

## Effect of Persimmon Leaf Extract on Phthalic Anhydride-induced Allergic Response in Mice

Ji Ye Mok<sup>1</sup>, In Hwa Jeon<sup>2</sup>, Jung-Keun Cho<sup>3</sup>, Ji Min Park<sup>2</sup>, Hyeon Soo Kim<sup>2</sup>,  
Hyun Ju Kang<sup>2</sup>, Hyung Soon Kim<sup>4</sup>, and Seon Il Jang<sup>1,2†</sup>

<sup>1</sup>Jeonju University Atopy & Health Research Institute, Jeonbuk 560-759, Korea

<sup>2</sup>School of Alternative Medicine & Health Science, and <sup>3</sup>Department of Radiological Science,  
College of Alternative Medicine, Jeonju University, Jeonbuk 560-759, Korea

<sup>4</sup>Moakgol Association, Jeonbuk 565-821, Korea

### Abstract

The purpose of this study was to investigate the anti-allergy activities of persimmon leaf extract (PLE) on a phthalic anhydride (PA)-induced allergic mouse model. A human leukemic mast cell line (HMC-1) was used to examine the inhibitory activity of PLE on the histamine release by human leukemic mast cells. PLE inhibited histamine release from HMC-1 cells in response to cross-linkage of high-affinity IgE receptor- $\alpha$  (Fc $\epsilon$ RI $\alpha$ ). Additionally, a PA-induced allergic mouse model was used to investigate the effects of PLE *in vivo*. Mice were orally administrated with or without PLE of single dose (250 mg/kg/day) for 31 days. Oral intake of PLE significantly inhibited passive cutaneous reactions. Oral administration of PLE to PA-induced allergic mice also led to a striking suppression of the development of contact dermatitis, ear swelling and lymph node weight. In addition, PA-specific IL-4 production of draining lymph node cells was markedly diminished by PLE oral administration, but not IFN- $\gamma$ . Furthermore, PLE treatment suppressed PA-induced thymus and activation-regulated chemokine (CCL17) and cutaneous T cell-attracting chemokine (CCL27) expressions in ear tissues. Based on these results, we suggest that PLE may have therapeutic potential as an effective material for management of irritant contact dermatitis or related inflammatory diseases.

**Key words:** persimmon leaf extract, phthalic anhydride, contact dermatitis, IgE, chemokines

### INTRODUCTION

Phthalic anhydride (PA) is a low-molecular-weight compound and an important industrial chemical, especially for the large scale production of plasticizers for plastics. Exposure to PA causes irritation and can lead to immune-mediated allergic diseases such as contact urticaria, rhinitis, conjunctivitis, and atopic dermatitis upon repeat exposure (1). These disorders affect 10~15% of Western populations, and their prevalence has doubled in the last 10~15 years (2). This increase has included allergic skin disorders such as atopic or contact dermatitis accompanied by irritant dermatitis in the site at which contact with an allergen has occurred (3). Such allergic responses are characterized by infiltration by specific cells, including inflammatory cells such as T lymphocytes, neutrophils, eosinophils, macrophages and mast cells (4-6). Activation of inflammatory cells by this infiltration is mediated by various cytokines and chemokines at the site of injury.

For example, interleukin (IL)-4, IL-5, and IL-13 pro-

duced by Type 2 helper (Th2) cells are responsible for increased serum IgE levels and the presence of blood eosinophilia (7). In particular, enhanced IL-4 is a hallmark of acute allergic skin disease (8) and inhibits ceramide synthesis, and exogenous applications of IL-4 impede recovery of the permeability barrier after acute perturbations (9). Additionally, chemokines such as keratinocyte-derived chemokine were recently found to be the most potent molecules involved in the regulation of selective recruitment of inflammatory cells. These mainly include thymus and activation-regulated chemokine (CCL17), which acts specifically on the chemokine receptor CCR4, and cutaneous T cell-attracting chemokine (CCL27), which acts specifically on the chemokine receptor CCR10 (10,11). The serum concentrations of these Th2 chemokines correlate with the severity of allergic skin diseases (12). Therefore, agents that regulate these inflammatory molecules could provide beneficial anti-inflammatory effects.

