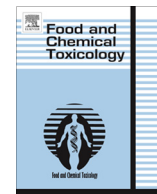




Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Saussurea lappa alleviates inflammatory chemokine production in HaCaT cells and house dust mite-induced atopic-like dermatitis in Nc/Nga mice

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ARTICLE INFO

Article history:

Received 5 September 2013

Accepted 31 October 2013

Available online xxx

Keywords:

Atopic dermatitis

Chemokines

Keratinocyte

Nc/Nga mice

Saussurea lappa

ABSTRACT

Saussurea lappa is a traditional herbal medicine used for to treat various inflammatory diseases. In this study, we investigated the protective effects of *S. lappa* against atopic dermatitis using human keratinocyte HaCaT cells, murine mast cell line MC/9 cells, and a house dust mite-induced atopic dermatitis model of Nc/Nga mice. Treatment with the *S. lappa* caused a significant reduction in the mRNA levels and production of inflammatory chemokines and cytokine, including thymus- and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC), regulated on activation, normal T-cell expressed and secreted (RANTES), and interleukin-8 (IL-8) in tumor necrosis factor- α /interferone- γ -stimulated HaCaT cells. *S. lappa* exhibited the significant reduction in histamine production in MC/9 cells. In the atopic dermatitis model, *S. lappa* significantly reduced the dermatitis score and serum IgE and TARC levels. In addition, the back skin and ears of *S. lappa*-treated Nc/Nga mice exhibited reduced histological manifestations of atopic skin lesions such as erosion, hyperplasia of the epidermis and dermis, and inflammatory cell infiltration. In conclusion, an extract of *S. lappa* effectively suppressed the development of atopic dermatitis, which was closely related to the reduction of chemokines and cytokine. Our study suggests that *S. lappa* may be a potential treatment for atopic dermatitis.

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1. Introduction

Atopic dermatitis is a chronic inflammatory skin disease that is characterized by the presence of eczematous lesions, pruritus, and redness. The prevalence of atopic dermatitis has increased substantially in the last decade. This disease develops mainly in childhood, and approximately 10–20% of children suffer from atopic dermatitis worldwide (Eichenfield et al., 2012). In particular, most cases of atopic dermatitis present during the first year of life and 80% of patients display the symptoms of atopic dermatitis within the first 5 years of life (Blume-Peytavi and Metz, 2012). Atopic dermatitis appears to have a variety of causes, such as genetic factors, environmental pollution, and immunological responses (Hinz et al., 2006; Bieber, 2008).

Despite numerous preclinical and clinical studies, the pathogenesis of atopic dermatitis remains unclear. At present, the pathogenesis of atopic dermatitis is known to be related to keratinocyte dysfunction (Eichenfield et al., 2012). The stimulation of keratinocytes can cause the production of inflammatory chemokines,

including thymus- and activation-regulated chemokine/CCL17 (TARC), macrophage-derived chemokine/CCL22 (MDC), and regulated on activation, normal T-cell expressed and secreted/CCL5 (RANTES) (Saeki and Tamaki, 2006). These chemokines are specialized for the recruitment of effector cells, such as monocytes, granulocytes, and T lymphocytes, which facilitate the development of inflammatory response in atopic dermatitis lesions. In addition, interleukin (IL)-8 is another important mediator in atopic dermatitis. Previous studies demonstrated that IL-8 is closely associated with the severity of atopic skin lesions (Casas et al., 2011; Amarbayasgalan et al., 2013; Kim et al., 2013; Park et al., 2013). *Saussurea lappa* is a traditional herbal medicine that has been used to treat asthma, inflammation, rheumatism, coughs, tuberculosis, and many other diseases (Shah, 1982; Choi et al., 2012). It has also been used as an analgesic, digestive, aphrodisiac, and diuretic (Yaeesh et al., 2010). Previous studies have demonstrated that *S. lappa* possesses several pharmacological properties, such as antioxidative (Saleem et al., 2013), antiulcer (Shah, 1982), anticancer (Kim et al., 2012), antiviral (Chen et al., 1995), and hepatoprotective effects (Yaeesh et al., 2010). In particular, Sarwar and Enbergs (2007) reported that *S. lappa* may contribute to the suppression of immune-mediated inflammatory reactions. *S. lappa* was also found to decrease proinflammatory mediators in lipopolysaccharide (LPS)-stimulated

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murine macrophage cells (Choi et al., 2012). Of interest, our preliminary study revealed of *S. lappa* extract on proinflammatory cytokines including IL-6 and TNF- α in murine macrophages (data not shown).

In the present study, we investigated the effects of an extract of *S. lappa* on the levels of chemokines and cytokine in a human keratinocyte cell line, HaCaT cells, and production of histamine in murine mast cell line, MC/9 cells. Based on the results of *in vitro* experiments, we evaluated the protective effects of the *S. lappa* extract against atopic dermatitis using a house dust mite-induced atopic dermatitis model in Nc/Nga mice. We measured the skin severity score, histological changes in the skin, including mast cell infiltration, and the plasma IgE level and TARC levels.

2. Materials and methods

2.1. Reagents and materials

Dried *S. lappa* (100 g) was extracted three times with 70% methanol (1 L) by refluxing for 90 min. The extract was filtered, evaporated to dryness, and freeze-dried using a freeze dryer (PVTFD100R, Ilshin Lab. Ltd., Korea). The yield was 28.6%. The *S. lappa* material used in this experiment was purchased from HMAX (Jecheon, Korea) in 2009. A specimen (2009-KIOM62) has been deposited in the Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine.

Biostir-AD[®], which is an ointment that contains house dust mite (*Dermatophagoides farinae*) extract, was purchased from Biostir Inc. (Kobe, Japan).

2.2. Cell culture

The human keratinocyte HaCaT cells and murine mast cell line MC/9 were obtained from CLS Cell Lines Service GmbH (Eppenheim, Baden-Württemberg, Germany) and the American Type Culture Collection (Rockville, MD, USA), respectively. The HaCaT cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco Inc., Grand Island, NY, USA), which was supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco Inc.), penicillin (100 U/mL), and streptomycin (100 μ g/mL), in a 5% CO₂ incubator at 37 °C. The MC/9 cells were cultured in DMEM supplemented with 0.05 mM 2-mercaptoethanol (Sigma-Aldrich, St Louis, MO, USA), 10% Rat T-STIM (Becton, Dickinson & Co., Franklin Lakes, NJ, USA), 10% heat-inactivated FBS (Gibco Inc.), penicillin (100 U/mL), and streptomycin (100 μ g/mL), in a 5% CO₂ incubator at 37 °C.

