Research Article

Heat-Processed *Scutellariae* Radix Enhances Anti-Inflammatory Effect against Lipopolysaccharide-Induced Acute Lung Injury in Mice via NF-κB Signaling

Yu Ock Shin, 1 Chan Hum Park, 2 Gyeong-Hwan Lee, 3 Takako Yokozawa, 2,4,5 Seong-Soo Roh, 2 and Man Hee Rhee 1

1 College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea
2 College of Korean Medicine, Daegu Haany University, Daegu 706-060, Republic of Korea
3 Jeollanamdo Development Institute for Korean Traditional Medicine, Jeollanamdo 529-851, Republic of Korea
4 Molecular Inflammation Research Center for Aging Intervention, Pusan National University, Busan 609-735, Republic of Korea
5 Graduate School of Science and Engineering for Research, University of Toyama, Toyama 930-8555, Japan

Correspondence should be addressed to Seong-Soo Roh; ddede@dhu.ac.kr

Received 12 February 2015; Revised 7 May 2015; Accepted 28 May 2015

Academic Editor: Alvin J. Beitz

Copyright © 2015 Yu Ock Shin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The present study was conducted to examine whether heat-processed *Scutellariae* Radix has an ameliorative effect on lipopolysaccharide- (LPS-) induced acute lung injury in mice. The effects of *Scutellariae* Radix heat-processed at 160°C (HSR) were compared with those of nonheat-processed *Scutellariae* Radix (NSR). The LPS-treated group displayed a markedly decreased body weight and significantly increased lung weight; however, the administration of NSR or HSR improved both the body and lung weights. The increased oxidative stress and inflammatory biomarker levels in the serum and lung were reduced significantly with HSR. The reduced superoxide dismutase and catalase increased significantly by both NSR and HSR. Also, the dysregulated oxidative stress and inflammation were significantly ameliorated by NSR and HSR. The expression of inflammatory mediators and cytokines by nuclear factor-kappa B activation was modulated through inhibition of a nuclear factor kappa B degradation. Also, lung histological change was markedly suppressed by HSR rather than NSR. Overall, the ameliorative effects of HSR were superior to those when being nonheat-processed. The representative flavonoid contents of *Scutellariae* Radix that include baicalin, baicalein, and wogonin were greater by heat process. These data reveal heat-processed *Scutellariae* Radix may be a critical factor involved in the improvement of lung disorders caused by LPS.

1. Introduction

Acute lung injury is a form of acute respiratory failure, which is characterized by increased pulmonary vascular permeability, pulmonary edema, excessive neutrophil migration, and the release of proinflammatory cytokines and mediators [1, 2]. Thus, it leads to marked morbidity and mortality [3]. The etiologies of acute lung injury are severe hypoxemia, bilateral infiltration of leukocytes, pulmonary edema, pneumonia, and trauma. In particular, the incidence of acute lung injury in the US, with 200,000 newly diagnosed cases per year, is very high [1]. Therefore, highly effective drugs and therapies are required for the treatment of acute lung injury.

Lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, has been generally used in an experimental lung injury model [4, 5]. LPS has been reported to activate macrophages to produce proinflammatory cytokines and mediators. Also, it promotes the production of reactive oxygen species (ROS), such as superoxide (O2−), hydrogen peroxide (H2O2), and hydroxyl radicals in macrophages [6]. Nitric oxide (NO) induced after LPS challenge is a potent inflammatory mediator that reacts with O2− and produces peroxynitrite (ONOO−). ONOO− is linked to cell death and lung injury. The excessive production of ROS suppresses the innate antioxidant system. So, the oxidant/antioxidant systems become unbalanced [7].
**Scutellariae Radix (Scutellaria baicalensis Georgii)** has been used to treat high fever, diarrhea, jaundice, hypertension, and bacterial and viral infections as herbal medicine in China, Japan, and Korea. Traditionally, herbal medicine is comprised of a complex mixture of biologically active components, and some undergo heat processing such as air drying, steaming, roasting, and baking to enhance the therapeutic efficacy [8]. These processing methods increase their biological activity through the chemical alteration of components [9, 10] and also improve the bioactive gradients through increasing the total polyphenol contents, including flavonoids [II, 12]. However, *Scutellariae Radix* has yet to be reported regarding its chemical profiling and biological activity following heat-processing. Therefore, we measured the contents of three flavonoids, baicalin, baicalein, and wogonin, of nonheat-processed and heat-processed *Scutellariae Radix* and evaluated the anti-inflammatory activities of these forms of *Scutellariae Radix* on LPS-induced acute lung injury in mice.

