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Antiallergic Effect of Two Variants of *Artemisia princeps* Pampanini

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Abstract – Antiallergic activities of two variants of *Artemisia princeps* Pampanini SJ-1 (named as Sajabalssuk) and SS-1 (named as Sajuarissuk) cultivated in Ganghwado, which contain high content of eupatilin compared to those cultured by other places, were investigated to evaluate the possibility as inhibitors against allergic diseases. Ethanol and supercritical fluid extracts of SJ-1 and SS-1 inhibited the release of β-hexosaminidase from RBL-2H3 cells, although their water extracts were inactive. These extracts potently inhibited lipopolysaccharide-induced NO production of RAW264.7. However, these extracts almost did not scavenge free radicals. Oral administration of these extracts to mice inhibited passive cutaneous anaphylaxis reaction induced by IgE, and acute dermatitis induced by 12-*O*-tetradecanoylphorbol-13-acetate. However, these extracts did not inhibit chronic dermatitis. Scratching behaviors, vascular permeability, and writhing syndromes were weakly inhibited by these extract at a dose of 50 mg/kg. Based on these findings, we believe that SJ-1 and SS-1 can improve IgE-induced allergic diseases such as rhinitis and asthma.

Keywords – *Artemisia princeps* Pampanini, Sajabalssuk, Sajuarissuk, antiallergic effect, passive cutaneous anaphylaxis reaction, acute dermatitis

Introduction

Allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and psoriasis afflict up to 20% of the human population in most countries (Wuthrich, 1989). The etiology of allergy is based on IgE-mediated pharmacological processes of a variety of cell populations such as mast cell and basophils (Stevens, 1989). Degranulation of mast cells and basophils with antigen-crosslinked IgE releases histamine, prostaglandins, leukotrienes, and cytokines (Stevens and Austen, 1989; Plaut *et al.*, 1989). These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally cytokine-induced reaction causes tissue inflammation such as psoriasis. Psoriasis is a chronic and inflammatory skin disorder. Psoriasis patients have been shown to have interferon-γ producing Th1 bias in lesion skin and peripheral blood and are thought to develop cytokine net works of Th1 cell, resulting in keratinocyte hyperplasia and angiogenesis (Austin *et al.*, 1999; Nicoloff, 1991; Ovigne *et al.*, 2001). Anti-histamines, steroids and immunosuppressants have been used against allergic diseases (Schafer-Korting *et al.*, 1996; Sakuma *et al.*, 2001; Simons, 1992). However, improving these diseases is too difficult. Therefore, herbal medicines have been advanced for allergic diseases, and its effectiveness has received increasing attention (Bielory, 2004; Yang *et al.*, 2001).

Artemisia princeps Pampanini (Family Asteraceae) has long been used for the treatment of inflammation, diarrhea, gastric ulcer, and many circulatory disorders (Kim *et al.*, 1997). It contains eupatilin, acacetin, and eudesmane as main components (Ryu *et al.*, 2005), *A. princeps* Pampanini SJ-1 (local name Sajabalssuk) and SS-1 (local name Sajuarissuk), cultivated in Ganghwado, contain high content of eupatilin, compared to that of other places, such as China. Recently we reported that antiallergic effect of SJ-1 and SS-1.

During the screening program to discover antiallergic agents from natural products, these SJ-1 and SS-1 were found to show inhibitory activity against in vivo passive cutaneous anaphylaxis (PCA) reaction induced by IgE. Therefore, we fermented *A. princes* Pampanini SJ-1 and

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SS-1 with lactic acid bacteria and investigated their antiallergic activities.

Experimental

Materials – Oxazolone, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), betamethasone compound 48/80, egg albumin, *p*-nitrophenyl-N-acetyl-β-D-glucosaminide, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Evans blue were purchased from Sigma Co. (U.S.A). The Griess reagent was purchased from Promega Co. (USA). RAW 264.7 cells were obtained from the Korean Cell line Bank.

