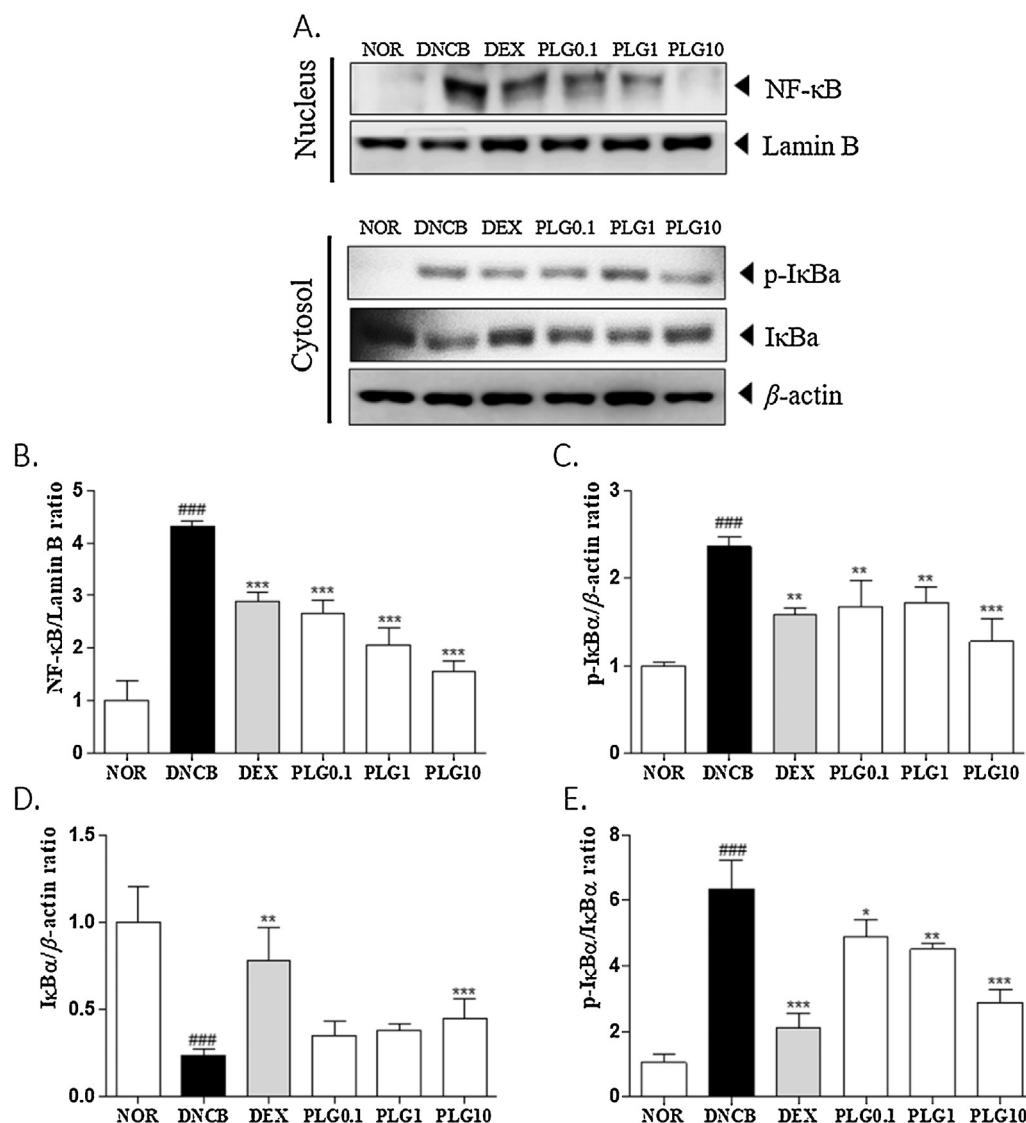


Fig. 5. The effects of PLG on the expressions of NF- κ B, phospho-I κ B α and I κ B α of mice with AD-like skin lesions. (A) NF- κ B signaling were performed on the dorsal skin by western blotting assay. Bar graphs show relative (B) NF- κ B (C) phospho-I κ B α (D) I κ B α and (E) phospho-I κ B α /I κ B α ratios. ### $p < 0.001$ vs. NOR, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. DNCB. NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μ M and DNCB; positive group), and PLG0.1, PLG1 and PLG10 (treated with PLG 0.1, 1, 10 μ M and DNCB, respectively). Data from three independent experiments ($n = 3$).

3.5. Effect of PLG on the expressions of NF- κ B, I κ B α and phospho-I κ B α

To examine whether the NF- κ B signaling could be modulated by PLG treatment, the expression of NF- κ B, I κ B α and phospho-I κ B α was performed on the dorsal skin by western blotting assay (Fig. 5A). In Fig. 5B, the expression of NF- κ B in the dorsal skin of DNCB-induced AD was significantly higher than the NOR group (about 4.3 fold). The increase in NF- κ B expression by DNCB was significantly decreased in the application of PLG and dexamethasone (reduction rate: PLG0.1: 38.7%, PLG1: 52.3%, PLG10: 63.9% and DEX: 33.2%). To further clarify the effect of PLG on down-stream signaling of NF- κ B signaling pathway, the expressions of phospho-I κ B α and I κ B α were confirmed. PLG group significantly reduced phospho-I κ B α and increased I κ B α expressions as compared with DNCB group (Fig. 5C, D). The ratio of phospho-I κ B α /I κ B α ratio in PLG group was decreased in a concentration-dependent manner as compared with the DNCB group (reduction rate: PLG0.1: 22.87%, PLG1: 28.9%, PLG10: 54.5% and DEX: 66.9%, Fig. 5E).