### 2. Materials and Methods

#### 2.1. Materials

LPS from *Escherichia coli* serotype 0127:B8 (purity > 99%; made up of a hydrophobic lipid (lipid A, which is responsible for the toxic properties of the molecule), a hydrophilic core polysaccharide chain, and a hydrophilic O-antigenic polysaccharide side chain), phenylmethylsulfonyl fluoride (PMFSF), and dithiothreitol (DTT) were purchased from Sigma Aldrich Co., Ltd., St. Louis, MO, USA. The protease inhibitor mixture and ethylenediaminetetraacetic acid (EDTA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 

2',7'-Dichlorofluorescein diacetate (DCFH-DA) was obtained from Molecular Probes (Eugene, OR, USA). ECL Western Blotting Detection Reagents and horseradish peroxidase- (HRP-) conjugated secondary anti-histone, and goat anti-rabbit and goat anti-mouse IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The solvents, such as acetonitrile, methanol, formic acid, and water, were of high purity high-performance liquid chromatography (HPLC) grade and obtained from Merck (Darmstadt, Germany). The standard materials of baicalin, baicalein, and wogonin for HPLC were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

#### 2.2. Plant Materials and Their Heat Processing. *Scutellariae Radix* was purchased from Ominherb Co. (Youngcheon, Korea). A voucher herbarium specimen has been deposited at the Herbarium of Daegu Haany University and was identified by Professor S.S. Roh, the herbarium leader of Daegu Haany University. Dried slices of *Scutellariae Radix* were heat-processed at 160°C (internal temperature) for 7 min using a roasting machine (Genesis Co., Ltd., Kyungki-do, Korea). The material was pulverized and extracted by 60-Hz ultrasonic waves at room temperature for 3 h, and the solvent was evaporated *in vacuo* to give an extract with a yield of 22.4%, by weight, of the original *Scutellariae Radix*. Non-heat-processed material was also pulverized and extracted by the same method as described above, giving an extract with a yield of 22.8%.

#### 2.3. Analysis of Baicalin, Baicalein, and Wogonin.

The 70% ethanol extract of each sample (10 mg) was dissolved in 10 mL of 50% methanol with multivortexing, and filtered through a Dismic-13 JP membrane filter (Advantec Toyko, Tokyo, Japan; pore diameter: 0.2 μm). We injected 20 μL of the sample into a reverse-phase HPLC using a Phenomex Gemini NX C18 (4.6 × 150 mm, 3-μm pore size), with a column temperature of 35°C. Mobile phase component A = 0.1% formic acid (aq.) and B = acetonitrile. The gradient conditions were as follows: 0 min, 0% B; 3 min, 0% B; 5 min, 10% B; 7 min, 10% B; 12 min, 20% B; 17 min, 30% B; 22 min, 30% B; 31 min, 60% B; 35 min, 60% B; 40 min, 95% B; 43 min, 95% B; 45 min, 50% B. The flow rate was 0.6 mL/min. The UV absorbance from 277 nm was monitored using an Agilent 1200 series with a multimwavelength detector (Agilent Technologies, San Jose, CA, USA). All peaks were assigned by carrying out coinjection tests with authentic samples and comparing them with the UV spectral data. The conditions of major compounds (baicalin, baicalein, and wogonin) were detected from nonheat-processed *Scutellariae Radix* and 160°C heat-processed *Scutellariae Radix* extracts. The measurement was repeated three times for each sample. Representative HPLC results are illustrated in Figure 1. The amount of each flavonoid was as follows: nonheat-processed *Scutellariae Radix*: 146.7 mg/g baicalin, 11.1 mg/g baicalein, 1.7 mg/g wogonin; 160°C heat-processed *Scutellariae Radix*: 154.5 mg/g baicalin, 38.6 mg/g baicalein, 6.1 mg/g wogonin.

#### 2.4. Experimental Animals and Treatment

Experiments were performed according to the “Guidelines for Animal Experimentation” approved by Daegu Haany University. Six-week-old male ICR mice were purchased from Samtako (Osan, Korea). Losonczy et al. [13] reported that male mice may be sensitized to LPS-induced shock and that the sensitivity of males to endotoxin is associated with an attenuated, not exaggerated, and total rate of NO synthesis. So, this experiment was conducted using male mice. The animals were maintained under a 12 h light/dark cycle and housed with a controlled temperature (24°C) and humidity (55 ± 5%). After adaptation (1 week), the mice were divided into four groups of equal number (n = 8, each), avoiding any intergroup differences in body weight. The normal and vehicle-treated LPS groups were given water using a stomach tube, while the other groups were orally administered nonheat-processed *Scutellariae Radix* or heat-processed *Scutellariae Radix* at a dose of 100 mg/kg body weight daily using a stomach tube for 3 consecutive days. The mice were given intraperitoneal LPS at 20 mg/kg body weight. At 24 h after LPS challenge, blood samples were collected by cardiac puncture from anesthetized mice. The serum was immediately separated from the blood...
samples by centrifugation. Subsequently, the lung was perfused through the artery with ice-cold physiological saline (0.9% NaCl, pH 7.4), removed, quickly frozen, and kept at −80°C until analysis.

2.5. Measurement of Immune Response-Associated Secreted Factors in the Serum. Proinflammatory biomarkers in the serum, including MCP-1 and IL-6, and regulated on activation normal T cells expressed and secreted (RANTES), were assayed using the multiplexed bead-based immunoassay Milliplex Map, MPXMCYTO70KPMX32 (Millipore, Billerica, MA, USA).