Plant material – The following 18 kinds of dried *Artemisia princeps* Pampanini were donated by Ganghwado, Kyunggi, Korean, which were identified by Prof. Ryu, S.N. (Department of agricultural Science Korea National Open University, Seoul 110-791, Korea), and their extracts as tested samples: *A. princeps* Pampanini SJ-1 brewed for 1 year (SJ-1-1), *A. princeps* SJ-1 brewed for 2 year (SJ-1-2), *A. princeps* SJ-1 brewed for 3 year (SJ-1-3), *A. princeps* SS-1 brewed for 1 year (SS-1-1), *A. princeps* SJ-1 brewed for 2 year (SS-1-2), and *A. princeps* SJ-1 brewed for 3 year (SS-1-3) were extracted with distilled water or 80% ethanol under reflux or supercritical fluid extraction (SFE: 5500 psi, 55 °C, 20 min) and then concentrated under vaccum.

Assay of antioxidant activity – DPPH radical scavenging activity of samples was measured according to the method of Xiong *et al.* (1996).

Assay of inhibitory activity against β-hexosaminidase release of RBL-2H3 cells – The inhibitory activity of test samples against the release of β-hexosaminidase from RBL-2H3 cells was evaluated according to Choo *et al.* (2003). RBL-2H3 cells were grown in DMEM supplemented with 10% fetal bovine serum and L-glutamine. Before the experiment, cells were dispensed into 24 well plates at a concentration of 5×10^5 cells per well, and using a medium containing 0.5 μg/ml of mouse monoclonal IgE, the cells were sensitized by incubation overnight at 37 °C in 5% CO_2. They were then washed with 500 μl of siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, 40 mM NaOH) and incubated in 160 μl of siraganian buffer containing 5.6 mM glucose, 1 mM $CaCl_2$ and 0.1% BSA for additional 10 min at 37 °C. Then cells were exposed to 40 μl of test materials for 20 min, followed by the treatment with 20l of antigen (DNP-HSA, 1 μg/ml) for 10 min at 37 °C to activate cells and to evoke allergic reactions (degranulations). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 2000 rpm for 10 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) for 1 h at 37 °C. The reaction was stopped by adding 200 μl of 0.1 M Na_2CO_3/$NaHCO_3$. Absorbance was measured by using an ELISA reader at 405 nm.

Assay of NO production of LPS-induced RAW264.7 cells – RAW264.7 cells were stimulated with LPS (1 μg/ml) for 16 h with or without tested agents, and the cells washed twice with PBS. They were then incubated with test agents for 16 h. Briefly, cells were centrifuged, and 150 μl of the supernatant mixed with 150 μl of Griess reagent and incubated 10 min at room temperature (light protected). The absorbance was measured using an ELISA reader at 540 nm, and compared to a standard calibration curve prepared from sodium nitrite (Choo *et al.*, 2003).

IgE assay of U266 cells induced by IL-4 – IgE assay of U266 cells induced by IL-4 was performed according to the previously reported method (Kim *et al.*, 2001).

Animals – Male and female ICR mice (20 - 22 g) and male BALB/c mice (18 - 22 g) were supplied from Charles River Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20 - 22 °C, a relative humidity of 50 ± 10% humidity, a frequency of air ventilation 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 procedures relating to the animals and their care conformed to the international guidelines 'Principles of Laboratory Animals Care' (NIH publication no. 85 - 23, revised 1985).

Passive cutaneous anaphylaxis (PCA) reaction – An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al.* (2003). The male ICR mice were injected intradermally with 10 μg of anti-DNP-HSA 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 μl of 3% Evans blue PBS containing 200 μg of DNP-HSA *via* the tail vein. The test agents were orally or intraperitoneally 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).

TPA-induced dermatitis – A TPA-induced dermatitis was measured according to the previous method of Reynolds *et al.* (1998). Each group contained 6 male ICR mice (20 - 25 g). TPA (3 mg/20 ml acetone) was applied to the inner and outer mice ear surfaces every day for 3 days to induce subchronic dermatitis every day for 3 days. Test agents, dissolved in an oil-based vehicle, were topically applied to the same site at 1 and 12 h after TPA treatment. The normal group received the vehicle alone. The control group received TPA and the vehicle. On the third day, the test compounds were treated 1 h after the TPA treatment. The thicknesses of both ears of the mice was measured using a Digimatic Micrometer (Mitsutoyo Co., Tokyo, Japan) 3 h after the final treatment of the test compounds.

