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Inhibition of Lung Inflammation by *Acanthopanax divaricatus* var. *Albeofructus* and Its Constituents

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Abstract

In order to find potential therapeutic agents on lung inflammatory conditions, the extracts of *Acanthopanax divaricatus* var. *albeofructus* were prepared and its constituents were isolated. They include lignans such as (+)-syringaresinol (**1**), acanthoside B (**2**), salvadoraside (**3**) and acanthoside D (**4**), lariciresinol-9-*O*-β-D-glucopyranoside (**5**) and phenylpropanoids such as 4-[(1E)-3-methoxy-1-propenyl]phenol (**6**), coniferin (**7**), and methyl caffeate (**8**). The extracts and several constituents such as compound **1**, **6** and **8** inhibited the production of inflammatory markers, IL-6 and nitric oxide, from IL-1β-treated lung epithelial cells and lipopolysaccharide (LPS)-treated alveolar macrophages. Furthermore, the extracts and compound **4** significantly inhibited lung inflammation in lipolysaccharide-treated acute lung injury in mice by oral administration. Thus it is suggested that *A. divaricatus* var. *albeofructus* and its several constituents may be effective against lung inflammation.

Key Words: *Acantopanax divaricatus* var. *albeofructus*, Lung inflammation, Acanthoside, Macrophage

INTRODUCTION

Airway inflammatory diseases include bronchitis and chronic obstructive pulmonary disease (COPD). In clinical practice, several classes of drugs such as antitussives, steroids, expectorants, and antibiotics have been used to treat and control these disorders. But it is still difficult to successfully control COPD since these diseases have a complex origin and etiology. Many cytokines/chemokines produced by lung epithelial cells, alveolar macrophages, and recruited neutrophils and macrophages play a role in inflammatory lung diseases, especially in COPD. Oxidative stress caused by nitric oxide (NO) and O_2 radical is another factor that accelerates the disease process. In addition, matrix degradation enzymes such as matrix metalloproteinases abrogate the elasticity and integrity of the alveolar sacs, resulting in dyspnea (Jeffery, 2001; Barns, 2014). Therefore, the need for new therapeutic agents with different mechanism(s) of action from the currently used drugs are desperately needed. To accomplish this goal, the potential therapeutic effects of many compounds and plant products are being evaluated worldwide.

As parts of these efforts, many plant products have been examined to find a potential therapeutic agent against lung inflammation in our laboratory. By these preliminary screening procedures, *Acanthopanax divaricatus* var. *albeofructus* (Araliaceae) was found to possess inhibitory action against lung inflammation. *Acanthopanax* family comprises *A. senticosus*, *A. sessiliflorum*, *A. koreanum*, *A. chiisanensis*, and *A. divaricatus* var. *albeofructus*. These plants have been frequently used in traditional herbal medicine for strengthening bones and cartilage, and as an anti-inflammatory agent and anti-fatigue remedy (Kim, 1998; Bae, 2000). Among these, *A. divaricatus* var. *albeofructus* is widely cultivated in Korea. The related species, *A. koreanum*, was shown to possess anti-inflammatory and immunoregulatory activities in an animal model of endotoxic shock (Jung *et al*., 2013). In addition, many constituents, including diterpenes and triterpenes, isolated from *A. koreanum* were demonstrated to possess anti-inflammatory activity. For instance, triterpenoids inhibited lipopolysaccharide (LPS)-treated cytokine production in bone marrow-derived dendritic cells (Kim *et al*., 2010; Kim *et al*., 2011; Nhiem *et al*., 2011). The diterpene derivative, acanthoic acid, inhibited experimental murine colitis (Kang *et al*., 2010). On the other hand, the protective action against ischemia-reperfusion injury of rat

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liver is the only reported pharmacological action of *A. divaricatus* var. *albeofructus*. (Lim *et al*., 2013a). No investigation has been carried out to establish the effect of *A. divaricatus* var. *albeofructus* and its constituents on inflammatory disorders to date. In the present study, the effects of *A. divaricatus* var. *albeofructus* and its constituents are investigated to establish the inhibitory activity and a therapeutic potential against pulmonary inflammation.

MATERIALS AND METHODS

Chemicals

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dexamethasone, IL-1β and lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) were purchased from Sigma Chem. (St. Louis, MO). 2-Amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT) was obtained from Tocris Cookson Ltd. (UK). RPMI and other cell culture reagents including FBS were products of Thermo Scientific Hyclone (UT, USA). Protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA, USA).

Animals

Male ICR mice (male, 18-22 g, specific pathogen-free) were obtained from KOATECH (Korea). Animals were fed with standard lab. chow and water ad libitum. The animals were maintained in animal facility (KNU) at 20-22°C under 40-60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation, KNU (KW-140929-1). In addition, the ethical guideline described in the Korean Food and Drug Administration guide for the care and use of laboratory animals was followed throughout the experiments.

Plant materials

The stems of *Acanthopanax divaricatus* var. *albeofructus* (Araliaceae) were purchased from a local retailer in 2012 and authenticated by Dr. J. H. Lee (Dongguk University, Gyoungju, Korea). The voucher specimen (CNU 12114) was deposited in the Herbarium of College of Pharmacy, Chungnam National University.