3.6. Effect of PLG on the expressions of MAPKs signaling

To further assess the inflammatory response, MAPKs signaling pathways in dorsal skin were analyzed by western blotting assay (Fig. 6A). Repeated DNCB exposure caused phosphorylation of MAPKs compared with the NOR group (phospho-ERK1/2: 2.1 fold, phospho-p38: 2.4 fold, phospho-JNK: 2.8 fold). PLG administration suppressed the phosphorylation of ERK, p38 and JNK in dorsal skin compared to the DNCB group. Specifically, phospho-ERK1/2 and -JNK expression showed a concentration-dependent reduction rate by PLG (Fig. 6B–D).

4. Discussion

AD is characterized by the infiltration of inflammatory cells, epidermal hypertrophy and hyperkeratosis in skin lesions [17]. In this study, topical elicitation of DNCB successfully induced AD-like skin, indicated by typical symptoms such as hemorrhage, excoriation, erosion and dryness. Treatment of PLG significantly alleviates the severity of AD in DNCB-induced AD-like skin. In addition, epidermal hyperkeratosis and dermal edema were observed in the DNCB group, whereas these changes were diminished in the PLG group.

Intense pruritus and excessive scratching are key factors of the development and the chronicization of AD [18]. Since ‘itch-scratch’ vicious cycle plays a critical role in AD, the reduction of itch sensation is a valuable approach in the treatment of AD [19]. Recent studies have showed an important role of neurogenic system on understanding the pathogenesis of AD [20]. NGF is not only a neurotrophic factor but also a peripheral inflammatory mediator related to pain and itch [21]. In the present study, PLG treatment significantly reduced the severe scratching behavior in AD-induced mice. In addition, enhanced NGF expression in AD-induced mice was down-regulated in a dose-dependent manner by PLG treatment.