Oxazolone-induced dermatitis – An oxazolone-induced dermatitis was measured according to the previous method of Fujii *et al.* (2002). Each group contained 6 female ICR mice (20 - 25 g). 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 then applied to both sides of the mouse ear, every 3 days, starting from 7 days after sensitization. The ear thickness was measured using a Digimatic Micrometer 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

Behavioral experiments – Before the experiment, the male BALB/c mice were put into acrylic cages (22 × 22 × 24 cm) for about 10 min for acclimation. The behavioral experiments were performed according to the method of Sugimoto *et al.* (1998). The rostral part of the skin on the back of mice was clipped, and 50 μg/50 μl of compound 48/80 for each mouse was intradermally injected with the use a 29 gauge needle. The scratching agent was dissolved in saline and then used. Control mice received a saline injection in the place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and, for the observation of scratching; their behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by 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 for 1 s, and a series of these behaviors was counted as one incident of scratching for 60 min. The samples were orally administered 1 h before the scratching agent.

Assay of vascular permeability – The increase in vascular permeability caused by scratching agents was assessed as reported previously (Sugimoto *et al.*, 1998). After the intradermal injection of 50 μg/50 μl of compound 48/80 into the rostral part of the back of each male mouse, 0.2 ml of 1% saline solution of Evans blue was injected intravenously. The samples were orally administered 1 h before the scratching agents. Mice were sacrificed 60 min later by cervical dislocation and the scratching agent-injected site excised. The skin specimen was dissolved in 1 ml of 1 M KOH solution by overnight incubation, and 4 ml of a mixture of 0.2 M phosphoric acid solution-acetone (5 : 13) was added. After vigorous shaking, the precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

Assay of analgesic activity – The analgesic activity was determined by using acetic acid-induced ICR mice (Suba *et al.*, 2005). The samples were orally administered prior to intraperitoneal administration of 0.7% acetic acid. The writhing produced in the mice was observed for 10 min (from 10 min to 20 min after administration of acetic acid).

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

Results

Inhibitory activity of SJ-1 and SS-1 in β-hexosaminidase release of RBL-2H3 cells and IgE production of U266 cells – To evaluate antiallergic effect of SJ-1 and SS-1, their inhibitory effect in β-hexosaminidase release (degranulation) of RBL-2H3 cells induced by IgE was investigated (Table 1). Eighty percent ethanol extracts of SJ-1 and SS-1 inhibited the degranulation of RBL-2H3 cells. Supercritical fluid extracted SJ-1 and SS-1 also inhibited the degranulation. However, water-extracted SJ-1 and SS-1 did not inhibit the degranulation. The inhibitory effect of SJ-1 and SS-1 extracts against degranultion of RBL-2H3 cells was not significantly different according brewed periods such as 1, 2 or 3 years.

The effect of these extracts in IgE production of U266 cells stimulated by IL-4 was also measured (Table 1). However, these extracts all did not inhibit the production of IgE.

DPPH radical-scavenging and NO production-inhibitory activities of SJ-1 and SS-1 – To understand the RBL-2H3 cell degranulation-inhibitory mechanism of SJ-1 and SS-1, firstly their antioxidant activity was measured (Table 2). Water extracts of SJ-1 and SS-1

Table 1. Inhibitory activity of SJ-1 and SS-1 in β-hexosaminidase release of RBL-2H3 cells and IgE production of U266 cells

sample[a]	extraction	IC_{50} (μg/ml)	
		degranulation of RBL-2H3 cells	IgE in U266 cells
SJ-1-1	80% ethanol	58.6	>10
SJ-1-2		60.0	>10
SJ-1-3		62.1	>10
SS-1-1		49.6	>10
SS-1-2		45.2	>10
SS-1-3		54	>10
SJ-1-1	water	>100	>10
SJ-1-2		>100	>10
SJ-1-3		>100	>10
SS-1-1		>100	>10
SS-1-2		>100	>10
SS-1-3		>100	>10
SJ-1-1	supercritical fluid extraction (SFE)	62.9	>10
SJ-1-2		68.4	>10
SJ-1-3		68.4	>10
SS-1-1		54.5	>10
SS-1-2		47.0	>10
SS-1-3		37.1	>10

