Antiallergic and Antipsoriatic Effects of Korean Red Ginseng

Eun-Ah Bae[1], Myung Joo Han[1,#], Yong-Wook Shin[2] and Dong-Hyun Kim[2]

[1]Department of Food and Nutrition, and [2]College of Pharmacy, Kyung Hee University,
1 Hoegi, Dongdaemun-ku, Seoul 130-701, Korea
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Abtract : Antiallergic and antipsoriatic effects of Korean Red Ginseng (KRG, steamed root of *Panax ginseng* C.A. Meyer, Family Araliaceae) were measured. Orally administered KRG water extract potently inhibited passive cutaneous anaphylaxis (PCA). KRG water extract also showed the potent inhibition in oxazolone-induced mouse dermatitis, and suppressed mouse ear swelling by 39% at 16 days at a dose of 0.1%. KRG water extract reduced the levels of mRNA of cyclooxygenase (COX)-2, IL-1β, TNF-α and IFN-γ increased in oxazolone-applied mouse ears, however, did not inhibit that of IL-4. KRG water extract also inhibited iNOS and COX-2 mRNA expression level of RAW264.7 cell induced by lipopolysaccharide. Based on these findings, we suggest that KRG can improve atopic and contact dermatitis by the regulation of IL-1β and TNF-α produced by macrophage cells and interferon-γ produced by Th1 cells.

Key words : Korean Red Ginseng, antiallergic activity; antipsoriatic activity.

INTRODUCTION

Korean Red Ginseng (KRG, the steamed root of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently used as a crude substance taken orally in Asian countries as a traditional medicine. The major components of raw ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton.[1,2] Many kinds of saponins, such as ginsenosides Rb1, Rb2, Rc and Rf, have been isolated. The ginsenosides Rg3, Rf and Rh2 are genuine saponins in KRG and heat-processed ginseng[3,4]. Ginsenosides Rg3 and Rh2 were produced from protopanaxadiol ginsenosides by steaming to prepare red ginseng.[5] These ginsenosides have been reported to show various biological activities including anti-inflammatory activity, antiallergic, endothelium-independent aorta relaxation and anti-tumor effects.[6-9] Particularly, Sugiyama *et al.* reported that ginsenoside Rg3 suppressed histamine release from mast cells due to stimulation with compoumd 48/80 *in vitro*[10], and ginsenoside Rb1 and Rc also inhibited partly the release histamine and leukotrienes during the activation of guinea pig lung mast cells *in vitro*[11]. We also reported the antiallergic and anti-inflammatory effect of ginsenoside Rh1, antiallergic and passive cutaneous ana-

phylaxis reaction (PCA)-inhibitory effects of compound K (20-*O*-β,-D-glucopyranosyl-20(*S*)-protopanaxadiol) and antiallergic effect of ginsenoside Rh2[12-14]. However, antiallergic and antipsoriatic effects of KRG have not been thoroughly studied.

Therefore, PCA reaction-inhibitory, antiallergic and antiposiatic effects of KRG water extract were measured.

MATERIALS AND METHODS

Materials

p-Nitrophenyl-N-acetyl-β-D-glucosaminide, Freund's complete adjuvant, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue, disodium cromoglycate (DSCG), trichloroacetic acid, and betamethasone, were purchased from Sigma Chemical Co. (U.S.A). RG water extract was donated from KT&G (Korea).

Animals

Female ICR mice (20-22 g) were supplied from Orient Charles River experimental animal breeding center (Korea). All animals were housed in wire cages, fed with usual laboratory chow (Orient Charles River feed production Co.) and water *ad libitum*.

Passive Cutaneous Anaphylaxis (PCA) Reaction

An IgE-dependent cutaneous reaction was measured

#To whom correspondence should be addressed.
(Tel) +82-2-961-0553; (Fax) +82-2-957-5030
(E-mail) mjhan@khu.ac.kr

according to the previous method of Katayama *et al.*[15] The male ICR mice (25-30 g) were injected intradermally with 10 μg of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each mouse received an injection of 200 μl of 3% Evans blue PBS containing 200 μg of DNP-HSA *via* the tail vein. The test agents were administered 1 h prior to DNP-HSA injection. Thirty min after DNP-HSA injection, the mice were sacrificed and their dorsal skins were removed for measurement of the pigment area. After extraction with 1 ml of 1.0 N KOH and 4 ml of a mixture of acetone and 0.6 N phosphoric acid (13:5), the amount of dye was determined colorimetrically (the absorbance at 620 nm).