2.6. Measurement of Nitrite (NO$_2^-$) and Nitrate (NO$_3^-$) Levels in the Serum. NO$_2^-$ and NO$_3^-$ levels were measured primarily following the method of Misko et al. [14]. Briefly, serum was filtered through an Ultrafree-MC microcentrifuge filter unit (Millipore, Bedford, MA, USA) for 1 h at 14,000 rpm to remove hemoglobin released by cell lysis. As NO$_3^-$ in serum is mostly oxidized to NO$_2^-$ by reaction with the iron-heme center of hemoglobin, the resulting NO$_2^-$ was first reduced to NO$_3^-$ by incubation with nitrate reductase and measured by a microplate assay method based on the Griess reaction [15].

2.7. Measurement of ONOO$^-$ Level in the Serum. The ONOO$^-$ level was evaluated using the method of Kooy et al. [16] with minor modifications. Serum was added to the rhodamine solution [50 mM sodium phosphate buffer, 90 mM sodium chloride, 5 mM diethylenetriaminepentaacetic acid, and dihydrothrodamine (DHR) 123], and then the fluorescence of rhodamine 123, the reduced form of DHR 123, at 485 nm excitation and 535 nm emission was measured every 5 min for 30 min with a fluorescence plate reader.

2.8. Measurement of ROS Level in the Serum and Lung. ROS levels in the serum and lung were measured employing the method of Ali et al. [17]. Lung tissues were homogenized on ice with 1 mM EDTA-50 mM sodium phosphate buffer (pH 7.4), and then 25 mM DCFH-DA was added to homogenates or serum. After incubation for 30 min, the changes in fluorescence values were determined at an excitation wavelength of 480 nm and emission wavelength of 535 nm.

2.9. Assessment of NO and ONOO$^-$ Generation in the Lung. NO was measured by assaying the lung tissue, using the method of Green et al. [15]. Each sample was mixed with an equal volume of Griess reagent (Promega Corporation, Madison, WI, USA) and incubated at room temperature for 10 min. Absorbance was measured at 540 nm with an ELISA reader. The nitrite level in the samples was determined by comparison against a sodium nitrite curve using the method of Misko et al. [14]. ONOO$^-$ was measured by the method of Kooy et al. [16]. Each sample was mixed with rhodamine buffer (pH 7.4) and 5 mM DHR 123. After incubation for 5 min at 37°C, the fluorescence intensity of the oxidized DHR 123 was measured with a microplate fluorescence reader at excitation and emission wavelengths of 485 and 530 nm, respectively.

2.10. Preparation of Nuclear and Postnuclear Fractions. Nuclear protein extraction was performed according to the method of Komatsu [18]. In brief, lung tissues were
homogenized with ice-cold lysis buffer containing 5 mM Tris-HCl (pH 7.5), 2 mM MgCl₂, 15 mM CaCl₂, and 1.5 M sucrose, and then 0.1 M DTT and protease inhibitor mixture solution were added. After centrifugation (10,500 x g for 20 min at 4°C), the pellet was suspended with extraction buffer containing 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (pH 7.9), 1.5 mM MgCl₂, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, and then 0.1 M DTT and protease inhibitor mixture solution were added. The mixture was placed on ice for 30 min. The nuclear fraction was prepared by centrifugation at 20,500 x g for 5 min at 4°C. The postnuclear fraction was extracted from the lung of each mouse, as described below. In brief, lung tissue was homogenized with ice-cold lysis buffer (pH 7.4) containing 137 mM NaCl, 20 mM Tris-HCl, 1% Tween 20, 10% glycerol, 1 mM PMSF, and protease inhibitor mixture solution. The homogenate was then centrifuged at 2,000 x g for 10 min at 4°C. The protein concentration in each fraction was determined using a Bio-Rad protein kit (Bio-Rad Laboratories, Hercules, CA, USA).

2.11. Immunoblotting Analyses. For the determination of NF-κB65 and histone, 10 μg of protein from each nuclear fraction was electrophoresed through 12% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE). Separated proteins were transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies to NF-κB65 and histone overnight at 4°C. After the blots were washed, they were incubated with anti-rabbit or anti-mouse IgG HRP-conjugated secondary antibody for 1 h at room temperature. Also, 10–15 μg of protein of each postnuclear fraction of SOD, catalase, IκBα, IκBβ, COX-2, iNOS, MCP-1, ICAM-1, TNF-α, IL-6, and β-actin was electrophoresed through 8–15% SDS-PAGE. Each antigen-antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with Sensi-Q 2000 (Lugen sci, Gyeonggi-do, Korea). Band densities were determined using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone or β-actin. The protein levels of groups are expressed relative to those of normal mice.

2.12. Histological Examination of Lung Tissue. For microscopic evaluation, the lung was cut to isolate the middle segment. This segment was fixed in 10% neutral-buffered formalin and after embedding in paraffin, cut into 2 μm sections and stained using hematoxylin and eosin (H/E) for microscopic evaluation. The stained slices were subsequently observed under an optical microscope and analyzed using the i-Solution Lite software program (Innerview Co.).