Traditionally, persimmon (*Diospyros kaki*) leaf tea is believed to be a health-promoting beverage, similar to

†Corresponding author. E-mail: [sonjjang@jj.ac.kr](mailto:sonjjang@jj.ac.kr)  
Phone: +82-63-220-3124, Fax: +82-63-220-2054

green tea in Korea and Japan (13). Recently, it was reported that flavonoids from persimmon leaf inhibited the adhesion between lymphocytes and neurons (14); however, there is no report on persimmon leaf's protective effect against PA-induced skin inflammation. Therefore, the purpose of this study was to investigate the protective effect of PLE on PA-induced inflammation in HMC-1 cells and mouse models.

## MATERIALS AND METHODS

### Plant material

Persimmon (*D. kaki*) leaf materials used in the present study were collected on 20 Jun 2010 from Bugui-myeon and Jeongcheon-myeon, Jinan-gun (Jeonbuk, Korea). The plant was identified and authenticated by Prof. Hong Jun Kim at the College of Oriental Medicine, Woosuk University. A voucher specimen (PL-10-06) has been deposited in the author's laboratory (Prof. Seon Il Jang).

### Preparation of the extract

The dried persimmon leaf (250 g) was steeped in boiling distilled water (3 L) for 5 minutes and filtered through a nylon mesh (pore size, 40  $\mu\text{m}$ ). After centrifugation at  $3,500\times g$  for 30 minutes at  $4^{\circ}\text{C}$ , the supernatant of the persimmon leaf extract (PLE) was freeze-dried and kept at  $-20^{\circ}\text{C}$ . The contaminated endotoxin level in persimmon leaf was less than 1 ng/mL.

### Reagents

Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO-BRL (Invitrogen Co., Grand Island, NY, USA), Phthalic anhydride, penicillin-streptomycin, hematoxylin & eosin (H&E), Congo red or toluidine blue were purchased from Sigma (St. Louis, MO, USA). CCL2 and CCL27 enzyme-linked immunosorbent assay (ELISA) kits for human and mouse were obtained from R&D Systems (Minneapolis, MN, USA). Anti-mouse CCL17 and anti-mouse CCL27 mAb conjugated with phycoerythrin (PE) were obtained from BD Biosciences (San Jose, CA, USA). Anti-Fc $\epsilon$ RI $\alpha$  mAb was purchased from Kyokuto Pharmaceutical Industry Co., Ltd. (Tokyo, Japan). Other chemicals for reagent grade were purchased from Sigma (St. Louis, MO, USA).

### Cells

Human leukemic mast cells, HMC-1, were kindly provided by Dr J. H. Butterfield (Mayo Clinic, Rochester, MN, USA). Cells were cultured with DMEM containing 10% heat-inactivated FBS, penicillin G (100 IU/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ) and incubated at  $37^{\circ}\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$  and 95% air. The medium was changed every 2 days. In this ex-

periment, cells were cultured to 70~80% confluence in a round plate (10 cm in diameter) and expanded by sub-culturing into four plates.

### Histamine release assay

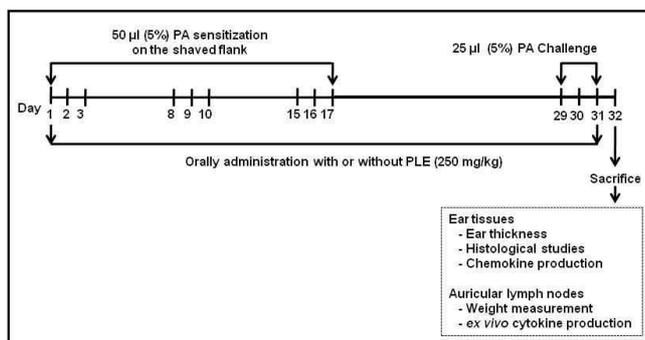
HMC-1 cells were suspended in a histamine release buffer (30 mmol/L Tris-HCl (pH 7.6), 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L  $\text{CaCl}_2$ , 1 mmol/L  $\text{MgCl}_2$ , and 0.03% BSA). The first incubation of 100  $\mu\text{L}$  of HMC-1 cells ( $10^5$  cells/mL) was performed with 100  $\mu\text{L}$  of persimmon leaf extract (250  $\mu\text{g}/\text{mL}$ ) in an ice bath for 30 minutes. After centrifugation at  $800\times g$  for 5 minutes at  $4^{\circ}\text{C}$ , 200  $\mu\text{L}$  of histamine release buffer was added to the cells, which were then incubated with 15  $\mu\text{g}/\text{mL}$  Anti-Fc $\epsilon$ RI $\alpha$  mAb at  $37^{\circ}\text{C}$  for 30 minutes. After centrifugation at  $800\times g$  for 5 minutes at  $4^{\circ}\text{C}$ , the histamine contents of the supernatant and cell lysate were measured by means of ELISA. Positive and negative controls were used to indicate histamine content obtained with or without anti-Fc $\epsilon$ RI $\alpha$  mAb.