Contact Hypersensitivity

An oxazolone-induced dermatitis was measured according to the previous method of Fujii *et al.*[16]. Female ICR mice were sensitized by application of 100 μl of 1.5% oxazolone in ethanol to the abdomen. Then a total of 20 μl of 1% oxazolone in a mixture of acetone and olive oil (4:1) was applied to both sides of the mouse ear every 3 days starting from 7 days after sensitization. Ear thickness was measured using a Digimatic Micrometer (Mitsutoyo Co., Tokyo, Japan) 72 h after each application of the oxazolone, test agents were applied in a total volume of 20 μl to both sides of the ear 30 min before and 3 h after each application of oxazolone

RT-PCR Analysis

Ear tissue extract for RT-PCR analysis were performed by the modified method of Chi *et al.*[17]. Briefly, ears were excised 6 h after the last application of oxazolone, freezed in liquid nitrogen and homogenized by a mortar and pestle prechilled in liquid nitrogen.

Total RNA was extracted by using TRI reagent according to the manufacturer's instructions, and treated with RNase-free DNase. The concentration of RNA content was determined by measuring the absorbance at 260 and 280 nm and stored at -70°C until RT-PCR analysis. The RT-PCR was performed with AccPower® RT/PCR Premix (Bioneer, Seoul, Korea). The primers were designed as described by UniSTS database: COX-1, forward primer 5'-CTTTTATC-CTCCCAGGATTTGG-3 and reverse primer 5'-GCTAA-ATACTTTGACACCGG-3' (product size 231 bp); COX-2 (UniSTS 254306), forward primer 5'-TGTATCCCCCCA-CAGTCAAAGACAC-3 and reverse primer 5'-GTGCTC-CCGAAGCCAGATGG-3' (product size 146 bp); IL-1β

forward primer 5'-ATGGCAACTGTCCCTGAACT-3 and reverse primer 5'-GTCGTTGCTTGTCTCTCCTT-3' (product size 508 bp); IFN-γ (UniSTS 160031), forward primer 5'-CTTTAACAGCAGGCCAGACA-3' and reverse primer 5'-GCGAGTTATTTGTCATTCGG-3' (product size 144 bp); IL-4 (UniSTS 143568), forward primer 5'-CCGAT-TATGGTGTAATTTCCTATGCTG-3' and reverse primer 5'GGCCAATCAGCACCTCTCTTCCAG-3' (product size 111 bp); tumor necrosis factor (TNF)-α (UniSTS 209165), forward primer 5'-GATTTTATTTGTTTAAAA-GCAGATATC-3' and reverse primer 5'-CATCCTAAG-TCTACACAGGATCT-3' (product size 206bp); GAPDH (UniSTS 225899), forward primer 5'-ACCACAGTC-CATGCCATCAC-3' and reverse primer 5'-TCCACCAC-CCTGTTGCTGTA-3' (product size 452 bp). The amplification was performed at 94°C for 30 - 60 s, and 49 - 62°C for 30 - 40 s, and 72°C for 30 - 60 s with 30 cycles for COX-1, IL-1β, TNF-α and GAPDH, and 32 cycles for other genes, in 20 μl reaction mixture. The RT-PCR products were electrophoresed on 2% agarose gel in TBE buffer, stained with ethidium bromide and photographed under UV light. The GAPDH gene was used as an internal control. The signal intensity of each RT-PCR product was estimated by Shimazu 9301-PC scanner (Tokyo, Japan).

Histopathological Study

Mouse ears were excised 72 h after the last application of oxazolone and fixed in 10%-buffered formalin solution, embedded in paraffin by standard methods, cut into 5-μm sections, stained with hematoxylin-eosin, and then assessed under light microscopy.