2.13. Statistical Analysis. Data are expressed as mean ± SEM. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test (SPSS 11.5.1 for Windows, 2002, SPSS Inc., USA). Values of p < 0.05 were considered significant.

3. Results

3.1. Changes in Body and Lung Weights. Table 1 shows the changes in body and lung weights during the experimental period. The LPS-induced acute lung injury models displayed a markedly decreased body weight, and the decreased body weight was significantly increased by both the nonheat-processed and heat-processed Scutellariae Radix administrations. Challenge of animals with LPS resulted in significant increases in the lung weight (207% of normal value), suggestive of pulmonary edema and infiltration. On the other hand, pretreatment with nonheat-processed or heat-processed Scutellariae Radix was effective for preventing LPS-induced increases in the lung weight (139 and 111% of normal value, resp.).

3.2. Biochemical Features of Serum. Table 2 shows the effects of Scutellariae Radix on general biochemical parameters of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutellariae Radix. Similarly, the RANTES level in LPS-treated mice was markedly higher than in normal mice, but it was slightly decreased in Scutellariae Radix-administered groups. In addition, the ROS level of serum. The MCP-1 and IL-6 levels of the control mice were markedly increased compared to normal mice, but they were significantly decreased by the administration of heat-processed Scutella...
Table 2: Hematological analyses.

| Group         | MCP-1 (pg/mL) | IL-6 (pg/mL) | RANTES (pg/mL) | ROS (fluorescence/min/mL) | NO$_2^-$/NO$_3^-$ (μmol/mL) | ONOO$^-$ (fluorescence/mL) |
|---------------|---------------|--------------|----------------|--------------------------|-----------------------------|-----------------------------|
| Normal        | 0.4 $\pm$ 0.2** | 5.3 $\pm$ 1.4*** | 4.8 $\pm$ 0.7*** | 42.7 $\pm$ 3.4***       | 15.9 $\pm$ 2.4***          | 28.9 $\pm$ 0.5***          |
| LPS-treated   |               |              |                |                          |                             |                             |
| Veh.          | 494.9 $\pm$ 44.3 | 284.9 $\pm$ 33.8 | 370.4 $\pm$ 71.9 | 72.7 $\pm$ 2.4           | 191.6 $\pm$ 9.3            | 35.2 $\pm$ 0.5             |
| NSR           | 331.1 $\pm$ 36.8* | 218.2 $\pm$ 34.6 | 314.1 $\pm$ 32.7 | 62.8 $\pm$ 4.0           | 163.2 $\pm$ 8.9            | 32.1 $\pm$ 0.6**           |
| HSR           | 253.8 $\pm$ 84.2* | 163.5 $\pm$ 42.7* | 277.6 $\pm$ 38.8 | 58.6 $\pm$ 2.2*          | 99.8 $\pm$ 32.9*           | 30.7 $\pm$ 0.8**           |

Veh., vehicle-administered and LPS-treated mice; NSR, nonheat-processed Scutellariae Radix-administered and LPS-treated mice; HSR, heat-processed Scutellariae Radix-administered and LPS-treated mice. Data are the mean ± SEM. Significance: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ versus vehicle-administered and LPS-treated mice.

Figure 2: H/E staining of lung tissue. (a) Normal mice, (b) vehicle-administered and LPS-treated mice, (c) nonheat-processed Scutellariae Radix-administered and LPS-treated mice, and (d) heat-processed Scutellariae Radix-administered and LPS-treated mice. ×400.

level was significantly decreased by both the nonheat-processed and heat-processed Scutellariae Radix administrations, as shown in Table 2.

3.3. Histological Examination of Lung Tissue. Figure 2 shows the results of the histological examinations of lung tissue stained with H/E. Histological changes such as lung edema, an increased alveolar wall thickness, inflammatory cell aggregation, and moderate pulmonary hemorrhage were observed. The lesions of normal mice were not apparent. Moreover, pretreatment with nonheat-processed or heat-processed Scutellariae Radix markedly ameliorated the pulmonary injury. In particular, heat-processed Scutellariae Radix more effectively improved the pathological status compared with the nonheat-processed form.

3.4. ROS, NO, and ONOO$^-$ in the Lung. Table 3 shows the effect of nonheat-processed or heat-processed Scutellariae Radix on the generation of ROS, NO, and ONOO$^-$. The levels of ROS, oxidative stress biomarkers, in the lung of the vehicle-administered and LPS-treated mice were significantly elevated compared with normal mice. Nonheat-processed Scutellariae Radix administration showed a tendency to decrease the ROS levels in the lung (without significance), but those levels in the lung on receiving heat-processed Scutellariae Radix were significantly decreased. The NO
Table 3: Oxidative stress biomarkers in lung tissue.