### Animals

Seven-week-old Balb/c mice were purchased from Central Lab. Inc. (Seoul, Korea), the Korean branch of Charles River Japan (Kanagawa, Japan). Mice were maintained in an environmentally controlled rearing system and used for experiments at 8 weeks of age. All experiments in this study were performed in accordance with Jeonju University Institutional Animal Care and Use Committee guidelines.

### Experimental design

The mice were divided into four groups. Control and positive control groups (PA alone) were orally administered with 200  $\mu\text{L}$  saline, daily for 31 weeks. Also, active control group (PA plus EGCG) was administered with 50 mg/kg epigallocatechin-3-gallate (EGCG) and the last group (PA plus PLE) was orally administered with 250 mg/kg PLE (the determined optimum dose by our previous study) (15) daily for 31 weeks. Each experimental group consisted of five mice. Fig. 1 depicts the experimental protocol used. On days 1~3, 8~10 and 15~17, a 50  $\mu\text{L}$  aliquot of PA solution or solvent alone was applied to the dorsum of both ears of each mouse for dermal sensitization. On the day after challenge (day 32), all mice were anesthetized with pentobarbital sodium (75 mg/kg) and sacrificed. Blood samples were taken from the portal vein, and serum samples were assayed for IgE levels. Each animal's ear thickness was measured and then ears for each group were removed to investigate histological changes and chemokine expression. Also, each animal's auricular lymph nodes (LN) were removed to measure weight of LN and cytokine



**Fig. 1.** Schematic diagram of experimental protocol. Mice were sensitized on shaved flank skin with and without 50 µL of 10% phthalic anhydride (PA) on days 1~3, 8~10 and 15~17. On day 29, 30, and 31, mice were challenged with or without 25 µL of 10% PA on the dorsum of both ears. Control group received only saline for 31 days. Experimental groups received the single dose of persimmon leaf extract (PLE) (250 mg/kg body weight) or EGCG (50 mg/kg body weight) one time per day for 31 days.

production.

#### Measurement of ear thickness and skin severity score

The ear thickness and skin severity scores of the mice were measured to determine the degree of contact irritant dermatitis induced by PA treatment. Briefly, the ear thickness was also measured using a dial caliper (Mitutoyo, Japan) 24 hr after final PA treatment. In addition, changes in the skin on the dorsum of the ear and the back skin of mice were observed 24 hr after final PA treatment using the method described in a previous study (16). In this method, the degree of four characteristics, erythema/hemorrhage, edema, excoriation/erosion and dryness, was scored from 0 points (none) to 3 points (severe) depending on the severity of the dermatitis. Finally, the total skin score was calculated from the sum of the scores of the four characters.

#### Preparation of lymph node cell

Auricular LN cells were derived from the auricular region. Freshly isolated lymph node cells were made into single-cell suspensions, and contaminated erythrocytes were lysed by hypotonic shock with sterile distilled water. Cells ( $1 \times 10^6$ /well) were stimulated with soluble anti-CD3 (1 µg/mL) plus anti-CD28 (1 µg/mL) antibodies (BD Pharmingen, San Jose, CA, USA) in RPMI1640 medium (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% heat-inactivated FBS (Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B (Sigma-Aldrich, St. Louis, MO, USA), and 50 µM 2-mercaptoethanol. After 48 hours, cytokines were measured from supernatants using IL-4 and IFN-γ ELISA kit.