[a] SJ-1-1, *Artemisia princeps* SJ-1 brewed for 1 year; SJ-1-2, *A. princeps* SJ-1 brewed for 2 years; SJ-1-3, *A. princeps* SJ-1 brewed for 3 years; SS-1-1, *A. princeps* SS-1 brewed for 1 year; SS-1-2, *A. princeps* SJ-1 brewed for 2 years; and SS-1-3, *A. princeps* SJ-1 brewed for 3 years.

Table 2. DPPH radical scavenging and NO production-inhibitory activity of SJ-1 and SS-1

sample[a]	extraction	IC_{50} (μg/ml)	
		DPPH radical scavenging	NO production
SJ-1-1	80% ethanol	>50	10.3
SJ-1-2		>50	8.1
SJ-1-3		>50	10.2
SS-1-1		>50	5
SS-1-2		>50	5
SS-1-3		>50	5.7
SJ-1-1	water	33.4	5
SJ-1-2		27.5	5
SJ-1-3		26.4	5
SS-1-1		29.9	–[a]
SS-1-2		28.5	12.0
SS-1-3		31.1	9.9
SJ-1-1	supercritical fluid extraction (SFE)	>50	9.6
SJ-1-2		>50	5
SJ-1-3		>50	8.3
SS-1-1		>50	6.8
SS-1-2		>50	7.5
SS-1-3		>50	–

[a] SJ-1-1, *Artemisia princeps* SJ-1 brewed for 1 year; SJ-1-2, *A. princeps* SJ-1 brewed for 2 years; SJ-1-3, *A. princeps* SJ-1 brewed for 3 years; SS-1-1, *A. princeps* SS-1 brewed for 1 year; SS-1-2, *A. princeps* SJ-1 brewed for 2 years; and SS-1-3, *A. princeps* SJ-1 brewed for 3 years.
[b] Not detected.

scavenged the DPPH radicals. However, neither supercritical extract nor 80% ethanol extraction of SJ-1 and SS-1 scavenged the DPPH radicals.

The inhibitory activity of SJ-1 and SS-1 on NO production of RAW264.7 cells induced by LPS was also measured (Table 2). All extracts of SJ-1 and SS-1 inhibited NO production, with IC_{50} values of 5.0 - 12.0 μg/ml. The inhibitory activity of SJ-1 and SS-1 on NO production of RAW264.7 cells was not significantly different according brewed periods such as 1, 2 or 3 years and extracted methods.

Inhibitory activity of SJ-1 and SS-1 on passive cutaneous anaphylaxis reaction – Degranulation-inhibitory and RAW264.7 cell NO production-inhibitory activities of ethanol extracts of SJ-1 and SS-1 were not significantly different to those of their supercritic extracts. These biological activities of these extracts were not significantly different according to their brewed periods. Therefore, we used 80% ethanol extracts of SJ-1 and SS-1 brewed for 2 years for *in vivo* experiments. First, we measured the inhibitory activity of SJ-1 and SS-1on mouse passive

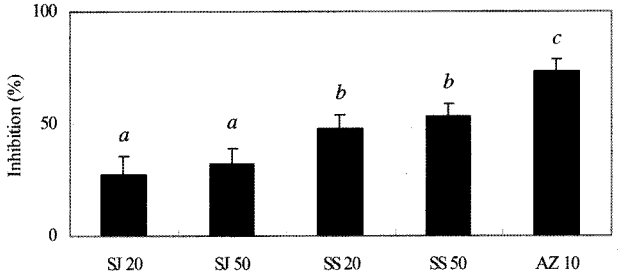

Fig. 1. Inhibitory effect of SJ-1 and SS-1 on IgE-induced passive cutaneous anaphylaxis in mice.
The SJ-1 and SS-1 used in in vivo study was SJ-1-2 (*Artemisia princeps* SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. SJ 20, orally administered 20 mg/kg of SJ-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SJ-1-2 ethanol extract; SS 20, orally administered 20 mg/kg of SS-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SS-1-2 ethanol extract; AZ, orally administered 10 mg/kg of azelastine. [a,b,c]The same letters are not significantly different. All values are mean ± S.D. (n = 5).

