

Antiallergic Effects of Fermented *Ixeris sonchifolia* and Its Constituents in Mice

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To evaluate the antiallergic effect of fermented *Ixeris sonchifolia* (IS, family Compositae), we prepared IS *kimchi*, isolated lactic acid bacteria (LAB) from it, fermented IS with these LAB, and investigated their antiallergic effects. IS *kimchi* inhibited the passive cutaneous anaphylaxis (PCA) reaction induced by an IgE–antigen complex as well as the scratching behavior induced by compound 48/80 or histamine more potently than IS. When IS was fermented with LAB isolated from IS *kimchi*, its antiallergic effects was also increased. Of LAB used for fermentation, *Lactobacillus brevis* more potently increased the antiallergic effects. Its main constituents, chlorogenic acid and luteolin, potently inhibited the PCA reaction induced by the IgE–antigen complex as well as the pruritis induced by compound 48/80 or histamine. These constituents inhibited the expression of proinflammatory and allergic cytokines, TNF- α and IL-4, and transcription factor NF- κ B activation induced by the IgE–antigen complex in RBL-2H3 cells, as well as the degranulation of RBL-2H3 cells induced by the IgE–antigen complex. Luteolin more potently inhibited these allergic reactions than chlorogenic acid. These findings suggest that the antiallergic effect of IS can be increased by LAB fermentation, and the fermented IS might improve allergic reactions such as pruritus, anaphylaxis, and inflammation.

Keywords: *Ixeris sonchifolia*, antiallergic effect, passive cutaneous anaphylaxis reaction, pruritus

and *Saccharomyces* sp. [16, 18]. These microbes transform some components of foods as well as convert sugars to alcohol and lactic acid. For example, lactic acid bacterial fermentation of ginseng produces lactic acid as well as compound K, which is transformed from ginsenoside Rb1, Rb2, and Rc, and exhibits potent cytotoxicity against tumor cells [1, 9, 10].

Ixeris sonchifolia (IS, family Compositae) is a bitter, perennial plant distributed and cultivated widely in Korea. It has been used as a food, such as an ingredient of *kimchi*, and traditional Chinese medicine for suppurative inflammation and pain symptoms [7, 11, 25]. Its representative constituents are cynaroside (luteolin-7-O- β -glycoside), zaluzanin, and ixerin. Of these constituents, flavonoids, such as luteolin, as well as sesquiterpene lactones, such as zaluzanin, exhibit cytotoxic effect against tumor cells [11, 13, 21, 25]. However, the antiallergic effects of IS on allergic reactions, such as anaphylaxis and scratching behavior, have not been studied.

As part of a screening program aimed at discovering antiallergic agents from folk vegetables, we tried to find out the inhibitory effects of IS extract on the passive cutaneous anaphylaxis (PCA) reaction and scratching behavior in mice. It exhibited a weak antiallergic effect. Therefore, we prepared IS *kimchi*, isolated lactic acid bacteria (LAB) from it, fermented IS with these LAB, and investigated their antiallergic effects, such as PCA reaction and scratching behaviors.

MATERIALS AND METHODS

Materials

Dexamethasone, compound 48/80, egg albumin, *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide, anti-dinitrophenol (DNP)–IgE, DNP–human serum albumin (HSA), Evans blue, luteolin, and chlorogenic acid were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Enzyme immunoassay kits for the TNF- α and IL-1 β were purchased

Fermentation produces beneficial products for humans. Therefore, fermentation has been used for products manufacture on an industrial scale. These processes are performed by microbes, such as *Bifidobacterium* sp., *Lactobacillus* sp.,

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from Pierce Biotechnology, Inc. Antibodies to NF- κ B (pp65 and p65) were from Santa Cruz Biotechnology. The protein assay kit was from Bio-Rad Laboratories. The acetonitrile and water used for the HPLC mobile phase were of HPLC grade, and those used for other purposes were of analytical grade.

Extraction of Plant Materials

The cultivar of IS, harvested at Suwon, Kyunggido, Korea in May 2007, was extracted twice with 70% aqueous ethanol for 2 h in a boiling water bath. After extraction, the sample solutions were combined, evaporated to dryness under a vacuum, and freeze-dried.