Culture of RAW264.7 Cells and Immunoblot

Immunoblot analyses of the iNOS, COX-2 and NF-kB were performed according to the method of Ishihara *et al.*[18] The RAW 264.7 cells were plated in 60 mm culture dishes (3×10⁶ cells), KRG and LPS* (1 μg/ml) added to the culture medium, and the cells incubated at 37°C for 6 - 20 h. The cells were lysed on ice for 15 min in an hypotonic buffer, containing 10 mM Tris (pH 8.0), 1.5 mM MgCl₂, 1 mM DTT, 0.1% NP-40, 5 μg/ml pepstatin A and 5 μg/ml aprotinin, and centrifuged at 12000×g and 4°C for 15 min. The supernatant was used as the cytosol fraction for the immuoblot assays for the iNOS and COX-2 protein expressions. The pelleted nuclei fractions for the Immunoblot assays of the NF-kB protein expression were resuspended in the extraction buffer, containing 10 mM Tris (pH 8.0), 50 mM KCl, 300 mM NaCl, 1 mM dithiothreitol, 5 μg/ml pepstatin A and 5 μg/ml aprotinin, and

then lysed on ice for 30 min. The lysed nuclei fraction was centrifuged at 12000×g and 4°C for 30 min. Protein expression levels of COX-2, iNOS, NF-kB and β-actin of the cell lysates (40 μg) were analyzed by the above immunoblot method.

Statistics

All the data were expressed as the mean ± S.E., and statistical significance was analyzed by one way ANOVA followed by Student-Newman-Keuls test.

RESULTS

Inhibition of KRG on PCA Reaction

PCA reaction in mice was induced by the intradermal injection of anti-DNP-HSA and KRG water extract was administered orally 60 min prior to challenge with DNP-HSA antigen and their inhibitory potency of PCA reaction was measured (Table 1). KRG water extract potently inhibited PCA reaction, and at doses of 100 and 500 mg/kg inhibited PCA reaction by 62 and 90%, respectively. It was more potent than DSCG, a commercial agent. However, when KRG was treated in RBL-2H3 cells induced by IgE, it did not inhibit the degranulation of RBL-2H3 cells (data not shown).

Table 1. Inhibitory effect of KRG and DSCG on PCA reaction

Group	Dose (mg/kg)	Inhibition (%)
KRG water extract	100	32±4.8
	500	61±5.1
Disodium cromoglycate	100	37±0.2

All agents were administered *p.o.* or *i.p.* prior to challege with antigen. Each experiment consisted of 6 observations.

Inhibition of KRG on Ear Thickness of Oxazolone-induced Mouse Ear Dermatitis

Therefore, we measured the effects of KRG on oxazolone-induced dermatitis mouse model by topical administration (Fig. 1). The ear applied with oxazolone to sensitized mice caused erythema (reddening of the skin), edema and/or induration, and sometimes abrasion. When ear thickness was measured as an index of skin inflammation, it increased as application was repeated, and reached its maximum 16 days after sensitization. Betamethasone used as a positive agent at concentration of 0.05% potently suppressed ear swelling with a suppressive rate of 78% at 16 days. The suppressive rate of KRG at doses of 0.1% was 39% at 16 days. For histopathological anal-
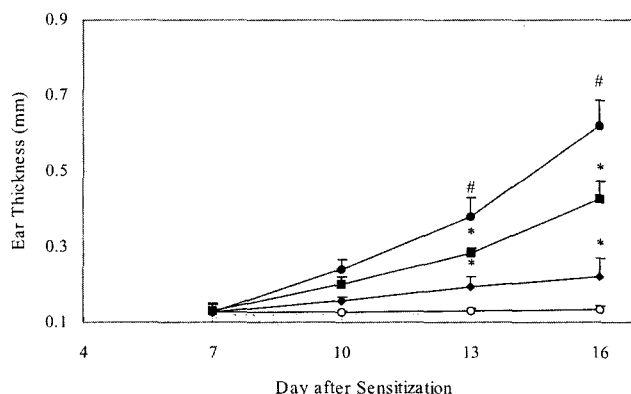

Fig. 1. Effects of KRG on the Ear Thickness of Mice Induced by Oxazolone. ○, vehicle alone (normal) control; ●, oxazolone alone treated control; ■, 0.1% KRG; ◆, 0.05% betamethasone. Values represent means S.E.D. for six mice. #Significantly different from the oxazolone treated (control) group (#P<0.05). *Significantly different from the oxazolone treated (control) group (*P<0.05).

ysis, we excised the ear at 16 days and stained it with hematoxylin-eosin (Fig. 2). The ear applied with oxazolone swelled so dramatically that the entire section could not be shown. KRG improved the ear injured by the application with oxazolone.