Extraction of the 70% ethanol and water extracts

The stems of *A. divaricatus* var. *albeofructus* (100 g) were dried and extracted with 70% (v/v) aqueous ethanol and water for 3 h. Evaporated and lyophilized to yield the 70% ethanol extract (ADE, 13.0 g) and the water extract (ADW, 14.2 g), respectively.

Extraction and isolation

Dried stems of *A. divaricatus* var. *albeofructus* (6.0 kg) were extracted three times with MeOH under reflux conditions. The MeOH extract (300.0 g) was suspended in H_2O (1.6 L) and partitioned with *n*-hexane and EtOAc (each 1.6 L×3). The EtOAc extract (40.0 g) was separated by silica gel column chromatography and eluted with a gradient of $CHCl_3$-acetone-MeOH (1:0:0-1:1:0.2) to yield seven fractions (Fr. A1-A7). The Fr. A2 was separated through a silica gel column and eluted with a gradient of *n*-hexane-EtOAc-MeOH (20:1:0.1-2:1:0.1) elution solvent to give six subfractions (Fr. A2.1-A2.6). The Fr. A2.3 was separated using a reverse-phase (RP) column chromatography with a MeOH-H_2O (1:1.5) eluent to give compound **6** (15.0 mg). Fr. A3 was further separated on a silica gel column using an eluent of *n*-hexane-EtOAc-MeOH (6:1:0.1-1:1:0.2), to yield eight subfractions (Fr. A3.1-A3.8). Further purification of Fr. A3.3 was conducted through a RP column using a gradient elution with MeOH-H_2O (1.5:1) to yield compound **8** (49.0 mg). Fr. A5 was separated through a silica gel column using a gradient elution with *n*-hexane-EtOAc-MeOH (2.3:1:0.1-1:1:0.1) to yield four fractions (Fr. 5.1-5.4). Fr. A5.2 was separated on a RP column and eluted with MeOH-H_2O (0.9:1) to provide compound **1** (60.0 mg).

The H_2O fraction (100.0 g) was separated on a column of highly porous polymer (Diaion HP-20) and eluted with H_2O and MeOH (1:0-3:1-1:1-1:3-0:1) to give five fractions (Fr. B1-B5). Fr. B2 was separated on a silica gel column with a gradient of $CHCl_3$-MeOH (10:1-0:1) to give six fractions (Fr. B2.1-B2.6). Fr. B2.3 was further separated on a RP column with a MeOH-H_2O (0.9:1-2:1) eluent to give compounds **4** (164.0 mg), **5** (8.0 mg), and **7** (15.0 mg). Fr. B3 was separated on a silica gel column chromatography with a gradient of $CHCl_3$-MeOH-H_2O (6.5:1:0.1-0:1:0) to give four subfractions (Fr. B3.1-B3.4). Fraction B3.2 was further chromatographed on silica gel chromatography column with EtOAc-MeOH (8:1) to yield compound **3** (52.0 mg). Fraction B3.4 was further separated on a RP column with MeOH-H_2O (0.8:1) to yield compound **2** (500.0 mg).

The structures of compounds **1**-**8** were identified based on spectroscopic data, chemical evidence and comparisons with previously reports (Fig. 1). Their structures were elucidated as (+)-syringaresinol (**1**, Cai *et al*., 2004), acanthoside B (**2**, Cai *et al*., 2004), salvadoraside (**3**, Zhang *et al*., 2010), acanthoside D (**4**, Cai *et al*., 2004), lariciresinol-9-*O*-β-D-glucopyranoside (**5**, Greca *et al*., 1993), 4-[(1*E*)-3-methoxy-1-propenyl]phenol (**6**, Ly *et al*., 2004), coniferin (**7**, Takano *et al*., 2006) and methyl caffeate (**8**, Lee *et al*., 2009).

A549 cell culture and measurement of IL-6 concentration

A549 cells, a human lung epithelial cell line, obtained from American type culture collection (Rockville, VA) were cultured with RPMI supplemented with 10% FBS, 1% L-glutamine and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a 5% CO_2 atmosphere at 37°C. After pre-incubation for 24 h, IL-1β (10 ng/ml) was added simultaneously with/without test compounds. Four hours later, media was collected and the concentration of IL-6 was determined from the media with an ELISA kit (eBioscience) according to the manufacturer's recommendation. The cell viability was checked using an MTT bioassay as previously described (Mossman, 1983).

The test compounds including the reference drug, dexamethasone, were dissolved in DMSO and properly diluted with complete RPMI media. The final concentration of DMSO in the cell culture was adjusted to 0.1% (v/v), and this concentration of DMSO did not affect the cell viability and the levels of IL-6 (data not shown).