Preparation of IS *Kimchi*

For the preparation of IS *kimchi*, IS was clearly washed with water and dipped in a 12% salt solution for 6 h. Then, IS were washed twice with tap water, drained, mixed with salts (2.5 g) and glutinous rice paste (2.5 g), and 95 g of IS, stored at 15°C for 2 days to ferment, and stored at 4°C until use in experiment.

For the preparation of IS *kimchi* fermented with LAB, the isolated LAB, grown for 3 days at 37°C in an MRS broth (Difco, Detroit, MI, U.S.A.), were harvested by centrifugation at 10,000 rpm for 15 min, and the collected cells (10^8 CFU/g) were then added to the above mixed *kimchi*, sterilized by heating at 65°C for 30 min, incubated at 15°C for 2 days, and stored at 4°C to ferment.

Isolation and Identification of LAB from IS *Kimchi*

IS *kimchi* (1 g) was homogenized in a stomacher blender for 2 min and then inoculated in MRS agar plates, and then anaerobically incubated at 37°C for 2 days. Of the colonies grown, ten colonies were identified by measuring Gram staining, carbohydrate utility, and 16S rDNA sequence analyses. 16S rDNA sequence analyses were performed using a Big Dye Terminator Cycle Sequencing kit with an automatic DNA kit from Applied Biosystems (Model 310, Perkin-Elmer, Foster City, CA, U.S.A.). The 16S rDNA sequences of the isolated strains were aligned with the 16S rDNA of LAB.

HPLC Analysis

The freeze-dried sample (10 mg) was dissolved in 1 ml of water and filtered through a 0.22- μ m membrane. The main constituents, luteolin and chlorogenic acid, were determined by an HPLC system consisting of a Younglin SP930D quaternary pump and a Younglin UV730D detector. The instrument was controlled and the data were processed by a Younglin AutoChro-3000 (1.0 build No. 5). The analytical column was a Develosil C30-UG-5 (250 \times 4.6 mm i.d., 5 μ m, 100 Å; Nomura Chemical, Japan) protected by a C18 Security Guard Cartridge (Phenomenex, Torrance, CA, U.S.A.). The elution solvent was a mixture of 0.4% acetic acid (A) and acetonitrile containing 0.4% acetic acid (B) used at a gradient profile of A to B from 100:0 for 3 min, from 100:0 to 0:100 for 3–15 min, and from 0:100 for 15–20 min. The flow rate and detection wavelength were 1.0 ml/min and 287 nm, respectively. A sample volume of 15 μ l was used for injection. The retention times of chlorogenic acid and luteolin were 11.767 and 14.317 min, respectively.

Animals

Male ICR (18–22 g, 4 weeks old) and male BALB/c (18–22 g, 5 weeks old) mice were supplied from the Charles River Orient Experimental Animal Breeding Center (Seoul, Korea). All the animals were housed in wire cages at 20–22°C, a relative humidity of

50% \pm 10%, air ventilation frequency of 15–20 times/h, and 12 h illumination (07:00–19:00; intensity, 150–300 Lux), fed standard laboratory chow (Charles River Orient Experimental Animal Breeding Center, Seoul Korea), and allowed water *ad libitum*. All the procedures relating to the animals and their care conformed to international guidelines, "Principles of Laboratory Animal Care" (NIH Publication No. 85–23, revised 1985).

PCA Reaction

An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al.* [5]. The male ICR mice were intradermally injected, with 10 μ g of anti-DNP IgE, into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later, each mouse received an injection of 200 μ l of 3% Evans blue in PBS, containing 200 μ g of DNP–HSA, *via* the tail vein. The test agents were orally or intraperitoneally administered 1 h prior to the DNP–HSA injection. Thirty minutes after the DNP–HSA injection, the mice were sacrificed and their dorsal skins removed, and the pigmented area was measured. After extraction with 1 ml of 1.0 M KOH and 4 ml of a mixture of acetone and 0.2 M phosphoric acid (13:5), the amount of dye was determined colorimetrically at 620 nm.

Scratching Behavior Frequency

Before the experiments, male BALB/c mice were placed in acrylic cages (22 \times 22 \times 24 cm) for about 10 min to become acclimatized. The behavioral experiments were performed according to the method of Sugimoto *et al.* [20]. The rostral part of the skin on the back of the mouse was clipped, and 50 μ g/50 μ l of compound 48/80 (or histamine) dissolved in saline intradermally injected into each mouse. Normal control mice received a saline injection in place of the compound 48/80. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and the scratching behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site with the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches per second, and a series of these behaviors was counted as one incident of scratching over a 60 min period. The test agents were orally administered 1 h before the scratching agent.