2.3. Cytotoxicity assay

The cell viability was assessed using a Cell Counting Kit-8 assay (CCK-8 from Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. HaCaT cells (1×10^3 cells/well) and MC/9 cells (4×10^4 cells/well) were incubated in 96-well plates with various concentrations of the *S. lappa* extract for 24 h. The CCK-8 reagent was added to each well, followed by incubation for an additional 4 h, and the absorbance was measured at 450 nm using a Benchmark plus microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The percentage of viable cells was calculated using the following equation: cell viability (%) = (mean absorbance in test wells/mean absorbance in control wells) $\times$ 100.

2.4. Measurement of chemokine and cytokine production

HaCaT cells (1×10^6 cells/well) were cultured in 6-well plates. After reaching a confluent state, the cells were washed and treated with *S. lappa* in 1 mL of serum-free medium that contained tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) (each 10 ng/mL; R&D Systems Inc., Minneapolis, MN, USA) for 24 h. The production of TARC, MDC, RANTES and IL-8 was determined using a commercial ELISA kits.

2.5. Measurement of histamine production

MC/9 cells (2×10^5 cells/well) were cultured in 48-well plates. After reaching confluence, MC/9 cells were treated with *S. lappa* extract in 0.5 mL of medium containing phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, St. Louis, MO, USA) and calcium ionophore A23187 (Sigma-Aldrich) for 24 h. The supernatants were harvested, histamine production was quantified using an commercial ELISA kit (Oxford Biomedical Research, Oxford, MI, USA).

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA was isolated using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). One microgram of total RNA was converted to cDNA with an iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA),

which contained oligo-dT primers, and diethyl pyrocarbonate-treated water was added to produce a final volume of 20 μ L, before incubating at 42 °C for 30 min. The PCR amplification used gene-specific primers for TARC (forward, 5'-ACT GCT CCA GGG ATG CCA TCG TTT TT-3'; reverse, 5'-ACA AGG GGA TGG GAT CTC CCT CAC TG-3'), MDC (forward, 5'-AGG ACA GAG CAT GGC TCG CCT ACA GA-3'; reverse, 5'-TAA TGG CAG GGA GCT AGG GCT CCT GA-3'), RANTES (forward, 5'-CCC CGT GCC GAG ATC AAG GAG TAT TT-3'; reverse, 5'-CGT CCA GCC TGG GGA AGG TTT TTG TA-3'), IL-8 (forward, 5'-GTG GCT CTC TTG GCA GCC TTC CTC AT-3'; reverse, 5'-TCT CCA CAA CCC TCT GCA CCC AGT TT-3') and GAPDH (forward, 5'-GTG ATG GCA TGG ACT GTG GT-3'; reverse, 5'-AAG GGT CAT CAT CTC TGC CC-3'). The RT-PCR reaction mixture comprised 1 μ L of cDNA and 1.56 μ L of γ Taq PCR master mix, which contained 1.5 mM MgCl₂, 0.1 μ M of each forward and reverse primer, and 7.44 μ L of water in a final volume of 10 μ L. The PCR reaction comprised 25 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min 30 s for all of the chemokines and IL-8, and 25 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min 30 s for GAPDH. The relative expression levels of TARC, MDC, RANTES, and IL-8 were adjusted based on the expression of GAPDH as a control.

2.7. Experimental animals

Specific-pathogen free male Nc/Nga mice (eight weeks old) were purchased from Central Laboratory Animal Inc. (Seoul, South Korea). The animals were maintained in an air-conditioned room and maintained at 24 $\pm$ 2 °C with 55 $\pm$ 15% humidity. The animals were housed one per cage and were allowed sterilized tap water and standard rodent chow (Samyang Feed Co., Wonju, Korea) *ad libitum*. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (Approval number: #12-047).

2.8. Induction of allergic dermatitis in Nc/Nga mice

Atopic dermatitis-like skin lesions were induced in 10-week-old male Nc/Nga mice using Biostir-AD, as described by the manufacturer (Lee et al., 2010). The animals were housed in conventional condition. Briefly, after shaving the upper back,

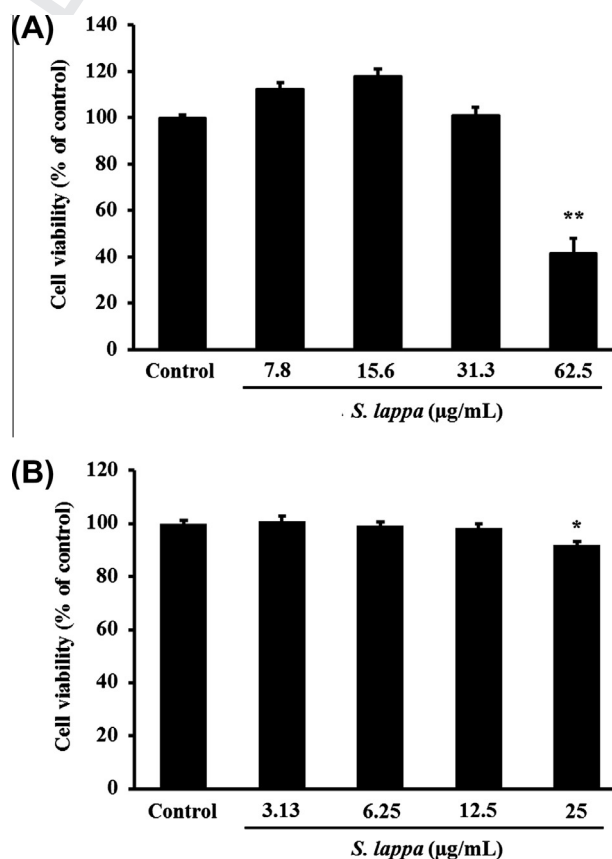


Fig. 1. Cytotoxicity of *Saussurea lappa* extract in HaCaT cells (A) and MC/9 cells (B). Cells were seeded into 96-well plates and treated with various concentrations of *S. lappa* extract for 24 h. Cell viability was assessed using a CCK-8 assay. The values were expressed as mean $\pm$ SD of three independent experiments. *, ** P < 0.05 and 0.01 vs. vehicle control cells.