MH-S cell culture and measurement of nitric oxide (NO) concentration

MH-S cells, a mouse alveolar macrophage cell line, obtained from ATCC were cultured with RPMI supplemented with 10% FBS, 1% L-glutamine and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a 5% CO_2 atmosphere

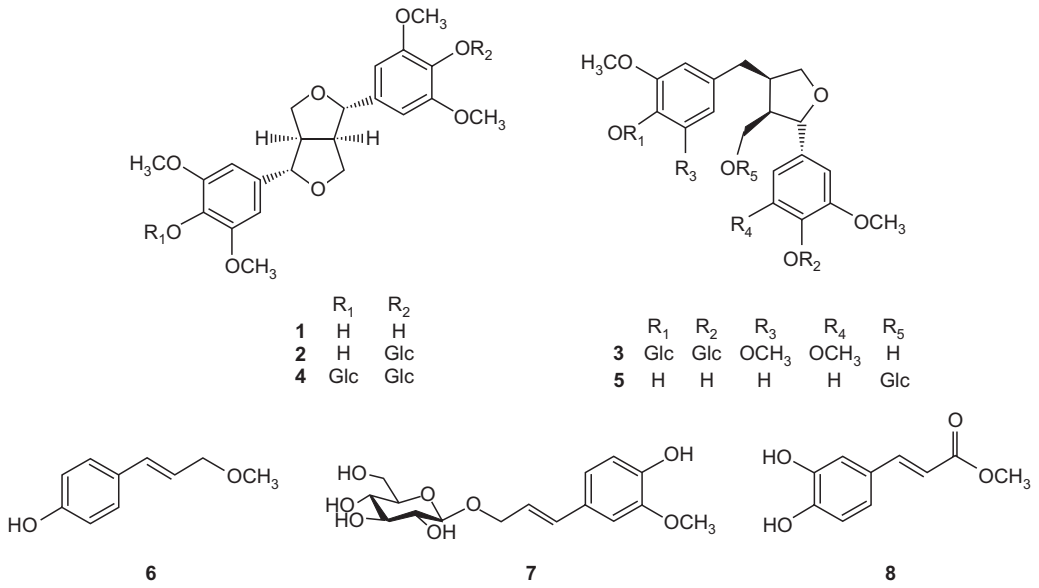

Fig. 1. Structures of the compounds **1**-**8** isolated the stems of *A. divaricatus* var. *albeofructus*.

at 37°C. As previously described (Ko *et al*., 2011), cells were plated in 96-well plates (2×10^5 cells/well). After pre-incubation for 24 h, test compounds and LPS (0.1 mg/ml) were added and the cells were incubated for 24 h unless otherwise specified. Media was collected and NO concentration was determined. To assess NO production, the stable conversion product of NO, nitrite (NO_2^-), was measured using Griess reagent and the optical density was determined at 550 nm.

The test compounds including the reference, AMT, were dissolved in DMSO and properly diluted with complete RPMI media. The final concentration of DMSO in the cell culture was adjusted to 0.1% (v/v), and this concentration of DMSO did not affect the cell viability and the levels of NO (data not shown).

LPS-induced airway inflammation in mice

In vivo activity was examined using a mouse model of airway inflammation, LPS-induced acute lung injury (Chapman *et al*., 2007; Lim *et al*., 2013b). The test compounds, including the reference drug, were dissolved in 0.3% carboxymethylcellulose (CMC) and were orally administered to mice at the indicated doses (n=10). The control and LPS treatment groups also received the same amount of CMC solution. The anesthetics (Avertin) was intraperitoneally injected. For inducing bronchitis, LPS (*E. coli* 0127:B8, 2 mg/kg, PBS) was administered intranasally to anesthetized mice (10 μl/mouse, 5 times) using a micropipette at 1 h after oral treatment with the test compounds according to the previously published procedures (Lim *et al*., 2013b). At 16 h after LPS treatment, mice were sacrificed (n=7), and bronchoalveolar lavage fluid (BALF) was collected via intratracheal cannulation after 700 μl of PBS was administered 3 times. In the BALF, the total cell number was counted with a haemocytometer, and the cells were differentially counted with FACS (BD Biosciences). For FACS analysis, cells were stained with following antibodies; allophycocyanin (APC) conjugated anti-Ly-6C (BD Bioscience), allophycocyanin-Cy7 (APC-Cy7) conjugated anti-CD11c (BD Bioscience), fluorescein isothiocyanate (FITC) conjugated anti-CD11b (BD Bioscience), phycoerythrin (PE) conjugated anti-F4/80 (eBioscience), phycoerythrin-Cy7 (PE-Cy7) conjugated anti-Ly-6G (BD Bioscience). After incubation with antibody, cells were washed with 2% FBS RPMI media. Cell pellets were resuspended and analyzed. CD11b, Ly-6C and Ly-6G are essential markers of neutrophils. CD11c and F4/80 are markers of alveolar macrophages, interstitial macrophages and dendritic cells. For the histological analysis, the remaining mice (n=3) were sacrificed and lung tissues were excised. Histological examination was carried out using the conventional methods of H&E staining.

Statistical analysis

Experimental values were represented as arithmetic mean ± SD. One way ANOVA followed by Dunnett's test was used to determine the statistical significance.