Assay of Degranulation of RBL-2H3 Cells Stimulated by IgE–Antigen Complex

The inhibitory activity of test agents against the release of β -hexosaminidase from RBL-2H3 cells was evaluated by a method reported previously [5]. Briefly, RBL-2H3 cells were dispensed into 24-well plates at a concentration of 5×10^5 cells/well using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μ g/ml), and 0.5 μ g/ml of mouse monoclonal anti-DNP IgE, and these were incubated overnight at 37°C in 5% CO₂ for sensitization of the cells. Then, the cells were washed twice with 500 μ l of Siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM MgCl₂, 25 mM PIPES, and 40 mM NaOH), and incubated in 160 μ l of Siraganian buffer containing 5.6 mM glucose, 1 mM CaCl₂, and 0.1% BSA for an additional 10 min at 37°C. Aliquots (40 μ l) of test sample solution were added to each well and incubated for 20 min, followed by the addition of 20 μ l of antigen (DNP–HSA, final concentration 1 μ g/ml)

at 37°C for 10 min to stimulate the cells to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 3,000 ×g for 2 min and 25-μl aliquots of the supernatant were transferred to 96-well plates and incubated with 25 μl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na₂CO₃/NaHCO₃, pH 10.0). The absorbance was measured using a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration 0.1%).

Reverse Transcription–Polymerase Chain Reaction (RT–PCR), Enzyme-Linked Immunosorbent Assay (ELISA), and Immunoblot RBL-2H3 cells (5×10⁵ cells), previously cultured in DMEM, were treated with 0.5 μg/ml of mouse monoclonal IgE to sensitize the cells. The cells were exposed to 40 μl of the test agents (dissolved in 0.5% dimethyl sulfoxide) for 20 min, followed by treatment with 40 μl of DNP–HSA (1 μg/ml) for 60 min at 37°C.

RT–PCR analysis was performed by the method of Shin *et al.* [17]. Briefly, the cells were collected, frozen 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. The RNA was 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 follows: IL-4, forward primer 5'-CCGATTATGGTGTAAATTCCTATGCTG-3' and reverse primer 5'-GGCCAATCAGCACCTCTCTTCCAG-3' (product size 111 bp); tumor necrosis factor (TNF)-α, forward primer 5'-GATTTTATTTGTTTAAAAGCAGATATC-3' and reverse primer 5'-CATCCTAAGTCTACACAGGATCT-3' (product size 206 bp); and GAPDH, forward primer 5'-ACCACAGTCC ATGCCATCAC-3' and reverse primer 5'-TCCACCACCCTGTTGC TGTA-3' (product size 452 bp). The amplification was performed at 94°C for 30–60 s, of 49–62°C for 30–40 s, and at 72°C for 30–60 s with 30 cycles for IL-4, TNF-α, and GAPDH, and 32 cycles for other genes, in a 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.

For the determination of IL-4 and TNF-α, the supernatant (50 μl) of RBL-2H3 cells treated with test agents was then transferred to 96-well ELISA plates, and their concentrations were then determined using commercial ELISA kits (Pierce Biotechnology, Inc., Rockford, IL, U.S.A.) [14].

For the immunoblot of NF-κB (pp65 and p65), the proteins of the supernatants of RBL-2H3 cells treated with test agents were subjected to electrophoresis on an 8%–10% sodium dodecyl sulfate–polyacrylamide gel, and then transferred to a nitrocellulose membrane. The pp65 and p65 of NF-κB were assayed using their corresponding antibodies according to a previously reported method [6]. Immunodetection was carried out using an enhanced chemiluminescence detection kit.

Statistical Analysis

All data were expressed as the mean±standard deviation, with statistical significance analyzed using one-way ANOVA followed by a Student–Newman–Keuls test.

RESULTS

As part of a screening program aimed at discovering antiallergic agents from folk vegetables, we tested to show the inhibitory effects of IS extract on IgE-induced passive cutaneous anaphylaxis (PCA) reaction and compound 48/80-induced scratching behavior in mice (Table 1). Therefore, to increase its antiallergic effect, we fermented IS like cabbage *kimchi* and measured its antiallergic effect. The antiallergic effect of fermented IS, IS *kimchi*, was more potent than that of the non-fermented one.