Effect of KRG on mRNA Levels of COXs and Some Cytokines in Oxazolone-induced Mouse Ear Dermatitis

The effect of KRG in mRNA levels of COX-1 and COX-2 of mouse ear dermatitis induced by oxazolone was investigated by using RT-PCR analysis (Fig. 3A). Oxazolone significantly induced mRNA levels of COX-2, however, did not induce that of COX-1. When KRG was treated in oxazolone-stimulated mouse, it did not affect the COX-1 mRNA level. KRG inhibited mRNA levels of COX-2. The effect of KRG in mRNA levels of TNF-α and IL-1β, which are produced by macrophage or monocyte, and IFN-γ and IL-4, which are by Th1 and Th2 cells, respectively, was also measured by using RT-PCR analysis (Fig. 3B). Oxazolone significantly induced these mRNA levels of TNF-α, IL-1β, IFN-γ and IL-4. KRG inhibited IL-1β, IFN-γ and TNF-α mRNA levels, however, did not reduce IL-4 mRNA level.

Effect of KRG on iNOS and COX-2 Protein Expression and NF-kB Activation in LPS-induced RAW264.7 Cells

Whether KRG could affect the iNOS and COX-2 protein expressions was also examined. Stimulation of the
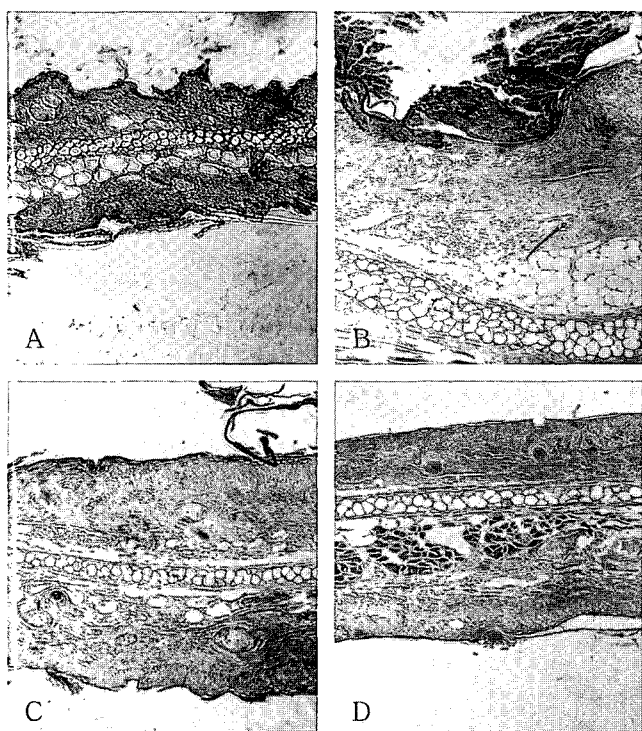
Fig. 2. Histopathological pictures of mouse ear after repeated application of oxazolone with or without KRB. Mouse ears were excised 72 h after the last application of oxazolone and stained with hematoxylin-eosin. As a negative control, mice were only sensitized with 1.5% oxazolone to obdomen followed by no application to the ear (A). 1 % Oxazolone was applied to both sides of the ear every 3 days starting from 7 days after sensitization, and vehicle (ethanol) (B). 0.1% KRG (C) or 0.05% betamethasone (D) was applied to the ear 30 min before and 3 h after each application of oxazolone.

Fig. 3. Effects of KRGS on COX-1, COX-2, IL-1β, IL-4, INF-γ, TNF-α and GAPDH mRNA Expression Levels in Oxazolone-induced Mouse Ear Dermatitis. A), RT-PCR products; (B), Relative density of RT-PCR products (COX-1, COX-2, IL-1β, IL-4, INF-γ or TNF-α/GAPDH). 1, normal control; 2, treated oxazoloe alone; 3, treated with 0.1% KRG with oxazolone; 4, treated 0.05% betamethasone with oxaxolone.

RAW 264.7 cells with LPS resulted in accumulation of the iNOS and COX-2 proteins, as determined by immunoblot analysis (Fig. 4). KRG reduced these levels, compared with the control cells stimulated with LPS. Therefore, the effect of KRG on the activation of the nuclear transcription factor, NF-kB, which induced both iNOS and COX-2, was also investigated. The NF-kB in the nuclei fraction was activated by LPS. However, the KRG inhibited the activation of the NF-kB in LPS-stimulated RAW 264.7 cells. The amounts of NF-kB protein correlated with the reduced accumulation of the iNOS and COX-2 proteins.