#### ELISA

IgE levels in the sera and cytokine levels in the culture supernatants were determined by sandwich ELISA using antibody pairs against murine IgE, IL-4 or IFN-γ from R&D Systems, following the manufacturer's instructions. Mouse chemokines were analyzed in the lysate of ear tissues. The tissues were lysed in 100 µL of protein extraction buffer (PRO-PREP, Intron, Seoul, Korea) with a sonicator (Ultra-Turrax-25, Ika-Werke, Staufen, Germany). Lysates were collected from the sonicator mixture using centrifugation and stored at -70°C until further analysis. Chemokine levels in the lysate were determined in appropriate dilutions by ELISA using antibody pairs against CCL17 and CCL27 from R&D Systems, following the manufacturer's instructions.

#### Histological analysis

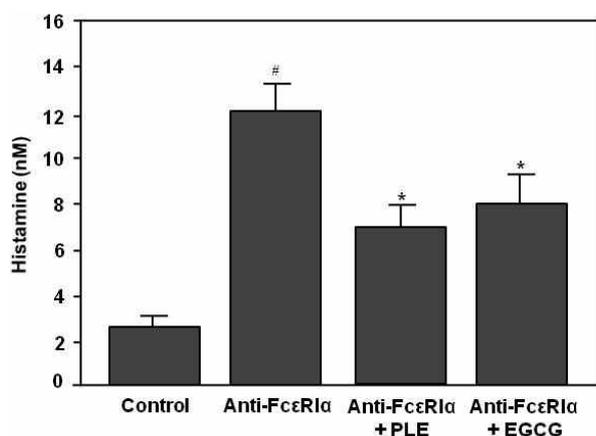
The ear tissues were fixed with 4% paraformaldehyde, embedded in paraffin and then 5 µm thin sections were made on saline coated micro slides (Muto-glass, Tokyo, Japan). The de-paraffinized skin sections were stained with H&E and examined by light microscopy (Olympus, Tokyo, Japan) to assess the histological changes. The sections for immunohistochemistry were de-paraffinized with xylene, rinsed with distilled water and then rinsed with PBS. The nonspecific antigen-antibody reactions were inhibited by one hour treatment with 10% normal goat serum. The sections were reacted with diluted solutions of each of the primary antibodies such as purified rat monoclonal antibodies against mouse CCL17 or CCL27 for overnight at 4°C. The sections were rinsed with distilled water and PBS and then were covered with a coverslip. They were examined by fluorescence microscopy (Olympus) to assess the molecule expressions.

#### Statistical analysis

Differences in data among the groups were analyzed by one-way ANOVA, and all values were expressed as mean ± SE. The differences between groups were considered to be significant at  $p < 0.05$ .

## RESULTS

Effect of PLE on histamine release from HMC-1 cells in response to FcεRI cross-linkage. First, to investigate whether PLE could inhibit histamine release, we used human leukemic mast cells, HMC-1. As shown in Fig. 2, histamine release was significantly increased in the anti-FcεRIα mAb alone group ( $p < 0.001$ ), compared to the control group. However, histamine level of PLE or EGCG treated group was significantly lower than that for anti-FcεRIα mAb alone group ( $p < 0.05$ ). The in-



**Fig. 2.** Inhibitory effect of PLE treatment on histamine release from HMC-1 cells stimulated with anti-FcεRIα mAb. Cells ( $10^4$ ) were incubated with 250 μg/mL PLE or 50 μg/mL EGCG on ice for 30 minutes and then incubated with 15 μg/mL anti-FcεRIα mAb at 37°C for 30 minutes. Histamine content of the supernatant was measured by means of ELISA. Control and anti-FcεRIα mAb alone groups indicate histamine content with or without anti-FcεRIα mAb. Data represent the mean  $\pm$  SE of 3 determinations. <sup>#</sup> $p < 0.001$  compared with the control group. <sup>\*</sup> $p < 0.05$  compared with the anti-FcεRIα alone group.

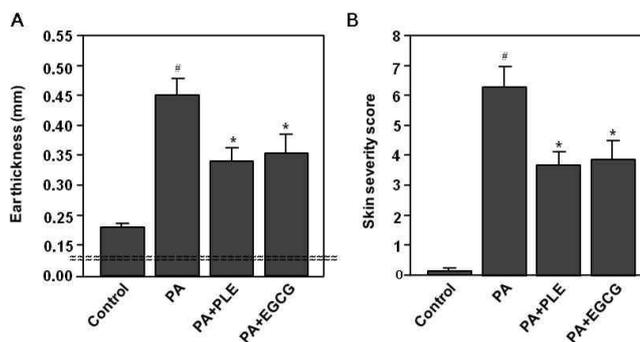
hibitory effect of PLE on histamine release was slightly higher than that for the EGCG group.