Therefore, to make antiallergic IS *kimchi*, we isolated Gram-positive and glucose-fermenting LAB from IS *kimchi* and identified them by 16S rDNA analysis. Two major strains were isolated and identified as *Lactobacillus brevis*

Table 1. Inhibitory effects of ethanol extracts of *Ixeris sonchifolia* (IS) with and without fermentation on scratching behaviors and PCA reaction in mice.

| Agent | Dose (mg/kg) | Inhibition (%) | | |
|---------------------------|--------------|-----------------------|---------------------|---------------------------|
| | | Pruritus ^a | | PCA reaction ^b |
| | | Compound 48/80 | Histamine | |
| Ethanol extract of IS | 50 | 20±3 ^c | 19±3 ^c | 20±5 ^c |
| | 100 | 28±3 ^{d,e} | 24±4 ^c | 35±6 ^d |
| Ethanol extract of kimchi | 50 | 24±3 ^{c,d} | 26±4 ^{c,d} | 24±2 ^c |
| | 100 | 33±4 ^c | 35±7 ^d | 38±5 ^d |
| Azelastine | 10 | 68±6 ^f | 73±11 ^c | 72±5 ^c |

^aScratching behavior frequency of the normal control, which was treated with saline alone, and the control group, which was treated with compound 48/80 or histamine, was counted for 1 h. The numbers of scratching behavior frequency of the saline-treated, compound 48/80-treated, and histamine-treated groups were 3±2, 245±23, and 82±5, respectively.

^bThe amounts of Evans blue extravasated from the dorsal skin (1×1 cm) of the control stimulated with the IgE-antigen complex and vehicle-treated groups were 24±4 and 11±3 μg, respectively.

^{c,d,e,f}Items with the same letter in each column were not significantly different.

Inhibition values indicate the mean±SD (n=5).

Table 2. Inhibitory effect of ethanol extract of *Ixeris sonchifolia* (IS) fermented by lactic acid bacteria on scratching behaviors and PCA reaction in mice.

| Agent | Dose (mg/kg) | Inhibition (%) | | |
|--|--------------|-----------------------|---------------------|---------------------------|
| | | Pruritus ^a | | PCA reaction ^b |
| | | Compound 48/80 | Histamine | |
| Ethanol extract (EE) of IS | 50 | 20±3 ^c | 19±3 ^c | 25±3 ^c |
| | 100 | 28±3 ^{d,e} | 24±4 ^{e,d} | 35±6 ^{d,e,f} |
| <i>Lactobacillus brevis</i> -fermented EE of IS | 50 | 24±3 ^{c,d} | 25±5 ^{c,d} | 31±5 ^{d,e} |
| | 100 | 41±7 ^{f,g} | 39±8 ^{d,e} | 43±6 ^f |
| <i>Leuconostoc mesenteroides</i> -fermented EE of IS | 50 | 26±3 ^{c,d} | 26±3 ^{d,e} | 29±1 ^{c,d} |
| | 100 | 37±7 ^{e,f,g} | 37±4 ^e | 39±5 ^{e,f} |
| Azelastine | 10 | 68±6 ^h | 86±4 ^f | 77±5 ^g |

^aScratching behavior frequency of the normal control, which was treated with saline alone, and the control group, which was treated with compound 48/80 or histamine, was counted for 1 h. The numbers of scratching behavior frequency of the saline-treated, compound 48/80-treated, and histamine-treated groups were 3±2, 245±23, and 82±5, respectively.

^bThe amounts of Evans blue extravasated from the dorsal skin (1×1 cm) of the control stimulated with the IgE-antigen complex and vehicle-treated groups were 23±5 and 10±4 µg, respectively.

^{c,d,e,f,g,h}Items with the same letter in each column were not significantly different ($P>0.05$).

Inhibition values indicate the mean±SD (n=5).

and *Leuconostoc mesenteroides* and named *Lactobacillus brevis* I25 and *Leuconostoc mesenteroides* KNUC03 with 99.5% and 99.8% similarities, respectively. IS was fermented with these LAB, and their inhibitory effects against IgE-induced PCA reaction and compound 48/80 or histamine-induced pruritus were tested (Table 2). Of these LAB, *Lactobacillus brevis* more potently increased the antiallergic effect of IS. *Lactobacillus brevis*-fermented IS inhibited IgE-induced PCA reaction as well as compound 48/80- or histamine-induced pruritic reactions.