DISCUSSION
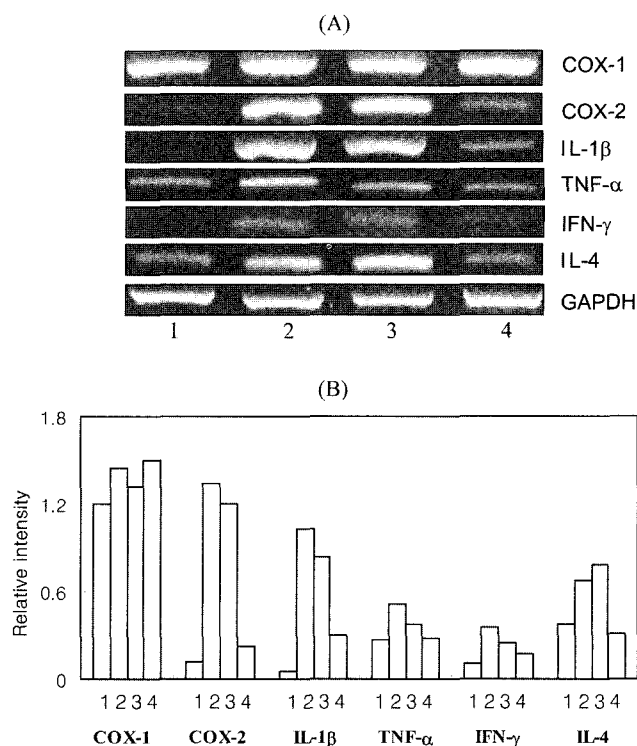
Allergic diseases such as asthma, allergic rhinitis, atopic

dermatitis and food allergy afflict up to 20% of the human population in most countries[19]. The etiology of allergy reactivity is based on IgE-medicated pharmacological processes of a variety of cell populations such as mast cell and basophils[20]. Degradation of mast cells and basophils with antigen-crosslinked IgE releases histamine, prostaglandins, leukotrines and cytokines affecting lymphocytes, macrophages, eosinophils and neutrophils. Finally cytokine-induced reaction causes tissue damages such as chronic dermatitis or psoriasis. Therefore, antiallergic agents with antiinflammatory or antipsoriatic actions may be beneficial drugs for allergic diseases.

KRG potently inhibited PCA reaction induced by IgE and contact dermatitis treated by oxazolone. Nevertheless, when KRG was treated in RBL-2H3 cells induced by IgE, it did not inhibit the release of β-hexosamindase from RBL-2H3 cells (data not shown). This result suggests that KRG almost did not contain active ginsenosides and, if its
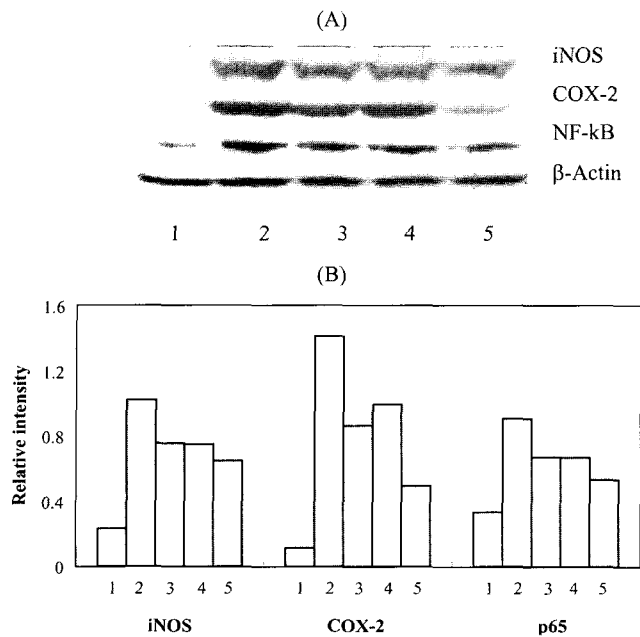
Fig. 4. Effects of KRGS and Its Ginsenosides in iNOS, COX-2 and NF-kB Protein Expression Levels of RAW264.7 Cells Induced by Lipopolysaccharides. (A), Immunoblot; (B), Relative density of immunoblots (iNOS, COX-2 or NF-kB/β-Actin). 1, normal control; 2, treated LPS alone; 3, treated 20 mg/ml KRG with LPS; 4, treated 4 mg/ml KRG with LPS; 5, treated 4 mg/ml betamethasone with LPS.