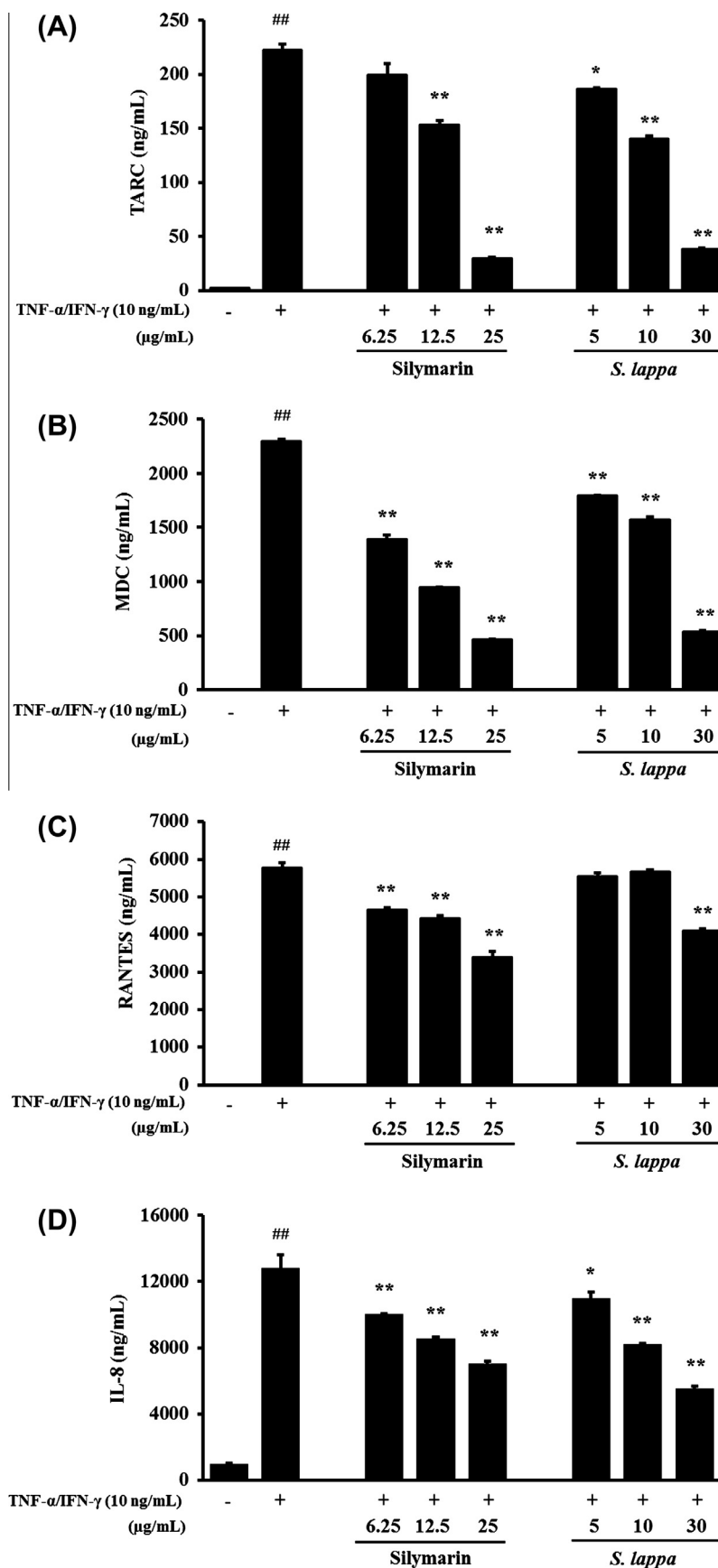


Fig. 2. Effects of *Saussurea lappa* extract on TNF- α and IFN- γ -stimulated productions of chemokines and cytokine in HaCaT cells. Productions of TARC (A), MDC (B), RANTES (C) and IL-8 (D) were measured using the culture supernatant from cells cotreated with *S. lappa* extract (5, 10, or 30 μ g/mL), and TNF- α and IFN- γ (each 10 ng/mL, TI) for 24 h. Silymarin (6.25, 12.5, or 25 μ g/mL) was used as a positive control. Values were expressed as mean $\pm$ SEM of three independent experiments. ## P < 0.01 vs. vehicle control cells; * P < 0.05 and P < 0.01 vs. TI-treated cells, respectively.