To understand why the LAB fermentation of IS increases the antiallergic effect, we measured the contents of the main constituents, luteolin and chlorogenic acid, which were determined by LC-MS/MS analysis (data not shown), before and after IS fermentation (Fig. 1). The contents of luteolin and chlorogenic acid were significantly increased by the fermentation, although the yield of each LAB-fermented IS extracted with 50% ethanol was not different (Fig. 1). The results suggest that their glycosides or conjugates may be transformed by LAB.

Next, we measured the inhibitory effects of the main constituents, luteolin and chlorogenic acid, of IS on IgE-induced PCA reaction in mice (Table 3). These constituents inhibited the PCA reaction. Luteolin showed good inhibition. Luteolin, at 10 and 25 mg/kg, inhibited the PCA reaction by 42% and 56%, respectively. Both compounds also inhibited scratching behaviors induced by compound 48/80 or histamine. Luteolin also showed good inhibition on scratching behavior in mice. Luteolin, at 10 and 25 mg/kg, also significantly inhibited compound 48/80-induced scratching frequency by 25% and 39%, respectively, as well as histamine-induced pruritus by 18% and 34%, respectively.

To investigate the antiallergic mechanism of luteolin and chlorogenic acid, we measured their degranulation-

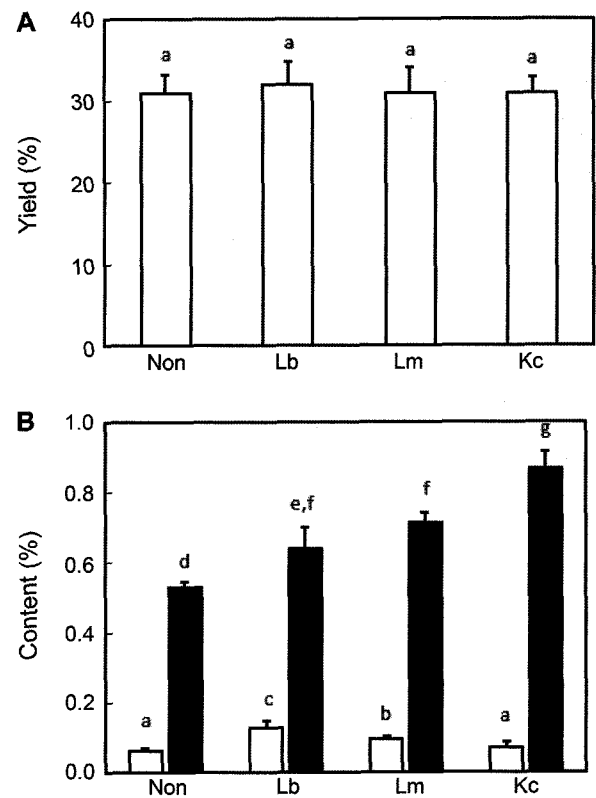


Fig. 1. The yield of 50% ethanol extract of IS fermented with and without lactic acid bacteria (A) and contents of luteolin (white bar) and chlorogenic acid (black bar) in it (B). Non, non-fermented; Lb, fermented with *Lactobacillus brevis*; Lm, fermented with *Leuconostoc mesenteroides*; Kc, naturally fermented like cabbage kimchi. The yield and content values indicate the mean±SD (n=3).

Table 3. Luteolin and chlorogenic acid inhibit mouse PCA reaction induced by the IgE-antigen complex and scratching behavior induced by compound 48/80.

| Agent | Dose (mg/kg) | Inhibition (%) | | |
|------------------|--------------|----------------------------------|-------------------|---------------------------|
| | | Scratching behavior ^a | | PCA reaction ^b |
| | | Compound 48/80 | Histamine | |
| Luteolin | 10 | 25±4 ^{c,d} | 18±4 ^c | 42±6 ^{c,d} |
| | 25 | 39±6 ^e | 34±3 ^d | 56±8 ^c |
| Chlorogenic acid | 10 | 20±3 ^c | 23±4 ^c | 34±5 ^c |
| | 25 | 29±3 ^d | 35±8 ^d | 49±10 ^{d,e} |
| Azelastine | 10 | 73±4 ^f | 68±4 ^e | 77±5 ^f |