Mast cells is a well-known inducer of IgE-mediated immediate hypersensitivity and allergic disorders [22]. IgE initiates the release of allergic and pro-inflammatory mediators by binding to mast cells [23]. The anti-histamine activity of PLG was assessed by Ortiz de Urbina in the guinea-pig ileums as the histamine H1-receptor antagonistic activity [13]. In addition, the anti-allergic effects of pulegone were reported by suppressing antigen-induced

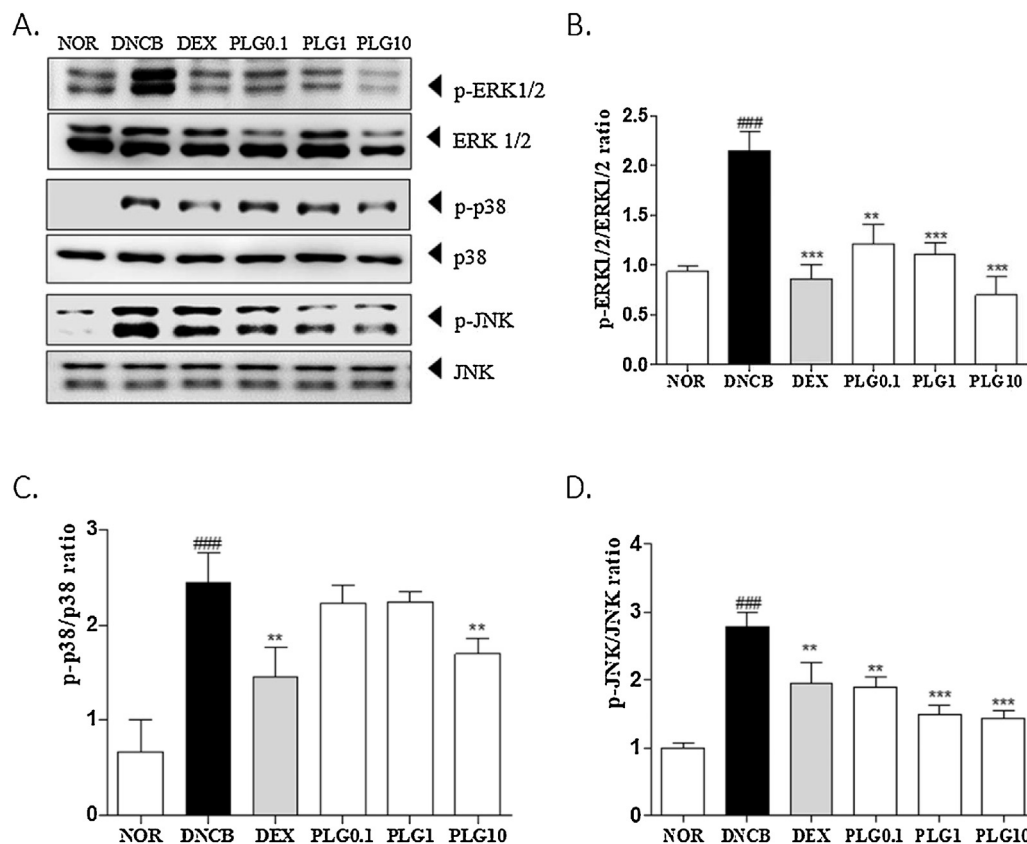


Fig. 6. The effects of PLG on the expressions of MAPKs signaling of mice with AD-like skin lesions. (A) MAPKs signaling were performed on the dorsal skin by western blotting assay. Bar graphs show relative (B) phospho-ERK1/2 (C) phospho-p38 and (D) phospho-JNK ratios. *** $p < 0.001$ vs. NOR, ** $p < 0.01$ vs. DNCB. NOR (treated with vehicle; normal group), DNCB (treated with vehicle and DNCB; negative group), DEX (treated with dexamethasone 10 μ M and DNCB; positive group), and PLG0.1, PLG1 and PLG10 (treated with PLG 0.1, 1, 10 μ M and DNCB, respectively). Data from three independent experiments ($n = 3$).

histamine release from rat peritoneal mast cells in type I allergic reactions [14]. Furthermore, pulegone treatment led to significant reduction of pro-inflammatory cytokines (IL-6, IL-8, and TNF- α) in PMA plus A23187-stimulated HMC-1 human mast cells [24]. Consistent with the previous studies, our study showed that PLG decreased the number of mast cells in dermis and normalized the level of IgE in serum. The results suggest anti-allergic and anti-inflammatory effects of PLG in mast cell, indicating the potential therapeutic efficacy of PLG for treatment of chronic inflammatory disorders.