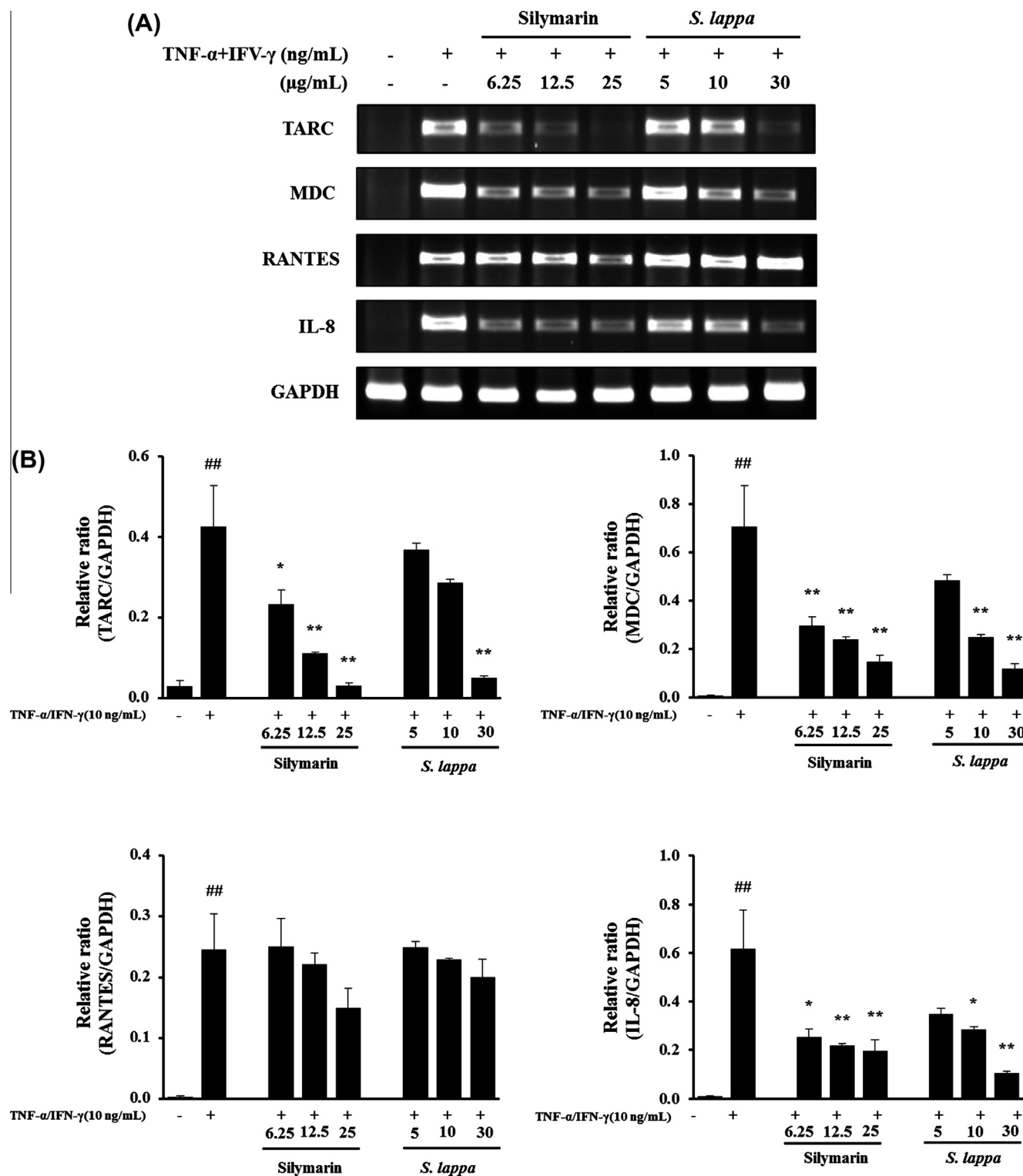


Fig. 3. Effect of *Saussurea lappa* extract on TNF- α and IFN- γ -stimulated chemokine and cytokine expression in HaCaT cells. (A) RT-PCR was performed to determine the mRNA expression levels of TARC, MDC, RANTES, and IL-8. (B) The intensities of the PCR bands. Values were expressed as mean $\pm$ SEM of three independent experiments. $^{##}P < 0.01$ vs. vehicle control cells; $^{*}P < 0.05$ and $^{*}P < 0.01$ vs. TI-treated cells, respectively.

200 μ L of 4% (w/v) sodium dodecyl sulfate was applied to the shaved dorsal skin and both surfaces of each ear for barrier disruption. After 2 h, 50 mg of Biostir-AD was applied topically twice per week for 6 weeks. The mice ($n = 35$) were allocated to five groups ($n = 7$ /group): normal control group (vehicle-treated mice; distilled water, 150 μ L/mouse, per os (p.o.)), Biostir group (Biostir-AD plus vehicle-treated mice; distilled water, 150 μ L/mouse, p.o.), prednisolone group (Biostir-AD plus prednisolone-treated mice; 3 mg/kg, p.o.), and *S. lappa* group (Biostir-AD plus

S. lappa-treated mice; 100 or 300 mg/kg, p.o.). The doses of *S. lappa* were based on the results of preliminary study. The procedure of preliminary study was same to present study. The skin severity significantly decreased in both 300 and 500 mg/kg of *S. lappa* compared with the animal with atopic dermatitis. However, 500 mg/kg-treated mice were observed the increases in liver weight, indicating liver toxicity. Based on the results of preliminary studies, we determined 100 mg/kg and 300 mg/kg of *S. lappa* as administration dosage. Prednisolone and *S. lappa*

were dissolved in distilled water and applied every day for 6 weeks. Prednisolone was used as a positive control. The mice were sacrificed under anesthesia via intraperitoneal injection with pentobarbital sodium (Entobar Inj., Hanlim Pharm. Co., Ltd., Korea) on the day of the experiment. During the autopsy, blood was collected from the posterior vena cava, and the back skin and each ear were excised for histopathological analyses.

2.9. Evaluation of skin severity

The relative severity of dermatitis was assessed macroscopically according to the Eczema Area and Severity Index (EASI) scoring system: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. The dermatitis score was defined as the sum of the scores for erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness (Hanifin et al., 2001). The mice were also photographed once each week.

2.10. Histological analysis

After sacrifice, the back skin and one ear of each mouse were fixed in 10% (v/v) neutral buffered formalin for 24 h at 4 °C. The tissue samples were embedded in paraffin and thin-sectioned (4 μm thickness). The sections were stained with hematoxylin and eosin (H&E) solution (Sigma–Aldrich, St. Louis, MO, USA) and mounted under cover slips using Dako mounting medium (Dako Cytomation, Glostrup, Denmark). The stained sections were performed using an image analyzer (Molecular Devices Inc., CA, USA). To measure the degree of mast cell infiltration, the skin sections were stained with toluidine blue, and the numbers of mast cells were counted in four sites.

2.11. Measurement of the plasma IgE and TARC levels

Blood samples were drawn from the mice, separated by centrifugation at 10,000g for 10 min at 4 °C, and stored at –80 °C. The IgE and TARC levels in the plasma were measured using an ELISA kit (IgE, BioLegend, San Diego, CA, USA; TARC, R&D Systems Inc.) according to the manufacturer's instructions.

2.12. Statistical analyses

The data were expressed as the mean ± SEM. All of the experiments were performed at least three times. One-way analysis of variance was used to detect significant differences between the control and treatment groups. Dunnett's test was used for multiple comparisons. The differences were considered significant at $P < 0.05$.

3. Results

3.1. *S. lappa* extract inhibits chemokine production in TNF-α and IFN-γ-stimulated HaCaT cells

First, we measured the cytotoxic effect of the *S. lappa* extract on HaCaT cells. Treatment with the *S. lappa* extract for 24 h at concentrations ranging from 7.8 to 31.3 μg/mL did not produce any significant cytotoxic effects (Fig. 1A). Thus, we used the *S. lappa* extract at 5, 10, or 30 μg/mL in the subsequent experiments.