| Group                  | ROS (fluorescence/min/mg protein) | NO (mM/mL)    | ONOO− (fluorescence/mg protein) |
|------------------------|----------------------------------|---------------|---------------------------------|
| Normal mice            | 88 ± 3***                        | 0.96 ± 0.17** | 1,075 ± 51***                   |
| LPS-treated mice       |                                  |               |                                 |
| Veh.                   | 147 ± 11                         | 1.87 ± 0.19   | 2,300 ± 84                      |
| NSR                    | 132 ± 5                          | 1.20 ± 0.10** | 1,430 ± 36***                   |
| HSR                    | 122 ± 6*                         | 0.63 ± 0.04***| 1,317 ± 47***                   |

Veh., vehicle-administered and LPS-treated mice; NSR, nonheat-processed Scutellariae Radix-administered and LPS-treated mice; HSR, heat-processed Scutellariae Radix-administered and LPS-treated mice. Data are the mean ± SEM. Significance: *𝑝 < 0.05, **𝑝 < 0.01, and ***𝑝 < 0.001 versus vehicle-administered and LPS-treated mice.

3.6. Oxidative Stress-Related Protein Expressions in the Lung. The protein levels of the oxidative stress-related proteins, such as IκBα, IκBβ, and NF-κBp65, were examined. In the vehicle-administered and LPS-treated mice, the protein expression of IκBα was significantly reduced and that of IκBβ was slightly reduced compared with the normal group (Figures 4(a) and 4(b)). Nonheat-processed Scutellariae Radix administration showed no difference compared with vehicle-administered acute lung injury mice, and heat-processed Scutellariae Radix led to a significant upregulation of IκBα protein expression. Moreover, the vehicle-administered and LPS-treated mice showed upregulation of the nuclear NF-κBp65 protein compared to normal mice. On the other hand, the administration

**Figure 3:** SOD (a) and catalase (b) protein expressions in lung tissue. N, normal mice; Veh., vehicle-administered and LPS-treated mice; NSR, nonheat-processed Scutellariae Radix-administered and LPS-treated mice; HSR, heat-processed Scutellariae Radix-administered and LPS-treated mice. Data are the mean ± SEM. Significance: *𝑝 < 0.05, **𝑝 < 0.01, and ***𝑝 < 0.001 versus vehicle-administered and LPS-treated mice.
of nonheat-processed and heat-processed *Scutellariae* Radix led to a significant downregulation of NF-κBp65 protein expression (Figure 4(c)).

### 3.7 Inflammation-Related Protein Expressions in the Lung

Next, we quantified COX-2, iNOS, MCP-1, ICAM-1, TNF-α, and IL-6 protein expressions (Figures 5 and 6).

The inflammation-related protein expressions in the vehicle-administered and LPS-treated mice were significantly augmented in the lung compared with normal mice. However, treatment with nonheat-processed and heat-processed *Scutellariae* Radix suppressed these proteins in the lung; especially, heat-processed *Scutellariae* Radix reduced these to nearly to normal levels in the lung.
Figure 5: COX-2 (a), iNOS (b), MCP-1 (c), and ICAM-1 (d) protein expressions in lung tissue. N, normal mice; Veh., vehicle-administered and LPS-treated mice; NSR, nonheat-processed *Scutellariae* Radix-administered and LPS-treated mice; HSR, heat-processed *Scutellariae* Radix-administered and LPS-treated mice. Data are the mean ± SEM. Significance: *p* < 0.05, **p** < 0.01, and ***p*** < 0.001 versus vehicle-administered and LPS-treated mice.

(Figures 5(a)–5(d) and Figure 6(a)), except for IL-6 protein expression (Figure 6(b)).

4. Discussion

Herbal medicine has long been used for the treatment of diverse diseases. These include heat-dissipating Chinese herbs. Herbs in this group can reduce heat, purge fire, dry dampness, stop bleeding, and excretion of toxic material. *Scutellariae* Radix, a heat clearing herb, has antipyretic, hepatoprotective, antihypertensive, diuretic, and antibiotic activities [19]. *Scutellariae* Radix is clinically used in two ways: nonheat-processed and heat-processed forms. Several studies have identified active flavonoids isolated from *Scutellariae* Radix: baicalin, baicalein, and wogonin [20, 21]. Flavonoids show medicinal and pharmacological activities against inflammation, allergy, viruses, and cancer [22, 23]. These three phenolic compounds were reported to have protective effects against LPS-induced acute lung injury in an animal model. However, there has been no comparative study on the effects of *Scutellariae* Radix according to heat
Figure 6: TNF-α (a) and IL-6 (b) protein expressions in lung tissue. N, normal mice; Veh., vehicle-administered and LPS-treated mice; NSR, nonheat-processed Scutellariae Radix-administered and LPS-treated mice; HSR, heat-processed Scutellariae Radix-administered and LPS-treated mice. Data are the mean ± SEM. Significance: * P < 0.05, ** P < 0.01, and *** P < 0.001 versus vehicle-administered and LPS-treated mice.

treatment. The HPLC analysis indicated that the total components of heat-processed Scutellariae Radix showed a more than 24.9% increase compared to those of nonheat-processed Scutellariae Radix. In the present study, we demonstrated that heat-processed Scutellariae Radix was more effective than nonheat-processed Scutellariae Radix in an LPS-induced acute lung injury model. This suggests that heat-processed Scutellariae Radix exhibits marked potential as a therapeutic agent for acute lung injury.