Alterations in secretion of Th2/Th1 and pro-inflammatory cytokines have been considered as a pathological factor of AD [25]. IL-4 plays a pivotal role in the induction of Th2 cytokine differentiation and B-cell class switching to IgE [26]. Overexpression of IL-4 in AD skin lesion induces allergic inflammation, resulting in pruritus and intraepidermal edema [27]. In addition, IFN- γ as the principal Th1 effector cytokine that plays a crucial role in the resolution of allergic-related immunopathology [28]. Previous research reported that elevation of IFN- γ expression was observed in chronic AD-like skin lesions of mice similarly to human AD lesions [29]. In this study, PLG suppressed the levels of IL-4 and IFN- γ in AD-like skin, demonstrating that inflammatory and allergic response may be controlled by PLG via the regulation of Th2/Th1 cytokines. TNF- α and IL-1 β is a well-known pro-inflammatory cytokine and have been described as pathogenic inducers of AD [30,31]. IL-6 also acts as both a pro-inflammatory and an anti-inflammatory cytokine and promptly and transiently produced in inflammation and infection responses. Therefore, elevated serum IL-6 levels have been correlated with a severity of AD [32]. In the present study, increased TNF- α , IL-1 β and IL-6 expression in DNCB-treated AD skin lesions was suppressed by topical application of PLG. These results imply that PLG may alleviate the pathogenesis of AD by regulating various inflammatory cytokines.

To clarify the further involvement of PLG in cytokine-regulating effect, we evaluated possible effects of PLG on potential upstream targets such as NF- κ B and MAPKs signaling. The transcription factor NF- κ B is a major protein complex involved in the regulation of various immune responses including the inducement of AD [33]. Therefore, most previous research proposed that NF- κ B has an especially important role in AD as well as other skin diseases [34]. NF- κ B are activated by IKK-mediated phosphorylation of I κ B and subsequent ubiquitin-dependent I κ B degradation [35]. Additionally, several evidence has shown that MAPKs can be involved in the regulation of NF- κ B transcriptional activity and the production of cytokines such as IL-4 and TNF- α [36]. Consequently, phosphorylation of ERK1/2, -p38 and -JNK induces NF- κ B activity transcription as well as pro-inflammatory cytokine [37]. These results showed that treatment with PLG effectively blocked DNCB-induced activation of NF- κ B through inhibiting the I κ B α degradation. Furthermore, phosphorylation of MAPKs was increased in AD-like skin, and effectively suppressed by PLG. The inhibition of NF- κ B and MAPKs signaling supported that PLG might be suppress Th2/Th1 and pro-inflammatory cytokine.

In conclusion, this study demonstrates that PLG is an effective phytochemical for treating AD including histopathological features, skin thickness and scratching. The anti-allergic effect of PLG was successfully confirmed by the analysis of IgE and mast cells. The anti-inflammatory effects of PLG seem to be mediated through the inhibition of NF- κ B and MAPKs signaling pathways. In addition, PLG inhibited cytokine production including IL-4, IFN- γ , IL-6, IL-1 β and TNF- α in AD-induced mice. Ultimately, the result shown that PLG ameliorated the exacerbation factors of AD via the regulation of inflammatory response. Therefore, PLG may be a valuable agent for the treatment of inflammatory skin diseases including AD.

Conflict of interest

The authors have no conflict of interest to declare.

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