The effects of the *S. lappa* extract on TARC, MDC, and RANTES production were assessed in TNF-α and IFN-γ (TI)-stimulated HaCaT cells. As shown in Fig. 2A, HaCaT cells treated with TI produced significantly more TARC (222.27 ± 5.73 ng/mL, $P < 0.01$) compared with the vehicle-treated cells (2.18 ± 0.29 ng/mL). By contrast, the *S. lappa* extract suppressed TI-stimulated TARC production in a dose-dependent manner. The levels were reduced by 153.81 ± 3.61 ng/mL ($P < 0.01$) and 29.96 ± 0.97 ng/mL ($P < 0.01$) with 10 and 30 μg/mL, respectively. MDC (2295.75 ± 22.33 ng/mL, $P < 0.01$) production in TI-treated cells was also increased compared with vehicle-treated cells, but the levels were reduced significantly after treatment with the *S. lappa* extract treatment ($P < 0.01$) (Fig. 2B). TI-treated cells produced significantly more RANTES (5773.62 ± 141.15 ng/mL, $P < 0.01$) compared with the vehicle-treated cells. The TI-induced increase in RANTES production was significantly inhibited by the *S. lappa* extract (4107.78 ± 33.71 ng/mL with 30 μg/mL, $P < 0.01$) (Fig. 2C). In addition, the level of IL-8 also was significantly reduced by the *S. lappa* extract in a concentration-dependent manner ($10,984.27 \pm 518.26$ ng/mL

with 5 μg/mL, $P < 0.05$; 8214.63 ± 36.88 ng/mL in 10 μg/mL, $P < 0.01$; 5522.16 ± 235.29 ng/mL in 30 μg/mL, $P < 0.01$) (Fig. 2D). The inhibitory effects of *S. lappa* were similar to those of silymarin, a positive control.

3.2. *S. lappa* extract inhibits the expression of chemokine and cytokine mRNA in TNF-α and IFN-γ-stimulated HaCaT cells

We investigated the effects of the *S. lappa* extract on the mRNA expression levels of TARC, MDC, RANTES, and IL-8. As shown in Fig. 3, the mRNA levels of TARC, MDC, and RANTES were enhanced after TI stimulation of the cells. By contrast, the *S. lappa* extract dramatically suppressed the expression of the TARC and MDC in a dose-dependent manner. Moreover, IL-8 was downregulated by *S. lappa* extract treatment of the TI-stimulated cells. The reductions in TARC, MDC and IL-8 mRNA by *S. lappa* extract were similar to those of silymarin-treated cells. Silymarin-treated cells exhibited the significant decreases in chemokine and cytokine mRNA expression. The level of RANTES mRNA showed little suppression as compared with those of TARC, MDC and IL-8.

3.3. *S. lappa* extract reduces the production of histamine in PMA and A23187-stimulated MC/9 cells

To determine the cytotoxicity of the *S. lappa* extract in MC/9 cells, the cells were exposed to various concentrations of the *S. lappa* extract for 24 h. Nontoxic concentrations of the *S. lappa* extract was determined and used for subsequent experiments (Fig. 1B). PMA and A23187-stimulated MC/9 cells exhibited a significant elevation of histamine compared with the vehicle-treated cells. In contrast, *S. lappa*-treated MC/9 cells showed the significant reduction in the histamine production compared with the PMA and A23187-stimulated MC/9 cells (Fig. 4).

3.4. *S. lappa* extract suppresses the severity of dermatitis and skin lesions in a house dust mite-induced atopic dermatitis Nc/Nga mouse model

The severity of dermatitis was evaluated once a week. The major clinical signs and symptoms developed immediately after the mite antigen was applied to the backs of the mice. The atopic dermatitis-like skin lesions worsened progressively for 4 weeks with the initial treatment. Repeated applications of Biostir-AD ointment induced skin dryness, followed by mild erythema,

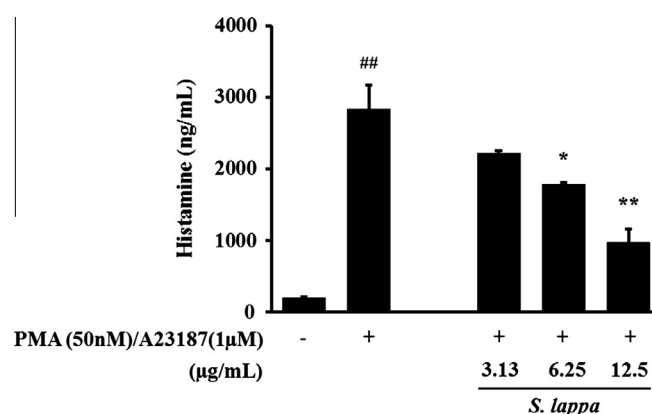


Fig. 4. Effect of *Saussurea lappa* extract on histamine production in MC/9 cells. The histamine production was evaluated using a commercial ELISA kits. Values were expressed as mean ± SEM of three independent experiments. ## $P < 0.01$ vs. vehicle control cells; * $P < 0.05$ and $P < 0.01$ vs. PMA and A23187-stimulated cells, respectively.

hemorrhage, and edema (Fig. 5A). Finally, the skin became thick with severe erythema, hemorrhage, edema, scarring, erosion, and excoriation. However, application of the *S. lappa* extract suppressed these skin symptoms. The dermatitis score for each group was determined by summing the EASI values for each mouse (Fig. 5B). In the *S. lappa* group (300 mg/kg), the dermatitis score was lower at 3 weeks compared with the Biostir group. This reduction of *S. lappa* group was similar to those of prednisolone

group. In particular, 300 mg/kg of *S. lappa* group more effective than the Biostir group.