RESULTS

Effects on IL-1β-treated IL-6 production in lung epithelial cells (A549)

The effects of the water and 70% ethanol extracts of the stems of *A. divaricatus* var. *albeofructus* were examined in a lung epithelial cell line, A549. When activated with IL-1β, human lung epithelial cells (A549) produced high levels of a pro-inflammatory cytokine, IL-6. The IL-6 concentration increased from the basal level of 0.02 ± 0.0 ng/ml to 1.91 ± 0.06 ng/ml after 4 h of incubation in the media (n=3). Under these conditions, the water extract significantly inhibited the production of IL-6 by 27% at 300 μg/ml (Fig. 2A). The 70% ethanol extract showed significant inhibition at 50-300 μg/ml, although the degree of inhibition was not strong. Dexamethasone (10 μM), a reference agent, showed strong inhibitory action (83.5% inhibition) against IL-6 production.

Based on these results, 8 major constituents were successfully isolated from the stems of A. divaricatus var. albeofructus. Among the isolated constituents tested, only (+)-syringaresinol (**1**, 43.6 % inhibition at 100 μM) and methyl caffeate (8, 38.6

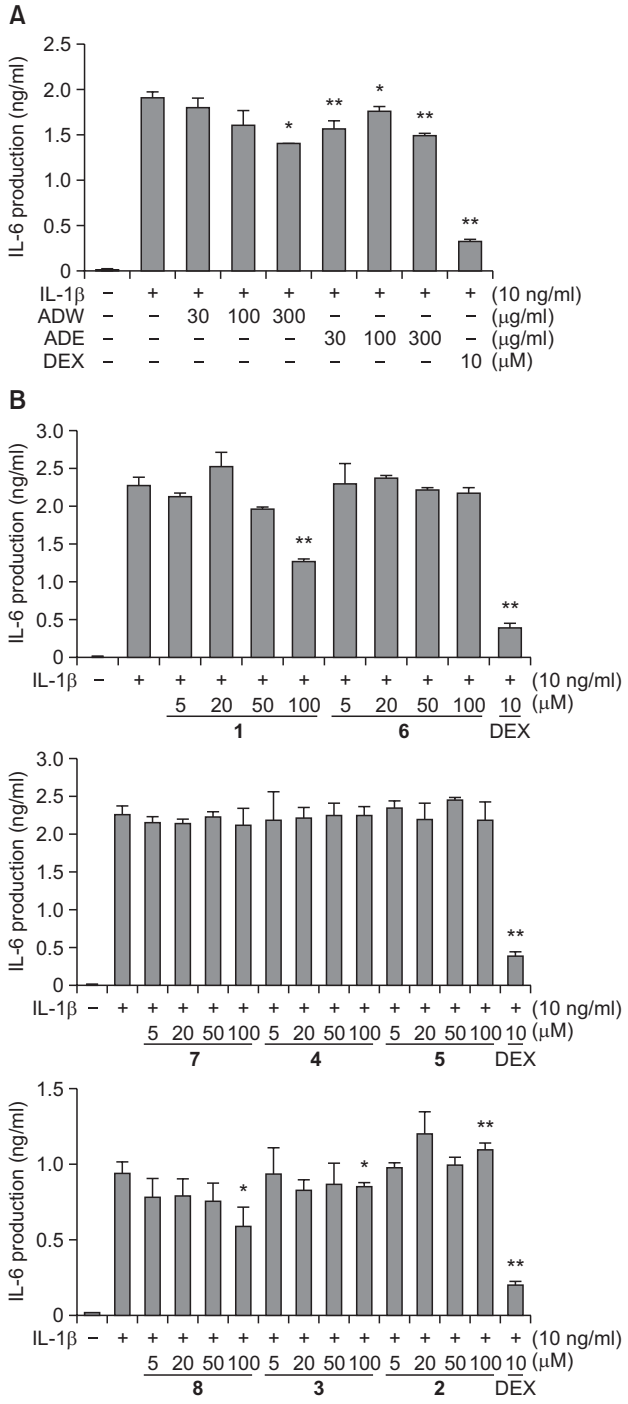

Fig. 2. Effects on IL-6 production from IL-1β-treated lung epithelial cells (A549). (A) Inhibition of the water and 70% ethanol extracts of *A. divaricatus* var. *albeofructus*, (B) Inhibition of the constituents isolated, IL-6 concentrations of the IL-1β-treated group were 2.25 ± 0.13 ng/ml and 0.95 ± 0.07 ng/ml, and those of the control group were 0.01 ± 0.0 ng/m. ADW (water extract), ADE (ethanol extract), DEX (dexamethasone), **p*<0.05, ***p*<0.01, Significantly different from the IL-1β-treated control group (n=3).