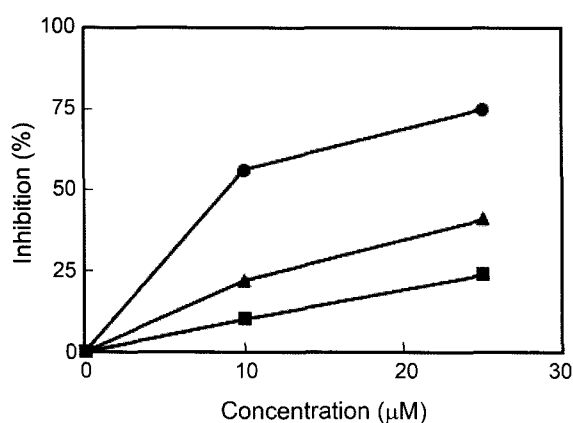
^aScratching behavior frequency of the normal control, which was treated with saline alone, and the control group, which was treated with compound 48/80 or histamine, was counted for 1 h. The numbers of scratching behavior frequency was 3±2 for saline, 245±23 for compound 48/80, and 85±4 for histamine.

^bThe amounts of Evans blue extravasated from the dorsal skin (1×1 cm) of the control stimulated with the IgE-antigen complex and vehicle-treated groups were 24±4 µg and 11±3 µg, respectively.

^{c,d,e,f}Items with the same letter in each column were not significantly different ($P>0.05$).

Inhibition values indicate the mean±SD (n=5).

inhibitory effect (Fig. 2). These agents all dose-dependently inhibited the release of β -hexosaminidase (degranulation) induced by an IgE-antigen complex in RBL-2H3 cells. Luteolin showed good inhibition. Luteolin and chlorogenic acid at 200 µM did not show cytotoxicity for RBL-2H3 cells. These agents inhibited IgE-antigen complex-induced TNF- α expression by 55% and 79%, respectively, and IL-4 expression by 45% and 69%, respectively (Fig. 3). Both compounds also inhibited IgE-antigen complex-induced activation of NF- κ B (p65), providing a mechanism for their inhibition of the inflammatory cytokines. The inhibitory


Fig. 2. Inhibitory effect of luteolin, chlorogenic acid, and azelastine against the degranulation of RBL-2H3 cells induced by the IgE-antigen complex.

RBL-2H3 cells were dispensed into 24-well plates, at a concentration of 5×10^5 cells per well, and sensitized using 0.5 µg/ml of IgE. They were then washed with 500 µl of Siraganian buffer, exposed to 40 µl of various concentrations of luteolin (●), chlorogenic acid (■), and azelastine (▲) for 20 min, and treated with 20 µl of antigen (DNP-HSA, 1 µg/ml) for 10 min at 37°C. The release of β -hexosaminidase from RBL-2H3 cells was measured. Inhibition values indicate the mean±SD (n=3).

effect of luteolin was more potent than that of chlorogenic acid.

DISCUSSION

Mast cells and basophils are critical participants in allergic diseases [19]. These cells express surface membrane receptors with high affinity and specificity for IgE, which is induced by IL-4 [15]. The interaction of antigen-bound IgE with surface membrane receptors causes the release of histamine, prostaglandins, leukotrienes, and cytokines [15]. These cytokine-induced reactions then cause tissue inflammation, anaphylaxis, and scratching behavior, all of which are increasing chronic health problems in many countries [23]. Macrophages activated by some cytokines, such as TNF- α , IL-1 β , and IL-6, play an important role in the regulation of inflammation and immune response [8]. Upon activation, macrophages release various growth factors, cytokines and lipid mediators, which promote inflammation by directing cellular migration to the site of inflammation. These proinflammatory cytokines are regulated by transcription factor NF- κ B activation [3].

In the present study, IS and its constituents, chlorogenic acid and luteolin, showed antiallergic effects *in vitro* and *in vivo*. Among them, luteolin potently inhibited the degranulation of RBL-2H3 cells stimulated with the IgE-antigen complex. Luteolin also inhibited the expression of IgE-switching cytokine IL-4 and proinflammatory cytokine TNF- α like the previous reports [4, 24], as well as the activation of its transcription factor NF- κ B in IgE-induced RBL-2H3 cells. Luteolin inhibited passive cutaneous anaphylaxis induced by IgE as well as pruritic reactions induced by compound 48/80 or histamine more potently than did chlorogenic acid. These results are supported by the