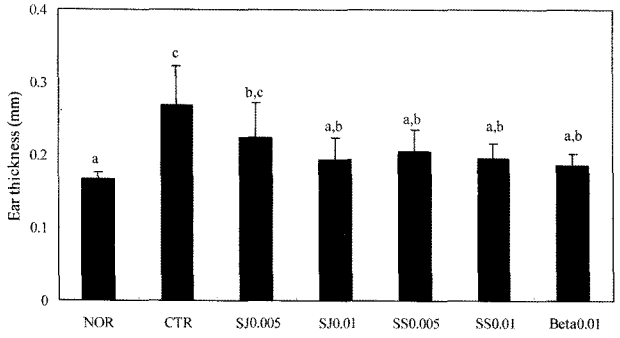

Fig. 2. Inhibitory effect of SJ-1 and SS-1 in TPA-induced acute dermatitis of mouse ears. The SJ-1 and SS-1 used in in vivo study was SJ-1-2 (*Artemisia princeps* SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. NOR, treated vehicle alone; CTR, treated TPA alone; SJ 0.005, treated TPA and 0.005% SJ-1-2 ethanol extract; SJ 0.01, treated TPA and 0.01% SJ-1-2 ethanol extract; SS 0.005, treated TPA and 0.005% SS-1-2 ethanol extract; SS 0.01, treated TPA and 0.01% SJ-1-2 ethanol extract; Beta 0.01, treated TPA and 0.01% betametasone. ^{a,b,c}The same letters are not significantly different. All values are mean ± S.D. (n = 5).

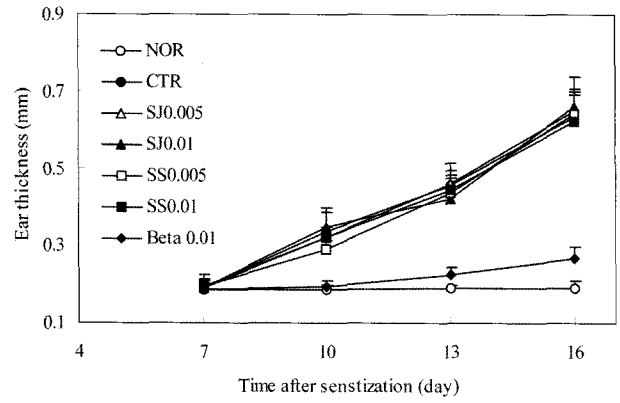

Fig. 3. Inhibitory effect of SJ-1 and SS-1 in oxazolone-induced chronic dermatitis of mouse ears. The SJ-1 and SS-1 used in in vivo study was SJ-1-2 (*Artemisia princeps* SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. NOR, treated vehicle alone; CTR, treated oxazolone alone; SJ 0.005, treated oxazolone and 0.005% SJ-1-2 ethanol extract; SJ 0.01, treated oxazolone and 0.01% SJ-1-2 ethanol extract; SS 0.005, treated oxazolone and 0.005% SS-1-2 ethanol extract; SS 0.01, treated oxazolone and 0.01% SJ-1-2 ethanol extract; Beta 0.01, treated oxazolone and 0.01% betametasone. All values are mean ± S.D. (n = 5).

cutaneous anaphylaxis reaction induced by IgE to evaluate *in vivo* antiallergic activity (Fig. 1). SJ-1 and SS-1 inhibited the PCA reaction induced by IgE, although these extracts weakly inhibited PCA reaction, compared to that of azelastine. SS-1 more potently inhibited PCA reaction than SJ-1.