saponins are orally administered, the ginsenosides may be metabolized by intestinal microflora and express the anti-PCA reaction like the previous reports[7,10]. We also confirmed that KRG did not exhibit the significant antihistamine effect against guinea pig ileum (data not shown), although Tachikawa et al. reported that ginsenoside Rg3 weakly inhibited histamine-induced ileum contractions of guinea-pig[10].

By our previous reports[12-14], ginsenoside Rh1, compound K and ginsenoside Rh2 showed more potent membrane stabilizing effect than those of DSCG, a commercial agent. These results suggest that the inhibitory action of these ginsenosides on the release of β-hexosamindase may be due to protection of the cytolytic response by anti-gen-IgE and these ginsenosides after all showed the most potent inhibitory activity on PCA reaction.

Contact dermatitis was accompanied by sustained swelling, predominant epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages, but not eosinophils. In the present study, the oxazolone-induced dermatitis was also accompanied by substained swelling and predominant epidermal hyperplasia as reported by Fujii et al.[16]. Interferon-γ and TNF-α, which are cytokines involved in chronic skin inflammatory disease[21-23], and COX-2, which is an acute marker of acute inflammatory disease were induced. COX-2, an inducible isoform of COX, is upregulated in skin inflammation and carcinogenesis. Hernandez et al. reproted that cylcooxygenase (COX)-2 was induced in angiogenesis-related diseases such as rheumatoid arthritis and psoriasis[24]. KRG significantly inhibit sustained swelling (thickness) of mice ear induced by oxazolone as well as mRNA levels of COX-2. KRG significantly inhibited those of TNF-α and IL-1β produced by macrophages. KRG also inhibited the increase of oxazolone-induced interferon-γ produced by Th1 cells, but did not inhibit the increase of IL-4 mRNA level produced by Th2 cells. KRG also inhibited protein expression of iNOS and COX2 and activation of NF-kB in RAW264.7 cells induced by LPS. Based on these findings, we suggest that KRG can improve chronic and inflammatory skin disorders contact dermatitis or psoriasis by the regulation of TNF-α, and IL-1β produced by macrophage cells and interferon-γ produced by Th1 cells.

Based on these findings, KRG may show extensive antiallergic and antipsoriatic activity properties and these ginsenosides can be a candidate for the therapeutic agent for allergy.

ACKNOWLEGEMENT

REFERENCES

1. Shibata, S., Fujita, M., Itokawa, H., Tanaka, O. and Ishii, T. : Panaxadiol, a sapongenin of ginseng roots (1). *Chem. Pharm. Bull.*, **11**, 759-764 (1963).

2. Tanaka, N., Tanaka, O. and Shibata, S. : Chemical studies on the oriental plant drugs. XXVIII. Saponins and sapogenins of ginseng; Stereochemistry of sapogenin of ginsenoside Rb1, Rb2 and Rc. *Chem. Pharm. Bull.*, **20**, 1212-1216 (1972).

3. Kitagawa, I., Yoshikawa, M., Yoshihara, M., Hayashi, T. and Taniyama, T. : Chemical studies on crude drug precession. I. On the constituents of ginseng radix rubura (I). *Yakugaku Zasshi* **103**, 612-622 (1983).

4. Kown, S.W., Han, S.B., Park, I.H., Kim, J.M., Park, M.K., and Park, J.H. : Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J. Chromatogr. A*, **921**, 335-339 (2001)

5. Kim, W.Y., Kim, J.M., Han, S.B., Lee, S.K., Kim, N.D., Park, M.K., Kim, C.K. and Park, J.H. : Steaming of ginseng at high temperature enhances biological activity. *J. Nat. Prod.* **63**, 1702-1704 (2000)

6. Wu, J.Y., Gardner, B.H., Murphy, C.I., Seals, J.R., Kensil, C.R., Recchia, J. and Beltz, G.A. : Newman, G.W. and Newman, M.J., Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. *J. Immunol.*, **148**, 1519-1525 (1992).