#### Effect of PLE on PA-induced ear thickness and skin severity score

Next, to investigate the skin irritation response in Balb/c mice, ear thickness and skin severity score were measured in mice following topical application of PA solution. The ear thickness was measured in the ears of mice using a thickness gauge. As shown in Fig. 3A, the ear thickness was significantly increased in the PA alone group ( $p < 0.001$ ), while it was not changed in the control group. In contrast, the ear thickness of PLE or EGCG treated group was significantly lower than those for PA alone group ( $p < 0.05$ ). The skin severity score was used to determine the degree of the erythema/hemorrhage, edema, excoriation/erosion and dryness on the flank and ear skin of mice. As shown in Fig. 3B, the skin severity scores increased significantly in PA alone group ( $p < 0.001$ ). However, the skin severity scores of PLE or EGCG treated group were significantly lower than those for PA alone group ( $p < 0.05$ ). The inhibitory effects of PLE on the changes in ear thickness and skin severity score were slightly higher than that for the EGCG group.

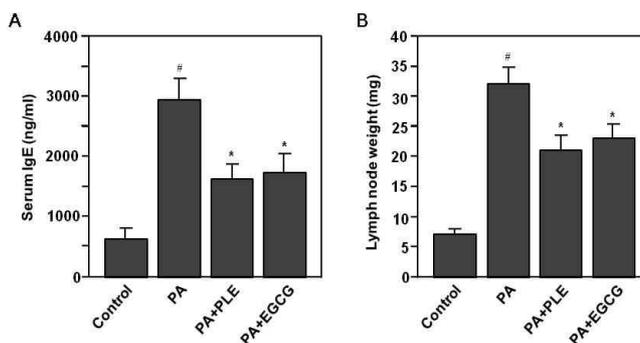
#### Effect of PLE on PA-induced serum IgE production and lymph node weight

We also investigated whether PLE might be effective for allergic response *in vivo* by measuring the serum IgE and auricular lymph node in PA treated mice. As shown in Fig. 4A, repeated topical application of the PA sol-



**Fig. 3.** Effect of PLE treatment on PA-induced changes in the ear thickness and the skin severity score. Mice were sensitized on shaved flank skin with and without 50 μL of 10% phthalic anhydride (PA) on days 1~3, 8~10 and 15~17. On day 29, 30, and 31, mice were challenged with or without 25 μL of 10% PA on the dorsum of both ears. Control group received only saline for 31 days. Experimental groups received the single dose of persimmon leaf extract (PLE) (250 mg/kg body weight) or EGCG (50 mg/kg body weight) one time per day for 31 days. (A) Ear thickness was measured using a dial caliper on day 32. (B) The total clinical severity score was defined as the sum of scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of five aspects: itch, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness on day 32. Data represent the mean  $\pm$  SE of 3 determinations. <sup>#</sup> $p < 0.001$  compared with the control group. <sup>\*</sup> $p < 0.05$  compared with the PA alone group.

ution induced a significant increase in serum IgE levels in mice, compared to those of the control group ( $p < 0.001$ ). However, the serum IgE level of PLE or EGCG



**Fig. 4.** Effects of PLE treatment on PA-induced changes in the serum level of IgE and the weight of auricular lymph node (LN). Mice were sensitized on shaved flank skin with and without 50 μL of 10% PA on days 1~3, 8~10 and 15~17. On day 29, 30, and 31, mice were challenged with or without 25 μL of 10% PA on the dorsum of both ears. Control group received only saline for 31 days. Experimental groups received the single dose of PLE (250 mg/kg body weight) or EGCG (50 mg/kg body weight) one time per day for 31 days. (A) Sera were obtained from each mouse group on day 32. Serum level of IgE was measured using the specific ELISA kit for IgE molecule. (B) Weight of auricular LN was measured using an electric balance on day 32. Data represent the mean  $\pm$  SE of 3 determinations. <sup>#</sup> $p < 0.001$  compared with the control group. <sup>\*</sup> $p < 0.05$  compared with the PA alone group.

treated group was significantly lower than that for PA alone group ( $p < 0.05$ ). In addition, PA treatment was found to induce an increase in the weight of auricular lymph node in mice, whereas the weight of auricular lymph node of PLE or EGCG treated group was significantly lower than that for PA alone group ( $p < 0.05$ ) (Fig. 4B).