3.5. *S. lappa* extract attenuates the atopic dermatitis-like lesions induced by house dust mite

The histopathological features were assessed using samples from the back skin and ears of Nc/Nga mice. As shown in Fig. 6, we

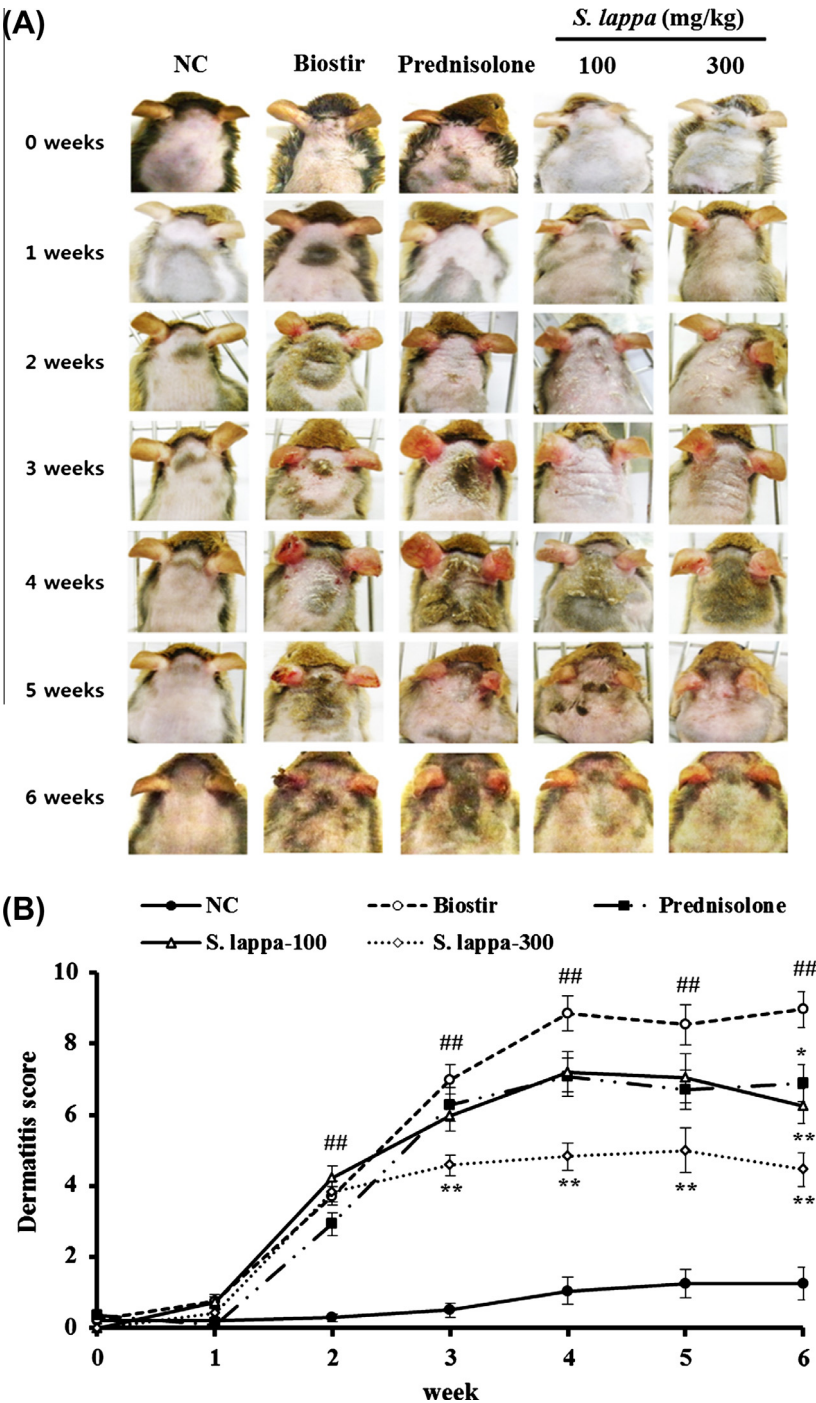


Fig. 5. Effects of *Saussurea lappa* extract on the development of atopic dermatitis, which was induced in Nc/Nga mice using a house dust mite extract. The house dust mite extract was administered to Nc/Nga mice to establish an atopic dermatitis animal model. The mice were allocated to five groups ($n = 7/\text{group}$): normal control group (vehicle-treated mice; distilled water, 150 $\mu\text{L}/\text{mouse}$, per os (p.o.)), Biostir group (Biostir-AD plus vehicle-treated mice; distilled water, 150 $\mu\text{L}/\text{mouse}$, p.o.), prednisolone group (Biostir-AD plus prednisolone-treated mice; 3 mg/kg, p.o.), and *S. lappa* group (Biostir-AD plus *S. lappa*-treated mice; 100 or 300 mg/kg, p.o.). The clinical features (A) and dermatitis scores (B) were assessed using the criteria described in Section 2. The features were monitored once each week for 4 weeks. The values are expressed as the mean $\pm$ SEM ($n = 7$). $^{***}P < 0.01$ vs. vehicle-treated mice; $^{*}P < 0.05$ and $P < 0.01$ vs. Biostir-AD-treated mice, respectively.

observed epidermal hyperplasia, parakeratosis, hyperkeratosis, dermal edema, and large numbers of infiltrated or inflammatory cells, such as mast cells, in the Biostir group. The symptoms were relieved by treatments with the *S. lappa* extract relative to the Biostir group (Fig. 6A). Increased numbers of mast cells were observed in the skin lesions of the Biostir group compared with the normal control group (Fig. 6B). Administration of the *S. lappa* extract decreased the numbers of mast cells compared with the Biostir group, but there was no significant difference. These results suggest that the *S. lappa* extract suppressed house dust mite-induced inflammation. The normal group exhibited no signs of inflammation.

3.6. *S. lappa* extract inhibits the IgE and TARC production in a house dust mite-induced atopic dermatitis Nc/Nga mouse model

The total IgE level in the plasma was determined *in vivo*. The Biostir group (413.17 ± 55.55 ng/mL, $P < 0.01$) had a significantly

higher the total IgE level compared with the normal control group, whereas the *S. lappa* group (82.27 ± 13.09 ng/mL in 100 mg/kg, $P < 0.01$; 75.31 ± 21.10 ng/mL in 300 mg/kg, $P < 0.01$) had a significantly lower IgE level than the Biostir group (Fig. 7A). In addition, The Biostir group (14.47 ± 1.31 ng/mL, $P < 0.01$) had a significantly higher the TARC production compared with the normal control group. In contrast, *S. lappa* groups (10.40 ± 1.41 ng/mL in 100 mg/kg group; 10.01 ± 0.76 ng/mL in 300 mg/kg group, $P < 0.05$) significantly lower TARC production than the Biostir group (Fig. 7B). These reductions by *S. lappa* administration were similar to those of prednisolone group (24.74 ± 5.29 ng/mL of IgE; 8.50 ± 1.05 pg/mL of TARC).

4. Discussion

Therapeutic approaches controlling for atopic dermatitis include corticosteroids, immunosuppressants, and ultraviolet light.