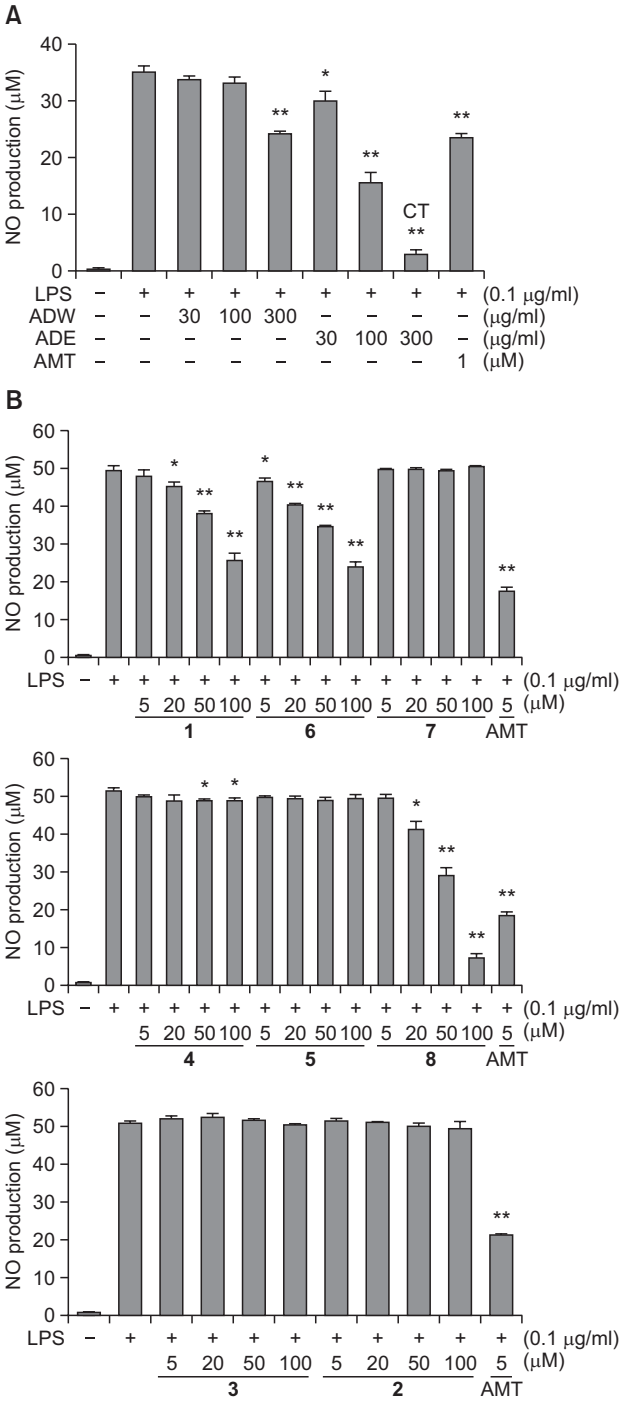

Fig. 3. Effects on NO production from LPS-treated alveolar macrophages (MH-S). (A) Inhibition of the water and 70% ethanol extracts of *A. divaricatus* var. *albeofructus*, (B) Inhibition of the constituents isolated, NO concentrations of the LPS-treated group were 49.2 ± 1.4 μM, 50.9 ± 1.2 μM and 50.8 ± 0.6 μM, while the control group showed 0.4 ± 0.1 μM, 0.6 ± 0.1 μM, 0.6 ± 0.1 μM of NO concentration, respectively. ADW (water extract), ADE (ethanol extract), AMT (iNOS inhibitor), **p*<0.05, ***p*<0.01, Significantly different from the LPS-treated control group (n=3).