#### Effect of PLE on PA-induced histological changes and chemokine expressions

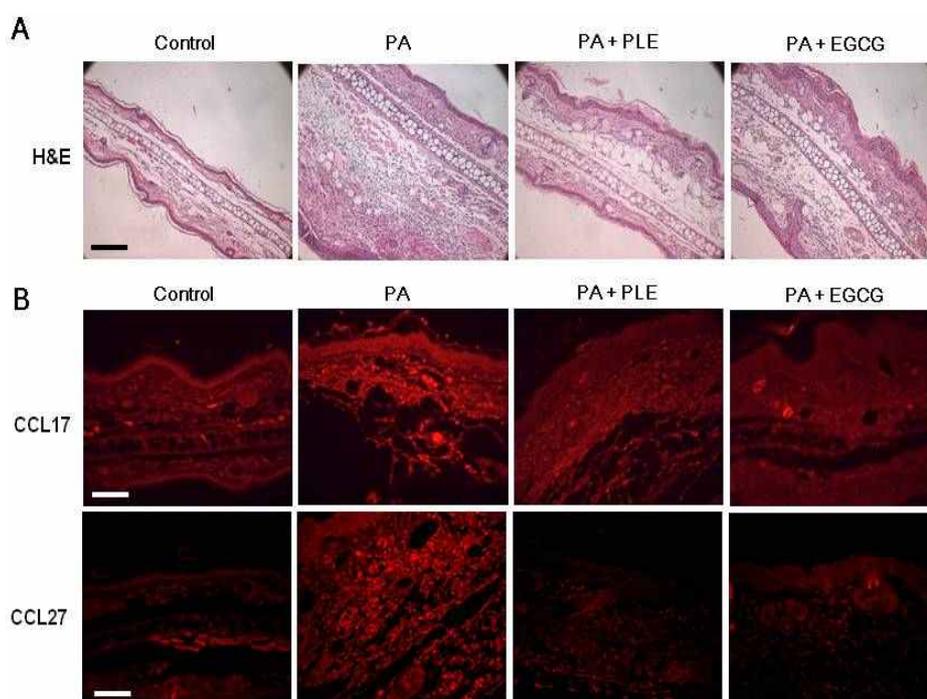
To evaluate the inhibitory effect of PLE on PA-induced skin lesions, we performed H&E staining on specimens of the ears of mice. As shown in Fig. 5A, sections of the ears in the PA alone group revealed a hyperplastic epidermis and edematous dermis, with a large number of infiltrated mononuclear cells together with some eosinophils. In contrast, sections of the ears of PLE or EGCG-treated groups showed markedly diminished epidermal hyperplasia and cellular infiltration into the dermis. We also conducted immunohistochemistry for CCL17 and CCL27 chemokines to assess whether oral administration of PLE has an effect on CCL17 and CCL27 expression in PA-induced skin lesions. As shown in Fig. 4B, CCL17 and CCL27 expressions were increased in the skin lesions in PA alone group. In contrast, they were markedly decreased with oral administration of the PLE or EGCG. The inhibitory effect of PLE on histological changes and chemokine expression were slightly higher than that of the EGCG group. These results demonstrate that PLE suppressed PA-induced histological changes and chemokine expression in mice treated with PA.