LPS or endotoxin causes a marked inflammatory and immune response in the host. LPS injection results in acute lung injury, which is widely used in animal models to investigate the mechanisms of endotoxin-related acute lung injury. LPS-induced acute lung injury was characterized by the loss of alveolocapillary membrane integrity, leakage of plasma protein, pulmonary edema, marked neutrophil infiltration, and release of proinflammatory cytokines and mediators. In the present study, we evaluated the body weight change, lung weight, and histological change to quantify the magnitude of pulmonary edema and inflammatory cell infiltration, which is a typical symptom of inflammation and a major characteristic of acute lung injury [2]. The administration of nonheat-processed Scutellariae Radix significantly decreased the body weight change, lung weight, and histological change. However, heat-processed Scutellariae Radix alleviated the symptoms more than nonheat-processed Scutellariae Radix.

Oxidative stress plays an important role in the development of LPS-induced acute lung injury and is associated with ROS formation, where the excessive production of ROS leads to an imbalance of the antioxidant system and finally causes cell damage. LPS is well known as a potent stimulator of iNOS expression, which further causes the overproduction of NO [24]. The iNOS-induced excessive NO plays an important role by directly inducing tissue dysfunction and ONOO⁻ formation. Inhibitions of ROS, NO, and ONOO⁻ are major factors to alleviate acute lung injury [25]. In our experiment, the levels of ROS, NO, and ONOO⁻ in the lung were significantly reduced regardless of nonheat-processed Scutellariae Radix treatment. Moreover, the administration of heat-processed Scutellariae Radix was much lower than that of nonheat-processed Scutellariae Radix. Based on the results obtained in this study, because heat-processed Scutellariae Radix inhibited NO production itself, it may be more effective than nonheat-processed Scutellariae Radix.

Antioxidant activities involving scavenging ROS are mediated by antioxidant enzymes such as SOD and catalase. These enzymes stimulate the repair of cells or their resistance to damage caused by the accumulation of ROS due to environmental stresses [26]. As a matter of fact, SOD and catalase are considered to be the first line of defense against free radical attack. Increasing free radicals including O₂⁻ generated by ROS accumulate in large amounts even inside cells. SOD is thoroughly utilized in scavenging O₂⁻ via dismutation reaction. This reaction produces H₂O₂ as a byproduct, which is also a potentially reactive radical. Then, catalase neutralizes H₂O₂ to form H₂O [27]. In this study, the oral administration of nonheat-processed Scutellariae Radix enhanced SOD and catalase activities. In particular, SOD and
catalase activities on the administration of heat-processed *Scutellariae* Radix were increased significantly higher than with nonheat-processed *Scutellariae* Radix. This suggests that heat-processed *Scutellariae* Radix may effectively scavenge oxyradicals during the inflammatory response in the presence of LPS-induced acute lung injury.

NF-κB is a transcriptional factor sequestered in the cytoplasm by the inhibitor protein IκBα. Upon activation by LPS, IκBα is rapidly phosphorylated and degraded, leading to the release of NF-κBp65. Then, NF-κB translocates to the nucleus and promotes the transcription of target genes such as TNF-α and IL-6 [23, 28, 29]. We investigated the degradation of IκBα, an inhibitor of NF-κB, and NF-κB activation. The results from the present study show that heat-processed *Scutellariae* Radix blocked the degradation of IκBα and prevented the translocation NF-κB in the lung. Namely, only heat-processed *Scutellariae* Radix markedly suppressed NF-κB activation through the inhibition of IκBα degradation. TNF-α and IL-6 appear in the early phase of the acute inflammatory response and play an important role in the pathophysiology of inflammation in acute lung injury [30, 31]. The elevated TNF-α and IL-6 protein expressions were significantly lowered with the administration of heat-processed *Scutellariae* Radix.

A previous study reported increases of pulmonary MCP-1 and ICAM-1 expressions in LPS-induced acute lung injury [32]. MCP-1 seems to play a primary role in many inflammatory states. It is expressed by various cells including monocytes/macrophages and controls recruitment [33]. With the administration of *Scutellariae* Radix, regardless of whether or not it received heat treatment, MCP-1 was reduced, but there was no significance. ICAM-1 protein is mainly located on the surface of endothelial cells. The level of expression of ICAM-1 on endothelial cells is increased following endothelial injury. The present experimental results show that the level of ICAM-1 protein expression was decreased on receiving heat-processed *Scutellariae* Radix.

The protein expressions of iNOS, which generates NO, and COX-2, which generates prostaglandin E2, contribute to the pathophysiological progression of acute lung injury [34]. LPS-induced increases in iNOS and COX-2 expressions in the lung are regulated by NF-κB activation. Nonheat-processed and heat-processed *Scutellariae* Radix pretreatment markedly suppress expressions of iNOS and COX-2 after LPS injection in the lung.