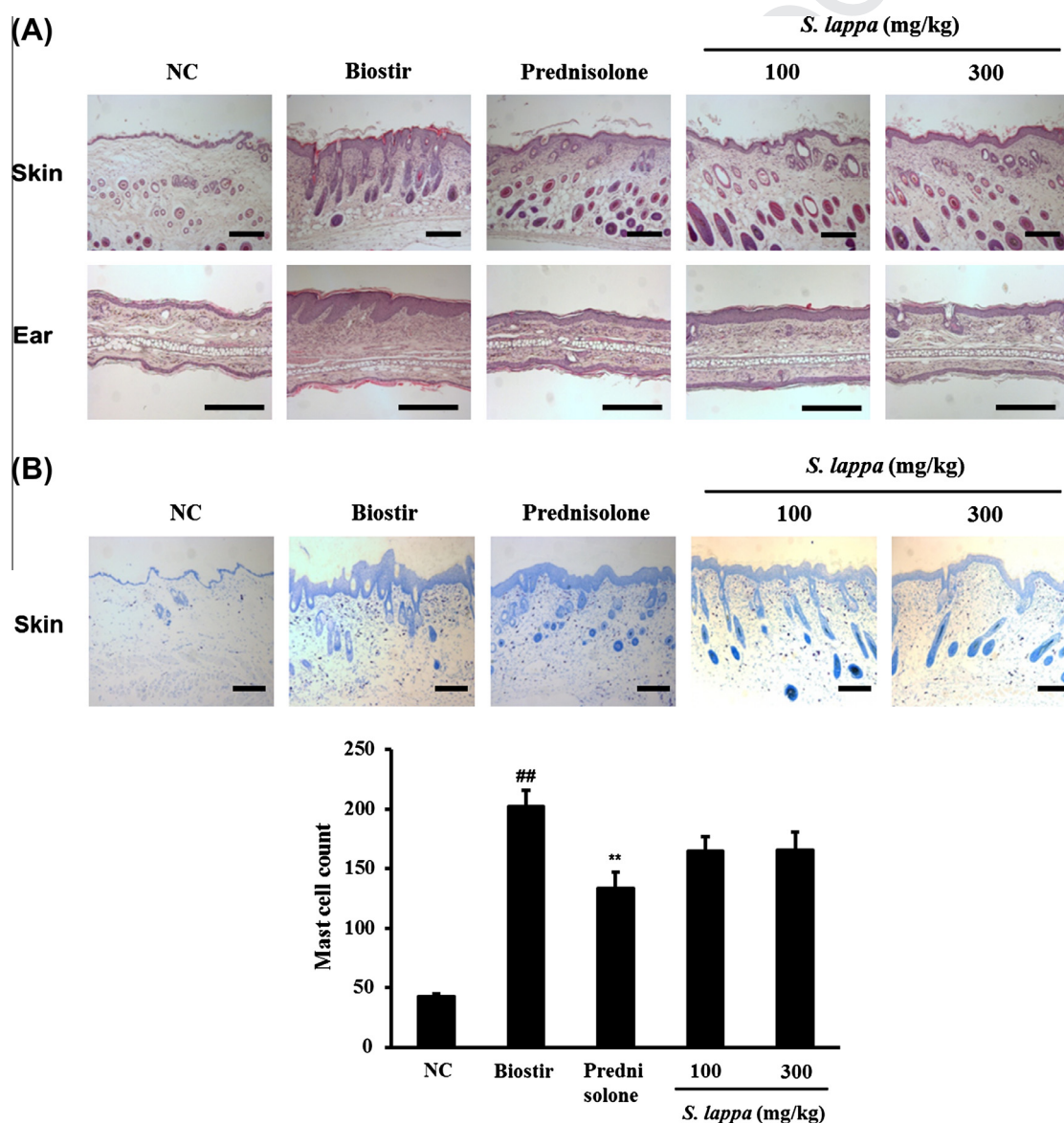


Fig. 6. Effects of *Saussurea lappa* extract on the histological features of house dust mite-induced atopic dermatitis in Nc/Nga mice. The skin and ear tissues were excised from the normal control group (vehicle-treated mice; distilled water, 150 μ L/mouse, per os (p.o.)), Biostir group (Biostir-AD plus vehicle-treated mice; distilled water, 150 μ L/mouse, p.o.), prednisolone group (Biostir-AD plus prednisolone-treated mice; 3 mg/kg, p.o.), and *S. lappa* group (Biostir-AD plus *S. lappa*-treated mice; 100 or 300 mg/kg, p.o.), fixed with 10% formaldehyde, embedded in paraffin, and cut into thin sections. The histological features (A) and mast cell counts (B) of the skin and ear lesions were determined after staining with hematoxylin and eosin, and toluidine blue, respectively. The values are expressed as the mean $\pm$ SEM ($n = 7$). ^{##} $P < 0.01$ vs. vehicle-treated mice; ^{**} $P < 0.01$ vs. Biostir-AD-treated mice. Scale bars: 100 μ m.

Traditionally, corticosteroids (ointments, creams, or injections) have been considered the most effective therapy for atopic dermatitis (Nakagawa, 2006). However, the long-term application of corticosteroids is limited because of their side effects, such as immunosuppression, stretch marks, thinning of the skin, and epidermal barrier dysfunction (Berke et al., 2012). Other therapies can also induce severe side effects such as malfunctions of the kidneys and liver, and skin cancer (Wooltorton, 2005; Jans et al., 2006). Thus, there is an urgent need to develop new therapies that target the underlying causes of atopic dermatitis. In the present study, we investigated the protective effects of an *S. lappa* extract on atopic dermatitis using HaCaT cells, MC/9 cells, and a murine model of house dust mite-induced atopic dermatitis.