#### Effect of PLE on PA-induced cytokine and chemokine productions

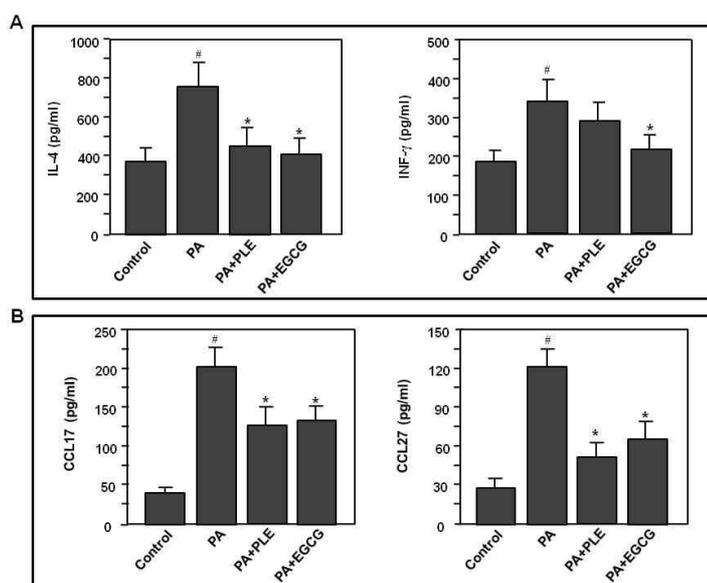
Finally, to examine whether PLE affects cytokines of auricular LN cells or chemokines of ear tissues, we measured cytokine levels of supernatants stimulated with anti-CD3 plus CD28 antibodies for 48 hr in PA-sensitized cells and also measured chemokine levels of ear tissue lysates of PA-sensitized mice. As shown in Fig. 6A, the levels of cytokines (IL-4 and IFN- $\gamma$ ) in the draining LN cells were significantly increased in PA-treated mice ( $p < 0.001$ ), compared to those of the control group. In contrast, IL-4 production of lymph node cells was markedly diminished by PLE or EGCG treatment ( $p < 0.05$ ), compared to those of PA alone group. However, IFN- $\gamma$  level of the PLE treated group, but not the EGCG group, was not changed. The levels of CCL17 and CCL27 were significantly increased in PA-treated mice ( $p < 0.001$ ), compared to those of the control group. In parallel with the protein expression of CCL17 and CCL27 in the ear tissue (Fig. 5B), PLE or EGCG treatment markedly suppressed PA-induced CCL17 and CCL27 productions in the lysate of ear tissues (Fig. 6B). These results demonstrate that PLE suppressed PA-induced Th2 cytokine production and Th2 chemokine expression in mice sensitized with PA chemical antigen.

## DISCUSSION

Irritant contact dermatitis accounts for 80% of all contact dermatitis reactions. It occurs as a result of xenobiotic chemicals penetrating the skin and chemically re-



**Fig. 5.** Effect of PLE treatment on PA-induced skin lesions in mice. Mice were sensitized on shaved flank skin with and without 50  $\mu$ L of 10% PA on days 1~3, 8~10 and 15~17. On day 29, 30, and 31, mice were challenged with or without 25  $\mu$ L of 10% PA on the dorsum of both ears. Control group received only saline for 31 days. Experimental groups received the single dose of PLE (250 mg/kg body weight) or EGCG (50 mg/kg body weight) one time per day for 31 days. (A) Sections were stained with hematoxylin and eosin, and then observed at an original magnification of  $\times 200$ , scale bar = 120  $\mu$ m. (B) CCL17 and CCL27 chemokine expressions were determined by immunochemical staining with anti-mouse CCL17 or CCL27 antibody. The sections were observed at an original magnification of  $\times 200$ , scale bar = 60  $\mu$ m.



**Fig. 6.** Effects of PLE treatment on cytokine production of auricular LN cells and chemokine production of ear tissue. Mice were sensitized on shaved flank skin with and without 50  $\mu$ L of 10% PA on days 1~3, 8~10 and 15~17. On day 29, 30, and 31, mice were challenged with or without 25  $\mu$ L of 10% PA on the dorsum of both ears. Control group received only saline for 31 days, experimental groups received the single dose of PLE (250 mg/kg body weight) or EGCG (50 mg/kg body weight) one time per day for 31 days. (A) At the last day of experiments, auricular LN cells were isolated and stimulated with anti-CD3 plus anti-CD28 antibodies for 2 days and then cytokine productions of supernatants were examined by ELISA. (B) At the last day of experiments, ear tissues were removed and lysed with tissue lysis buffer. CCL17 and CCL27 levels of the tissue lysate were measured using the each specific ELISA kit for CCL17 or CC27. Data represent the mean  $\pm$  SE of 3 determinations. <sup>#</sup> $p < 0.001$  compared with the control group. <sup>\*</sup> $p < 0.05$  compared with the PA alone group.

acting with self proteins, which results in a hapten-specific immune response (17). Exposure to PA causes irritation and can lead to allergic skin disorders such as atopic or contact dermatitis accompanied by irritant dermatitis in the site at which contact with an allergen has occurred (3).