7. Bae, E.A., Han, M.J., Choo, M.K., Park, S.Y. and Kim, D.H. : Metabolism of 20(S)- and 20(R)-ginsenoside Rg3 by human intestinal bacteria and its relation to in vitro biological activities. *Biol. Pharm. Bull.*, **25**, 58-63 (2002).

8. Kim, N.D., Kang, S.Y., Kim, M.J. and Park, J.H. : Schini-Kerth, V.B., The ginsenoside Rg3 evokes endothelium-independent relaxation in rat aortic rings: role of K+ channels. *Eur J Pharmacol.* **367**, 51-57 (1999)

9. Wakabayashi, C., Hasegawa, H., Murata, J. and Saiki, I. : *In vivo* antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. *Oncol. Res.*, **9**, 411-417 (1998).

10. Tachikawa, E., Kudo, K., Harada, K., Kashimoto, T., Miyate, Y., Kakizaki, A. and Takahashi, E., Effects of ginseng saponins on responses induced by various receptor stimuli. *Eur. J. Pharmacol.*, **369**, 23-32 (1999).

11. Ro, J.Y., Ahn, Y.S. and Kim, K.H., Inhibitory effect of ginsenoside on the mediator release in the guinea pig lung mast cells activated by specific antigen-antibody reactions. *Int. J. Immunopharmacol.* **20**, 625-641 (1998).

12. Park, E.K., Choo, M.K., Han, M.J. and Kim, D.H., Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. *Int. Arch. Allergy Immunol.* **133**, 113-120 (2004)

13. Choo, M.K., Park, E.K., Han, M.J. and Kim, D.-H., Antiallergic activity of ginseng and its ginsenosides. *Planta Med.* **69**, 518-522 (2003).

14. Park, E.K., Choo, M.K., Kim, E.J., Han, M.J. and Kim, D.H., Antiallergic activity of ginsenoside Rh2. *Biol. Pharm. Bull.* **26**, 1581-1584 (2003).

15. Katayama, S., Shionoya, H. and Ohtake, S. : A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.* **22**, 89-101 (1978).

16. Fujii, Y., Takeuchi, H., Tanaka, K., Sakuma, S., Ohkubo, Y. and Mutoh, S. : Effects of FK-506 (tacrolimus hydrate) on chronic oxazolone-induced dermatitis in rats. *Eur. J. Pharmacol.* **456**, 115-121 (2002)

17. Chi, Y.S., Lim, H., Park, H. and Kim, H.P. : Effect of wogonin, a plant flavone from Scutellariae Radix, on skin inflammation: in vivo regulation of inflammation-associated gene expression. *Biochem. Pharmacol.* **66**, 1271-1278 (2003)

18. Ishihara, T., Kohno, K., Ushio, S., Iwaki, K., Ikeda, M. and Kurimoto, M. : Tryptanthrin inhibits nitric oxide and prostaglandin E_2 synthesis by murine macrophages. *Eur. J. Pharmacol.* **407**, 197-204 (2000)

19. Bachert, C. and van Cauwenberge, P. : The WHO ARIA (allergic rhinitis and its impact on asthma) initiative. *Chem. Immunol. Allergy* **82**, 119-26 (2003).

20. Stevens, R.L. and Austen, K.F. : Recent advances in the cellular and molecular biology of mast cells. *Immunology Today* **10**, 381-386 (1989).

21. Abd-El-Aleem, S.A., Feguson, M.W., Appleton, I., Bhowmick, A., McCollum, C.N. and Ireland, G.W. : Expression of cyclooxygenase isoforms in normal human skin and chromic venous ulcers. *J. Pathol.* **195**, 616-623 (2001).

22. Bruch-Gerharz, D., Fehsel, K., Suschek, C., Michel, G., Ruzicka, T. and Kolba-Bachofen, V. : A proinflammatory activity of interleukin-8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. *J. Exp. Med.* **184**, 2007-2012 (1996).

23. Bruch-Gerharz, D., Ruzicka, T. and Kolb-Bachofen, V. : Nitric oxide in human skin: current status and future prospects. *J. Invest. Dermatol.* **110**, 1-7 (1998).

24. Hernandez, G.L., Volpert, O.V., Iniguez, M.A., Lorenzo, E., Martinez-Martinez, S., Grau, R., Fresno, M. and Redondo, J.M. : Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J. Exp. Med.* **193**, 607-620 (2001).