Inhibitory activities of SJ-1 and SS-1 against acute and chronic skin dermatitis models – To evaluate the

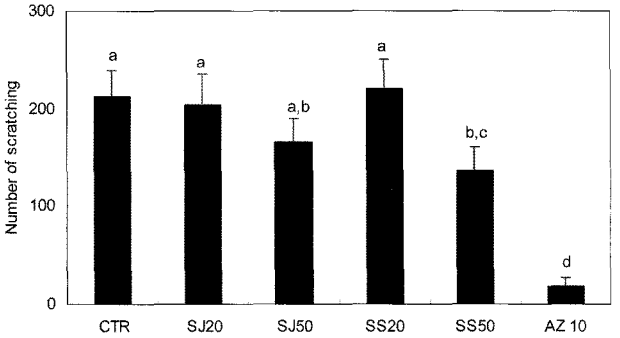

Fig. 4. Inhibitory effect of SJ-1 and SS-1 on compound 48/80-induced scratching behaviors in mice. The SJ-1 and SS-1 used in in vivo study was SJ-1-2 (*Artemisia princeps* SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. SJ 20, orally administered 20 mg/kg of SJ-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SJ-1-2 ethanol extract; SS 20, orally administered 20 mg/kg of SS-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SS-1-2 ethanol extract; AZ, orally administered 10 mg/kg of azelastine. ^{a,b,c,d}The same letters are not significantly different. All values are mean ± S.D. (n = 5).

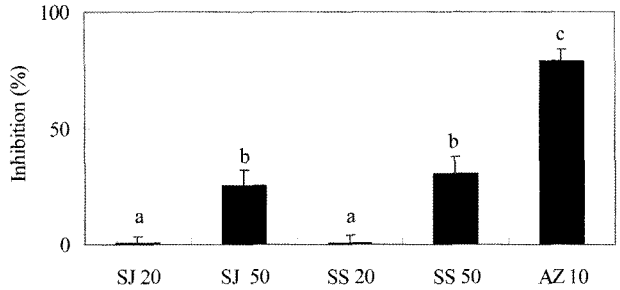

Fig. 5. Inhibitory effect of SJ-1 and SS-1 on compound 48/80-induced vascular permeability in mice. The SJ-1 and SS-1 used in *in vivo* study was SJ-1-2 (*Artemisia princeps* SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. SJ 20, orally administered 20 mg/kg of SJ-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SJ-1-2 ethanol extract; SS 20, orally administered 20 mg/kg of SS-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SS-1-2 ethanol extract; AZ, orally administered 10 mg/kg of azelastine. ^{a,b,c}The same letter are not significantly different. All values are mean ± S.D. (n = 5).

anti-inflammatory activity of SJ-1 and SS-1, their inhibitory activity on mouse acute dermatitis models induced by TPA was measured (Fig. 2). SJ-1 and SS-1 potently inhibited TPA reaction. SJ-1 and SS-1 at a dose of 0.01% inhibited the TPA reaction by 73 and 72%, respectively. The inhibitory potency of SJ-1 was not significantly different to that of SS-1. These inhibitory potencies was comparable to that of betamethasone (81% inhibition). However, these extracts did not inhibit chronic skin dermatitis of mice induced by oxazolone (Fig. 3).

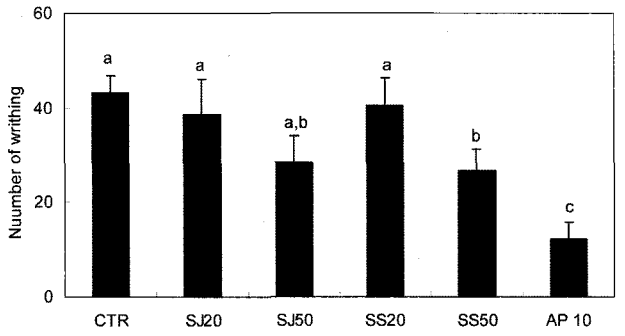

Fig. 6. Inhibitory effect of SJ-1 and SS-1 on acetic acid-induced writhing syndromes in mice. The SJ-1 and SS-1 used in in vivo study was SJ-1-2 (Artemisia princeps SJ-1 brewed for 2 years) and SS-1-2 (*A. princeps* SS-1 brewed for 2 years), respectively. SJ 20, orally administered 20 mg/kg of SJ-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SJ-1-2 ethanol extract; SS 20, orally administered 20 mg/kg of SS-1-2 ethanol extract; SJ 50, orally administered 50 mg/kg of SS-1-2 ethanol extract; AP, orally administered 10 mg/kg of antipyrine. ^{a,b,c}The same letter are not significantly different. All values are mean ± S.D. (n = 5).