First, to investigate whether PLE could inhibit histamine release from human leukemic mast cells, we stimulated HMC-1 cells with anti-Fc $\epsilon$ RI $\alpha$  antibody. The cross-linkage of Fc $\epsilon$ RI $\alpha$  by the anti-Fc $\epsilon$ RI $\alpha$  antibody in mast cells induces histamine release, as previously reported (18). The histamine level of the PLE treated group was significantly lower than that for anti-Fc $\epsilon$ RI $\alpha$  antibody alone group. For the inhibitory effect of PLE on histamine release, our result was similar to a previous report (19).

Next, we examined whether oral PLE administration ameliorates the development of irritant contact dermatitis in PA-sensitized mice and whether PLE alters IgE level of serum, cytokine production of the auricular LN cells, and chemokine expression of ear tissue in PA-sensitized mice. Allergic response to chemical allergens is generally acknowledged to be dependent on IgE-mediated mechanisms, and the hyper-production of IgE is one of the characteristic features of allergic response (20,21). The administration of PLE showed an inhibitory effect on serum IgE level and auricular LN weight induced by PA (Fig. 3). PLE also significantly reduced the clinical signs provoked by PA, such as erythema, edema, scaling and excoriation. A suppressive effect of PLE was similar to that of EGCG, which was used as an active control. It is also well known that the weight of the local lymph node increases in response to topical application of agents that have allergenic or sensitizing potential (22).

Balance between the Th1 and Th2 responses is regarded as important for efficient immune function, and imbalances lead to various immune diseases, such as autoimmune and allergic diseases (10). Th2-dominant phenotype is one of the aspects of human irritant contact dermatitis (23). Irritant contact dermatitis is typically characterized by the infiltration of inflammatory cells (6). It has been reported that several cytokines and chemokines could regulate the irritant contact dermatitis (24). IL-4 secreted from Th2 cells is an important factor for immunoglobulin class switching to IgE in B cells, but IFN- $\gamma$  strongly suppresses IgE production in B cells via suppression of CD40L mRNA expression in Th2 cells (25). We found that anti-CD3 plus anti-CD28 antibody-induced IL-4 production of draining auricular LN cells was markedly diminished by oral administration of PLE, but not IFN- $\gamma$  (Fig. 6A). These changes in the cytokine profile might contribute to IgE suppression. Since many studies demonstrated that CCL17 and CCL22 play a critical role in infiltration CCR4<sup>+</sup> lymphocytes into the skin area, the down-regulation of CCL17 and CCL22 production in keratinocytes may be an effective approach for treatment of inflammatory skin diseases (12,26). In addition, CCL27 is a skin-specific CC chemokine which is produced constitutively by keratinocytes and is highly upregulated in inflammatory skin conditions such as atopic dermatitis and allergic contact dermatitis (27). Therefore, CCL17 and CCL27 may contribute to the pathogenesis of a Th2-dominant disease, such as atopic dermatitis and allergic contact dermatitis. We found that PLE significantly inhibited the serum CCL17 and CCL27 levels in PA-treated mice (Fig. 5B). A suppressive effect of PLE was similar to that of EGCG used as an active control. These findings indicate that these cytokines and

chemokines play a role in determining the degree of skin irritation.

On the other hand, current treatments for inflammation include aspirin, nonsteroidal anti-inflammatory drugs and dexamethasone. These compounds tend to have adverse side effects, which may be ameliorated by the use of effective botanical alternatives (4). *D. kaki* is widely distributed in East Asia, and its leaf is a traditional plant medicine for the treatment of hypertension, angina and internal hemorrhage. PLE is used to promote maternal health that in Korea and Japan (13) and Persimmon leaf has been shown to contain bioactive compounds, including polyphenols, flavonoids, hydrocarbons, triterpene and steroids (28,29).

In conclusion, we have provided evidence that PLE has an inhibitory effect on the development of irritant contact dermatitis symptoms, especially ear thickness and skin symptoms, with alterations of Th2 cytokine and chemokines, as well as IgE level in PA-treated mice. These results suggest that PLE may be an effective material for management of irritant contact dermatitis.

#### ACKNOWLEDGEMENTS

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