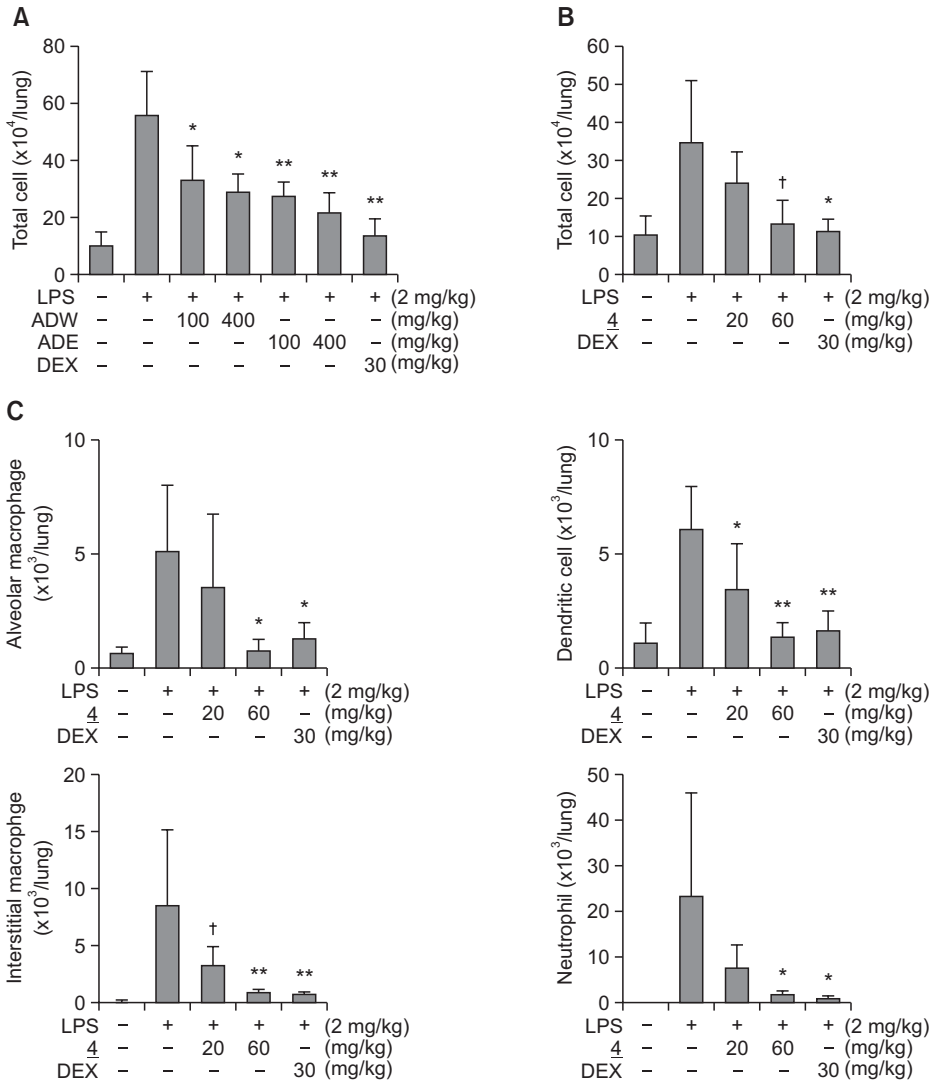

Fig. 4. Effects on mouse LPS-induced acute lung injury (ALI). LPS was intranasally treated to induce airway inflammation. Sixteen hours later, mice were sacrificed and BALF was obtained. All compounds were orally administered 1 h prior to LPS treatment. (A) Inhibition of total cell number in the BALF by the water and 70% ethanol extracts of *A. divaricatus* var. *albeofructus*, (B) Inhibition of total cell number in the BALF by acanthoside D, (C) FACS analysis of the cells in the BALF (acanthoside D-treated group), $^{\dagger}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, Significantly different from the LPS-treated control group (n=7). (D) Histological observation, x100. ADW (water extract), ADE (ethanol extract),, acanthoside D (**4**, AD), Dexamethasone (DEX), Arrows indicate that alveolar wall thickness is increased in the LPS-treated group.

% inhibition at 100 μM) showed significant inhibitory action (Fig. 2B).

Effects on LPS-induced NO production in alveolar macrophages (MH-S)

The effect on mouse alveolar macrophages (MH-S) was also measured. When treated with LPS, MH-S cells produced high amounts of NO via inducible NO synthase (iNOS, 35.2 ± 1.1 μM from the basal level of 0.3 ± 0.0 μM, n=3). Under this condition, the water extract significantly inhibited the production of NO by 31.9% at 300 μg/ml (Fig. 3A). And the 70% ethanol extract strongly inhibited NO production at 50-300 μg/ml, with some cytotoxicity at 300 μg/ml. AMT (iNOS inhibitor) was used as a reference agent, and showed significant inhibitory action (33.7% inhibition) on NO production at 1 μM as expected.

The inhibitory action of the 8 constituents was examined. Among the isolated constituents tested, (+)-syringaresinol (**1**), 4-[(1E)-3-methoxy-1-propenyl]phenol (**6**), and methyl caffeate (**8**) showed considerable inhibitory activity against NO production at 5-100 μM (Fig. 3B). The IC_{50} values for (+)-syringaresinol (1), 4-[(1E)-3-methoxy-1-propenyl]phenol (**6**), and methyl caffeate (**8**) were 102.4 μM, 93.1 μM and 56.6 μM, respectively. Acanthoside D (**4**) only slightly inhibited NO production (5.3% and 5.1% inhibition at 50 and 100 μM, respectively).

In all experiments including IL-6 and NO studies, no cytotoxicity of the isolated compounds and reference agents was observed at the indicated concentrations measured by MTT assay (data not shown), suggesting that the inhibitory action against lung inflammation is not associated with their cytotoxic