## 5. Conclusion

Heat-processed *Scutellariae* Radix leads to an increase in the contents of major flavonoids such as baicalin, baicalein, and wogonin. Heat-processed *Scutellariae* Radix has a protective effect against acute lung injury and exhibits stronger anti-inflammatory activity through the elevation of antioxidant enzymes and reduction of IκBα degradation and NF-κB activity compared to those of nonheat-processed *Scutellariae* Radix. This study suggests that heat processing of *Scutellariae* Radix may promote its lung-protecting potential through the inhibition of oxidative stress-sensitive mechanisms of the proinflammatory response, as shown in Figure 7.
Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

This study was supported in part by the Project, The Scientific Approach of Theory of Stir-Frying and Stir-Frying with Liquids, K14306, from the Korea Institute of Oriental Medicine.

References

[1] E. R. Johnson and M. A. Matthay, “Acute lung injury: epidemiology, pathogenesis, and treatment,” Journal of Aerosol Medicine and Pulmonary Drug Delivery, vol. 23, no. 4, pp. 243–252, 2010.

[2] C.-L. Tsai, Y.-C. Lin, H.-M. Wang, and T.-C. Chou, “Baicalein, an active component of Scutellaria baicalensis, protects against lipopolysaccharide-induced acute lung injury in rats,” Journal of Ethnopharmacology, vol. 153, no. 1, pp. 197–206, 2014.

[3] Z. Liu, Z. Yang, Y. Fu et al., “Protective effect of gossypol on lipopolysaccharide-induced acute lung injury in mice,” Inflammation Research, vol. 62, no. 5, pp. 499–506, 2013.

[4] K. H. Kim, M. J. Kwon, J.-Y. Choi et al., “Therapeutic effect of the tube of Alisma orientale on lipopolysaccharide-induced acute lung injury,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 863892, 10 pages, 2013.

[5] D. Wei and Z. Huang, “Antinflammatory effects of triptolide in LPS-induced acute lung injury in mice,” Inflammation, vol. 37, no. 4, pp. 1307–1316, 2014.

[6] C.-H. Lin, C.-H. Yeh, L.-J. Lin, S.-D. Wang, J.-S. Wang, and S.-T. Kao, “Immunomodulatory effect of Chinese herbal medicine formula sheng-fei-yu-chuan-tang in lipopolysaccharide-induced acute lung injury mice,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 976342, 12 pages, 2013.

[7] A. J. Reddy, J. D. Christie, R. Aplenc, B. Fuchs, P. N. Lanken, and S. R. Kleeberger, “Association of human NAD(P)(H)quinone oxidoreductase I (NQO1) polymorphism with development of acute lung injury,” Journal of Cellular and Molecular Medicine, vol. 13, no. 8B, pp. 1784–1791, 2009.

[8] H. H. Yoo, J. H. Park, and S. W. Kwon, “In vitro cytotoxic activity of some Korean medicinal plants on human cancer cell lines: enhancement in cytotoxicity by heat processing,” Phytotherapy Research, vol. 21, no. 9, pp. 900–903, 2007.

[9] C.-Z. Wang, H. H. Aung, B. Zhang et al., “Chemosensitizing effects of heat-processed Panax quinquefolius root on human breast cancer cells,” Anticancer Research, vol. 28, no. 5A, pp. 2545–2551, 2008.

[10] K. S. Kang, J. Ham, Y.-J. Kim, J. H. Park, E.-J. Cho, and N. Yamabe, “Heat-processed Panax ginseng and diabetic renal damage: active components and action mechanism,” Journal of Ginseng Research, vol. 37, no. 4, pp. 379–388, 2013.

[11] B. Jannat, M. R. Oveisi, N. Sadeghi et al., “Effect of roasting process on total phenolic compounds and γ-tocopherol contents of Iranian sesame seeds (Sesamum indicum),” Iranian Journal of Pharmaceutical Research, vol. 12, no. 4, pp. 751–758, 2013.

[12] Y. Wang, Y. Li, Y. Zhao, and L. Chang, “Effects of drying process of Yuanzhi (Radix Polygalae) on its bioactive ingredients,” Journal of Traditional Chinese Medicine, vol. 34, no. 2, pp. 206–213, 2014.

[13] G. Losonczy, T. Kriston, A. Szabó et al., “Male gender predisposes to development of endotox shock in the rat,” Cardiovascular Research, vol. 47, no. 1, pp. 183–191, 2000.

[14] T. P. Misko, R. J. Schilling, D. Salvemini, W. M. Moore, and M. G. Currie, “A fluorometric assay for the measurement of nitrite in biological samples,” Analytical Biochemistry, vol. 214, no. 1, pp. 11–16, 1993.

[15] L. C. Green, D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, and S. R. Tannenbaum, “Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids,” Analytical Biochemistry, vol. 126, no. 1, pp. 131–138, 1982.