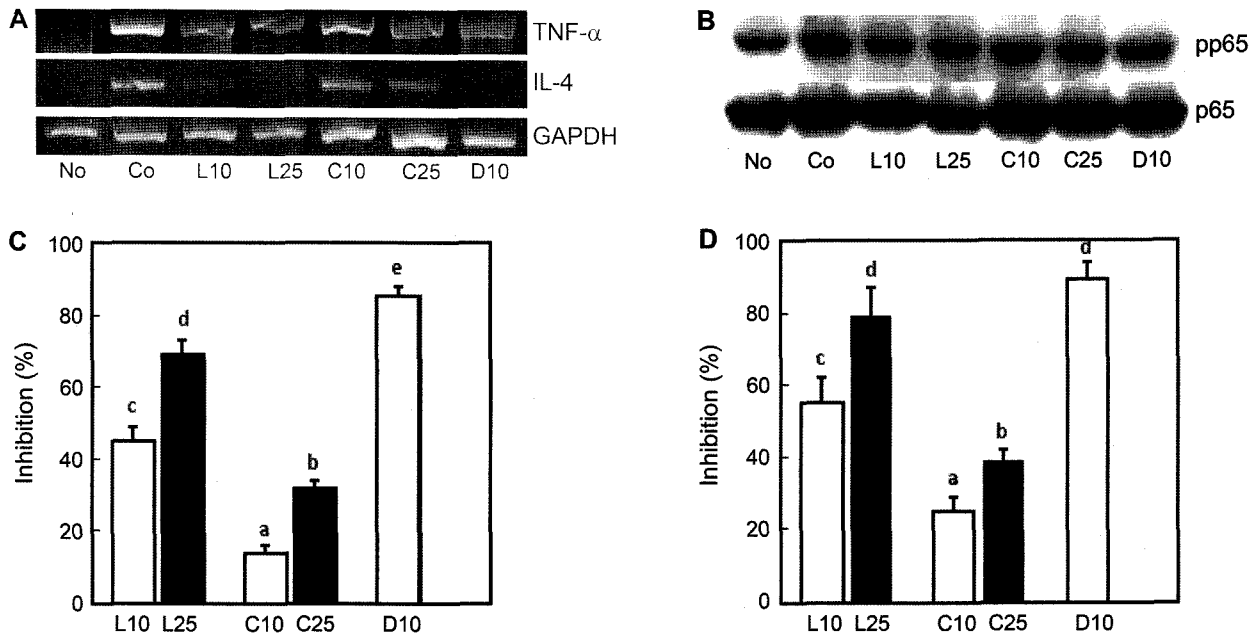


Fig. 3. Effects of luteolin (LT), chlorogenic acid (CA), and dexamethasone (DX) on the expression of TNF- α and IL-4 in RBL-2H3 cells induced by the IgE-antigen complex.

RBL-2H3 cells (5×10^5 cells) were treated with 0.5 μ g/ml of IgE for 1 h, and then exposed to 40 μ l of the test agents (open bar, 10 μ M; closed bar, 25 μ M; L10, 10 μ M luteolin; L25, 25 μ M luteolin; C10, 10 μ M chlorogenic acid; C25, 25 μ M chlorogenic acid; D10, 10 μ M dexamethasone) for 20 min, followed by treatment with 0.2 ml of dinitrophenol-human serum albumin (1 μ g/ml) for 1 h [RT-PCR (A) and immunoblot (B)] or 4 h [for ELISA: (C) IL-4 and (D) TNF- α] at 37°C. Immunoblots for pp65 and p65 and ELISAs for TNF- α and IL-4 were performed. The normal group (No) was treated with vehicle alone and the control group (Co) was with vehicle and IgE-antigen complex.

^{a,b,c,d,e}Items with the same letter in (C) and (D) were not significantly different.

previous reports that luteolin inhibited anaphylaxis and pruritus and improved ovalbumin-induced asthma [2, 12, 22]. The content of luteolin in IS was increased by the fermentation. Of LAB used for IS fermentation, *L. brevis* most potently increased luteolin and inhibited allergic reactions such as PCA and pruritus. These results suggest that the increased antiallergic effect of IS by LAB fermentation may be due to the increase of active constituents, such as luteolin.

Finally, the fermentation of IS by LAB is a beneficial technique to be able to increase the antiallergic effect of IS, and *L. brevis*-fermented IS may improve allergic reactions such as anaphylaxis, pruritus, and inflammation.

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