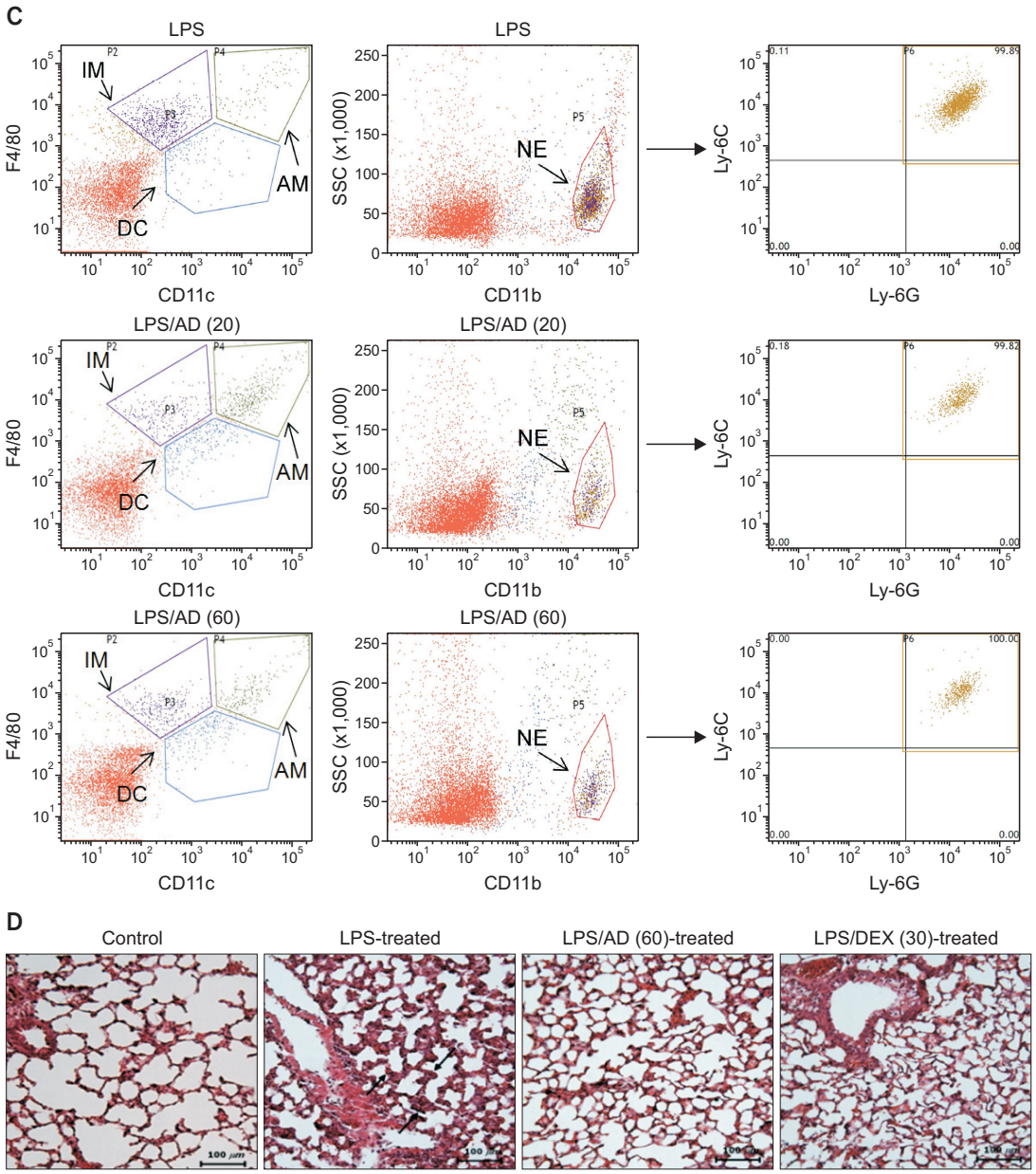

Fig. 4. Continued.

effects on the cells.

Inhibition of LPS-induced acute lung injury (ALI) in mice

Since the water and 70% ethanol extracts showed anti-inflammatory action in lung cells, *in vivo* activities of these two extracts were examined using an animal model of lung inflammation, ALI, according to previously reported studies (Chapman *et al*., 2007; Lim *et al*., 2013b). Intranasal administration of LPS produces an inflammatory response in their lungs. The most prominent change is the inflammatory cells recruited in the lung. In the BALF, the number of cells, including recruited neutrophils, is greatly increased by LPS treatment (Fig. 4A). Under this condition, when the extracts were orally administered to mice, the water extract reduced the total cell number in the BALF by 46.0% and 53.5% at 100 and 400 mg/kg, respectively. And the 70% ethanol extract strongly reduced the total number of cell in the BALF (59.4% and 73.8% reduction at 100 and 400 mg/kg). Since total cell number is one of the criteria used to judge the inflammatory response in the lung, these findings strongly suggest that *A. divaricatus* var. *albeofructus* possesses *in vivo* inhibitory activity against lung inflammation. In this experiment, dexamethasone (30 mg/kg) was used as a reference drug, and potently reduced (91.2%) the total cell number in the BALF as expected.

The above *in vitro* experiments have shown that (+)-syringaresinol (**1**) possesses anti-inflammatory action in lung cells. Since acanthoside D (**4**) is the most abundant major compound in the extract of A. divaricatus var. albeofructus,

and has the same chemical backbone structure as (+)-syringaresinol (**1**), this compound is selected for further *in vivo* study. When orally administered in the same ALI model, acanthoside D (**4**) potently reduced the total cell number in the BALF at 20 and 60 mg/kg by 43.8% and 88.5%, respectively (Fig. 4B). FACS analysis of the cells in the BALF further indicated that this compound reduced the recruitment of inflammatory cells, especially neutrophils, to the lung as shown in Fig. 4C. Moreover, acanthoside D (**4**) clearly alleviated the histological changes in the lung (Fig. 4D). This compound is revealed to reduce the infiltration of inflammatory cells and the increased thickness of the alveolar walls induced by LPS treatment. Dexamethasone strongly inhibited all of these inflammatory parameters. These results clearly indicate that the extracts and acanthoside D (**4**) possess *in vivo* inhibitory activity against lung inflammation.

DISCUSSION

The present investigation has clearly shown that the stems of *A. divaricatus* var. *albeofructus* and its several constituents possess inhibitory action against airway inflammation. *In vitro* experiments indicate that the extracts and its constituents, (+)-syringaresinol, 4-[(1E)-3-methoxy-1-propenyl]phenol and methyl caffeate, possess inhibitory action on lung inflammation via inhibiting cytokine and NO production. Particularly, acanthoside D exerted an inhibitory activity *in vivo*. Thus all these constituents are thought to be active principles of *A. divaricatus* var. *albeofructus*. Although the detailed mechanisms of action were not pursued, this is the first report to demonstrate inhibitory action against lung inflammation by *A. divaricatus* var. *albeofructus* and its constituents.