Inhibitory effect of SJ-1 and SS-1 on scratching behaviors induced by compound 48/80 – The inhibitory activity of SJ-1 and SS-1 in the compound 48/80-induced scratching behavior animal model was investigated (Fig. 4). SJ-1 and SS-1 extracts at a dose of 50 mg/kg inhibited the scratching frequency by 22 and 36%, respectively.

These extracts at a dose of 50 mg/kg also decreased the vascular permeability of skin induced by compound 48/80 by 25 and 30%, respectively (Fig. 5). The inhibitory activity of these extract against vascular permeability was proportion to their inhibitions against scratching behaviors.

Analgesic activity of SJ-1 and SS-1 – Analgesic activity of SJ-1 and SS-1 against acetic acid induced writhing syndromes in mice was measured. SJ-1 and SS-1 both weakly inhibited writhing syndromes. SJ-1 and SS-1 at a dose of 50 mg/kg inhibited writhing syndromes by 35 and 38%. The analgesic activity of SJ-1 was not significantly different to that of SS-1.

Discussion

Allergic reactions including rhinitis, asthma, anaphylaxis and psoriasis produced many inflammatory mediators and caused scratching, inflammation, pain and the increase of vascular permeability (Wuthrich, 1989; Stevens and Austen, 1989). Anti-histamines, NSAID, steroids and immunosuppressants are representative agents against these allergic diseases (Schafer-Korting *et al.*, 1996; Sakuma *et al.*, 2001; Simons, 1992; Friedman *et al.*, 2002). Among antihistamines, azelastine is an H1-receptor antagonist, but also decreases mediator release from mast cells and basophils. Disodium chromoglycate (DSCG) is a membrane stabilizer, whereas compound 48/80, a histamine releaser, is an activator of hyaluronidase (Cox, 1967). DSCG is mainly known to inhibit the release of chemical mediators from mast cells induced by the antigen-IgE antibody reaction. Steroids, betametasone and dexamethasone are clinically used in the treatment of psoriasis and other skin disorders as a potent corticosteroid. Corticosteroids are well known to have potent anti-inflammatory effects, but topical use can cause intense skin atrophy, one of the serious side effects limiting their uses for chronic skin diseases. Repeated application of corticosteroids on dorsal skin of rats also causes dramatic skin atrophy (Schafer-Korting *et al.*, 1996). FK-506 and cyclosporine A are a potent immunosuppressant currently used for preventing allograft rejection (Sakuma *et al.*, 2001). FK-506 also suppressed the increase in ear thickness and epidermal thickness. However, it also exhibited side effects, such as severe nephrotoxicity and neurotoxicity. Therefore, herbal medicines have been advanced for allergic diseases, and its effectiveness has received increasing attention. *A. princeps* SJ-1 and SS-1 cultivated in Ganghwado contains high content of eupatilin compared to those produced by other places (Ryu *et al.*, 2005). Particularly eupatilin exhibited potent anti-inflammatory activity for gastritis. Therefore, we evaluated antiallergic activities of APs cultivated in Ganghwado in the present study. Ethanol extracts of SJ-1 and SS-1 inhibited the release of β-hexosaminidase from RBL-2H3 cells, as potently as azelastine. These extracts potently inhibited NO production of RAW264.7 cells induced by LPS. However, these extracts almost did not scavenge DPPH radicals. These reports suggest that the inhibitory action of these extracts of APs on the release of β-hexosaminidase may be due to its ability to protect cells from the cytolytic response induced by antigen-IgE or compound 48/80. Oral administration of these extracts to mice inhibited PCA reaction induced by IgE and acute dermatitis induced by TPA. However, these extracts did not inhibit the chronic dermatitis. Scratching behaviors, vascular permeability and writhing syndromes were weakly inhibited by these extract at a dose of 50 mg/kg. Based on these findings, we believe that APs can improve IgE-induced allergic diseases such as rhinitis and asthma, however their topical applications may not improve these allergic diseases.

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