[16] N. W. Kooy, J. A. Royall, H. Ischiropoulos, and J. S. Beckman, “Peroxy nitrite-mediated oxidation of dihydroxaldamine 123,” Free Radical Biology and Medicine, vol. 16, no. 2, pp. 149–156, 1994.

[17] S. F. Ali, C. P. LeBel, and S. C. Bondy, “Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity,” Neurotoxicology, vol. 13, no. 3, pp. 637–648, 1992.

[18] S. Komatsu, “Extraction of nuclear proteins,” Methods in Molecular Biology, vol. 355, pp. 73–77, 2007.

[19] R. A. Muluye, Y. Bian, and P. N. Alemu, “Anti-inflammatory and antimicrobial effects of heat-clearing Chinese herbs: a current review,” Journal of Traditional and Complementary Medicine, vol. 4, no. 2, pp. 93–98, 2014.

[20] Z. Gao, K. Huang, X. Yang, and H. Xu, “Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellaria baicalensis Georgi,” Biochimica et Biophysica Acta, vol. 1472, no. 3, pp. 643–650, 1999.

[21] M. Li-Weber, “New therapeutic aspects of flavones: the anti-cancer properties of Scutellaria and its main active constituents wogonin, baicalein and baicalin,” Cancer Treatment Reviews, vol. 35, no. 1, pp. 57–68, 2009.

[22] C. Li, G. Lin, and Z. Zuo, “Pharmacological effects and pharmacokinetics properties of Radix Scutellariae and its bioactive flavonoids,” Biopharmaceutics & Drug Disposition, vol. 32, no. 8, pp. 427–445, 2011.

[23] L. R. Filgueiras Jr., J. O. Martins, C. H. Serezani, V. L. Capelozzi, M. B. A. Montes, and S. Jancar, “Sepsis-induced acute lung injury (ALI) is milder in diabetic rats and correlates with impaired NFκB activation,” PLoS ONE, vol. 7, no. 9, Article ID e44987, 2012.

[24] C. L. Speyer, T. A. Neff, R. L. Warner et al., “Regulatory effects of iNOS on acute lung inflammatory responses in mice,” The American Journal of Pathology, vol. 163, no. 6, pp. 2319–2328, 2003.

[25] C. J. Chu, N. Y. Xu, X. L. Li et al., “Rabdopsis japonica var. glauco caulis flavonoids fraction attenuates lipopolysaccharide-induced acute lung injury in mice,” Evidence-Based Complementary and Alternative Medicine, vol. 2014, Article ID 894515, 12 pages, 2014.

[26] W. Wu, X. Wan, F. Shah, S. Fahad, and J. Huang, “The role of antioxidant enzymes in adaptive responses to seethough blight infestation under different fertilization rates and hill densities,” The Scientific World Journal, vol. 2014, Article ID 502134, 8 pages, 2014.

[27] K. C. Srivastava, R. D. Austin, D. Shrivastava, and G. Pranavadhyani, “Oxidant-antioxidant status in tissue samples of oral leukoplakia,” Dental Research Journal (Izsfahan), vol. 11, no. 2, pp. 180–186, 2014.

[28] T. Lawrence and C. Fong, “The resolution of inflammation: anti-inflammatory roles for NF-κB,” The International Journal of Biochemistry & Cell Biology, vol. 42, no. 4, pp. 519–523, 2010.
[29] M. Wei, X. Chu, L. Jiang et al., “Protocatechuic acid attenuates lipopolysaccharide-induced acute lung injury,” Inflammation, vol. 35, no. 3, pp. 1169–1178, 2012.

[30] Y.-C. Li, C.-H. Yeh, M.-L. Yang, and Y.-H. Kuan, “Luteolin suppresses inflammatory mediator expression by blocking the Akt/NFκB pathway in acute lung injury induced by lipopolysaccharide in mice,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 383608, 8 pages, 2012.

[31] T. Meng, J. Yu, Z. Lei et al., “Propofol reduces lipopolysaccharide-induced, NADPH oxidase (NOX$_2$) mediated TNF-α and IL-6 production in macrophages,” Clinical and Developmental Immunology, vol. 2013, Article ID 325481, 9 pages, 2013.

[32] S.-P. Lin, X.-F. Sun, X.-M. Chen, S.-Z. Shi, Q. Hong, and Y. Lv, “Effect of aging on pulmonary ICAM-1 and MCP-1 expressions in rats with lipopolysaccharide-induced acute lung injury,” Nan Fang Yi Ke Da Xue Xue Bao, vol. 30, no. 3, pp. 584–587, 2010.

[33] T. Okuma, Y. Terasaki, N. Sakashita et al., “MCP-1/CCR2 signalling pathway regulates hyperoxia-induced acute lung injury via nitric oxide production,” International Journal of Experimental Pathology, vol. 87, no. 6, pp. 475–483, 2006.

[34] A. Feng, G. Zhou, X. Yuan, X. Huang, Z. Zhang, and T. Zhang, “Inhibitory effect of baicalin on iNOS and NO expression in intestinal mucosa of rats with acute endotoxemia,” PLoS ONE, vol. 8, no. 12, Article ID e80997, 2013.