Chemokines have a pivotal role in the development of atopic asthma. In particular, TARC, MDC, and RANTES are responsible for the initiation of atopic dermatitis. These are members of the CC chemokine group, which induces chemotaxis in T cells and leukocytes by interacting with the CC receptor 5, thereby leading to the development of atopic dermatitis (Pivarcsi and Homey, 2005; Gros et al., 2009). Chemokine production can be observed in various immune cells, including lymphocytes, dendritic cells, keratinocytes, mast cells, and eosinophils, which are activated by various stimuli (Saeki and Tamaki, 2006). In addition, keratinocytes are

well-known enhancers of the inflammatory response in atopic dermatitis. Keratinocytes stimulated with proinflammatory cytokines have been identified as important cellular sources of chemokines, including TARC, MDC, and RANTES, which affect T lymphocyte differentiation and the recruitment of leukocytes to atopic lesions (Albanesi, 2010). In addition, differentiated T lymphocytes and inflammatory cells produce proinflammatory cytokines, which are involved with the activation of eosinophils and mast cells, as well as the production of IgE (Carmi-Levy et al., 2011; Williams et al., 2012). Ultimately, these events aggravate atopic dermatitis lesions. Therefore, targeting chemokine production in atopic dermatitis is considered to be an important potential treatment for atopic dermatitis. In the present study, treatment with an *S. lappa* extract significantly reduced the increased production and mRNA expression of TARC, MDC, RANTES, and IL-8 in TI-stimulated HaCaT cells, thereby demonstrating its possible protective effects against atopic dermatitis. Silymarin treatment showed the reduction in mRNA expression of chemokines and cytokine. These findings were consistent with those of *S. lappa* treated cells.

The atopic dermatitis model based on Nc/Nga mice has been applied mainly to the investigation of atopic dermatitis pathogenesis and the efficacy of therapeutic drug candidates because of its similar clinical features to human atopic dermatitis. House dust mite-induced atopic dermatitis in Nc/Nga mice is characterized by pruritus, redness, and swelling of the skin, with elevated levels of proinflammatory cytokines, histamine, and IgE (Sung et al., 2012). Inflammatory cell infiltration, epidermis hypertrophy, and hyperkeratosis are important features of the skin lesions observed in Nc/Nga mice with house dust mite-induced atopic dermatitis (Lee et al., 2012). In our study, atopic dermatitis was induced in Nc/Nga mice using house dust mite and there were marked increases in their dermatitis scores and plasma IgE levels, as well as histopathological alterations in their skin lesions. However, administration of the *S. lappa* extract significantly reduced the elevated dermatitis scores and plasma IgE levels in house dust mite-induced atopic dermatitis. The *S. lappa* extract also attenuated the histopathological alterations related to atopic dermatitis and reduced the mast cell counts. These findings were similar to those of prednisolone treated mice. In particular, 300 mg/kg of *S. lappa* groups exhibited more effective or identical effects compared with the prednisolone group. In addition, we found that *S. lappa* significantly suppresses histamine production in PMA and A23187-stimulated MC/9 cells. Therefore, these findings indicate that the administration of *S. lappa* effectively suppresses house dust mite-induced atopic dermatitis.

Similar to our study, several recent studies have reported the *in vitro* or *in vivo* inhibitory effects of medicinal herbs against atopic dermatitis. For example, Jiang et al. (2009) and others (Lee et al., 2012; Sung et al., 2012; Yang et al., 2013) have reported that herbal remedies such as *Yokukansan*, *Catalpa ovata*, *Angelicae dahuricae*, and *Illicium verum* had protective effects against experimentally induced atopic dermatitis. Herbal remedies are attracting growing attention as potential agents for curing atopic dermatitis.

Overall, the present study demonstrated that an extract of *S. lappa* reduced the production of chemokines, i.e., TARC, MDC, RANTES, and IL-8 in TI-stimulated HaCaT cells, histamine production in PMA and A23187-stimulated MC/9 cells, and it also suppressed house dust mite-induced atopic dermatitis in an Nc/Nga mouse model. Our findings suggest that *S. lappa* has potent inhibitory effects against atopic dermatitis and could be developed into a useful therapeutic agent for treating atopic dermatitis.

Conflict of Interest

The authors declare that there are no conflicts of interest.

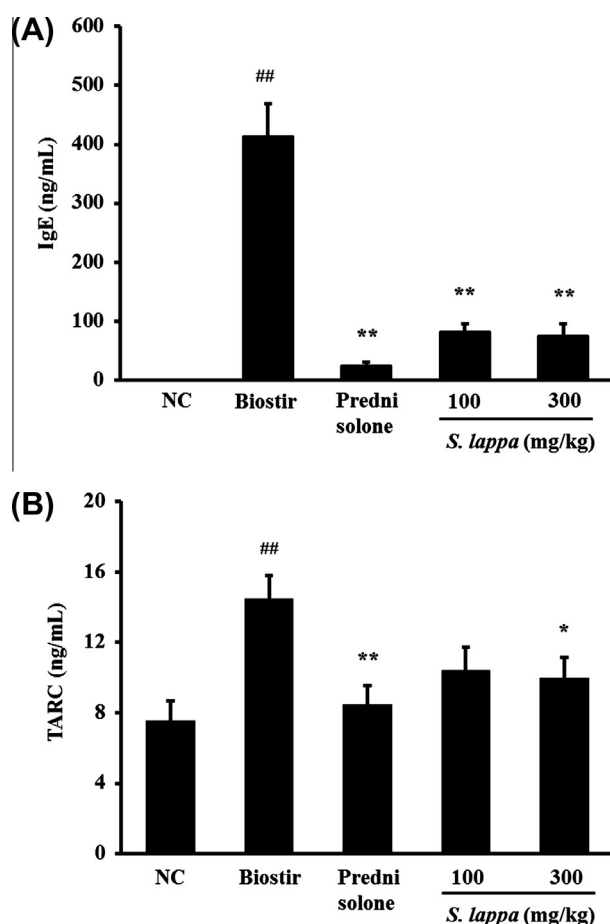


Fig. 7. Effects of *Saussurea lappa* extract on the plasma levels of IgE and TARC in Nc/Nga mice with house dust mite-induced atopic dermatitis. The IgE level (A) and TARC amount (B) was measured in plasma samples from the normal control group (vehicle-treated mice; distilled water, 150 μ L/mouse, per os (p.o.)), Biostir group (Biostir-AD plus vehicle-treated mice; distilled water, 150 μ L/mouse, p.o.), prednisolone group (Biostir-AD plus prednisolone-treated mice; 3 mg/kg, p.o.), and *S. lappa* group (Biostir-AD plus *S. lappa*-treated mice; 100 or 300 mg/kg, p.o.). Plasma concentration of IgE and TARC was measured by ELISA. Values are expressed as the mean $\pm$ SEM ($n = 7$). ^{##} $P < 0.01$ vs. vehicle-treated mice; ^{**} $P < 0.05$ and 0.01 vs. Biostir-AD-treated mice.

Acknowledgment

This work was supported by a grant from the Korea Institute of Oriental Medicine (No. K13030).

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