ALI is a mouse model of establishing airway inflammatory responses and it is frequently used for examining compounds' effects on lung inflammation including bronchitis (Chapman *et al*., 2007; Lim *et al*., 2013b). There are several markers of lung inflammation in this animal model; recruitment of inflammatory cells, proinflammatory cytokine concentration, levels of oxidative stress, and histological changes. Among these changes, inflammatory cell recruitment is the most important parameter. *A. divaricatus* var. *albeofructus* and acanthoside D reduce cell numbers in the BALF. Acanthoside D strongly reduces the number of neutrophils that infiltrate into the lungs as part of the inflammatory response. These findings strongly suggest that acanthoside D is the major active principle of *A. divaricatus* var. *albeofructus*.

Acanthoside D is most abundantly present in the extracts and possesses *in vivo* inhibitory action against lung inflammation, whereas the same compound did not considerably inhibit the production of inflammatory markers *in vitro*. The reason for this discrepancy is not clear at present. It is speculated that acanthoside D has high hydrophilic property, which may limit its penetration inside the cells because of its two glucose residues in addition to the (+)-syringaresinol backbone. This speculation may be supported, at least in part, by the finding that the aglycone form of acanthoside D, (+)-syringaresinol, possesses *in vitro* anti-inflammatory activities in lung cells (Fig. 2B, 3B). It is thought that acanthoside D is possibly absorbed into the body from the GI tract regardless of its sugar residues, and behaves as an active molecule inside the body after being hydrolyzed to its aglycone form. Based on this possibility, it is suggested that (+)-syringaresinol and its glycosides possess anti-inflammatory action against inflammatory lung conditions, although the *in vivo* activity of (+)-syringaresinol and acanthoside B has not been tested.

There have been several investigations concerning anti-inflammatory principles from the *Acanthopanax* family. Triterpenoids are active principles of *A. koreanum* inhibiting LPS-treated cytokine production in bone marrow-derived dendritic cells (Kim *et al*., 2010; Kim *et al*., 2011; Nhiem *et al*., 2011). Acanthoic acid from *A. koreanum* was found to inhibit experimental murine colitis (Kang *et al*., 2010). Several lignan derivatives, including acanthosessilin, (-)-sesamin, (+)-syringaresinol, and acanthoside B, which were isolated from *A. sessiliflorus* inhibited iNOS-induced NO production in RAW264.7 cells (Lee *et al*., 2013), while in our study (+)-sesamin and acanthoside B did not effectively inhibit iNOS-mediated NO production in MH-S cells. In particular, acanthoside D was previously found to show anti-arthritic activity in collagen induced arthritis in mice (He *et al*., 2014). All of these constituents may contribute to the pharmacological actions of the Acanthopanax family.

Lung inflammatory disorders are most common diseases that reduce the quality of life, especially in elderly patients. In particular, COPD is a group of chronic diseases that includes chronic bronchitis, chronic recurrent asthma, and emphysema. Even with several classes of drugs available, it is difficult to control the symptoms of COPD. In this respect, the efforts to develop a new efficient drug in this field are ongoing worldwide. Phosphodiesterase IV inhibitors and leukotriene receptor antagonists are a couple of examples of new drugs that have been developed (Jeffery, 2001; Reid and Pham, 2012). Although these new drugs alleviate the some of the symptoms in COPD, they have not been shown to be as effective as they were originally thought to be. The main reason for these disappointing results may be due to the complex etiology and origin of the disease (Al-Kassimi and Alhamad, 2013). It is likely that there are inflammatory factors, immunologic factors, and some physiological factors that are interrelated with each other that cannot be managed by these drugs. In this context, natural plant products have some advantages, in that they have complex constituents which may act on different parts of the cellular machinery and have a long history of clinical use in the same field. Examples are the extracts of *Hedera helix* (Guo *et al*., 2006), *Echinacea purpurea* (Sharma *et al*., 2006; Agbabiaka *et al*., 2008), and *Pelargonium sidoides* (Matthys and Funk, 2008), which are prescribed in European and Asian countries. And there have been many herbal medicines traditionally used to treat inflammatory lung disorders. The pharmacological activities of the root barks of *Morus alba*, whole plants of *Lonicera japonica*, and radix of *Broussonetia papyrifera* which have been used in Chinese medicine have been previously demonstrated *in vitro* and in animal models (Ko *et al*., 2011; Lim *et al*., 2013b). In this study, we have demonstrated the pharmacological activity of *A. divaricatus* var. *albeofructus*.

In conclusion, the water and 70% ethanol extracts of *A. divaricatus* var. *albeofructus* and its several constituents, including (+)-syringaresinol, acanthoside D, 4-[(1E)-3-methoxy-1-propenyl]phenol, and methyl caffeate inhibited inflammatory responses in the lung-related cells. Particularly, the 70% ethanol extract of *A. divaricatus* var. *albeofructus* and acanthoside D showed particularly strong inhibitory activity against LPS-induced lung inflammation when orally administered to mice,

indicating these compounds may have a therapeutic potential for treating inflammatory lung disorders.

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