**Original Article**

**Sarcandra glabra attenuates LPS-induced acute lung injury via inhibiting inflammation in rats**

Benchao Hou¹, Yanping Zhan¹, Haiyun Liu², Weicheng Liu³, Shibiao Chen¹

¹Department of Anesthesiology, The First Affiliated Hospital of Nanchang University, 17 Yongwaizheng Avenue, Nanchang 330006, Jiangxi Province, China; ²Department of Physiology, School of Basic Medical Jiangxi University of Traditional Chinese Medicine, 818 Xingwan Road, Nanchang 330004, Jiangxi Province, China

Received May 19, 2015; Accepted June 27, 2015; Epub February 1, 2016; Published February 15, 2016

**Abstract:** The effects of *Sarcandra glabra* (SGE) have been observed in acute lung injury (ALI) induced by LPS in mice. The present study aims at investigating the protective effects of SGE on LPS-induced ALI and the underlying mechanisms. Rats were randomly divided into six groups and respectively pretreated with lipopolysaccharide (LPS) (6 mg/kg weight) by intraperitoneal injection 8 h after given different dose of SGE (5, 10 g crude drug/kg weight) by different ways. SGE attenuated LPS-induced lung injury in rats, which was manifested by the improvement of histological characteristic in lung, neutrophil infiltration into the lungs which was assessed by MPO activity in lung homogenates, pulmonary edema which was assessed by lung index and W/D weight ratio, respiratory disorder which was assessed by oxygenation index. All of these results suggested that SGE may ameliorate ALI induced by LPS. Furthermore, SGE significantly reduced the release of TNF-α and IL-6 in lung. In addition, the expression of NF-κB and TLR4 was inhibited by SGE. In conclusion, these results indicated that SGE alleviates LPS-induced ALI through via inhibiting inflammation in rats.

**Keywords:** Sarcandra glabra, lipopolysaccharide, acute lung injury, inflammation

**Introduction**

Acute lung injury (ALI) caused by infestation, trauma and sepsis is a severe syndrome with high rates of morbidity and mortality in clinics [1, 2]. ALI is characterized by serious membrane damage to the alveolar-capillary barrier which leads to non-cardiogenic pulmonary edema, severe respiratory difficulties and intractable hypoxemia [3]. The pathogenesis of ALI is complex. Endotoxin is thought to be the most important pathogenic factor in the development of ALI [4]. The inflammatory response within the lung plays an important role during this period [5-10]. Firstly, inflammatory cells build up and are sensitized by inflammatory mediators in the lung [11]. Simultaneously, pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-6, nuclear factor kappa B and activated leukocytes, are released principally by inflammatory cells, which lead to systemic inflammatory response syndrome (SIRS) [12-15].

*Sarcandra glabra* (Thunb.) Nakai, a traditional Chinese medicine has been used for inflammatory disease treatment [16], such as rheumatism [17], thrombocytopenia [18], tumor [19], hypoglycemic [20], cellulitis, fracture and traumatic injury. Previous study have showed that SGE can ameliorate immunological hepatitis and acute inflammation via inhibiting inflammatory infiltration, Pulmonary edema, pulmonary permeability index in mice, which indicating that it may be a potential agent to treat ALI [21].

Despite rapid developments in therapeutic tool, the prognosis of patients with ALI/ARDS is still poor [22]. Consequently, searching for medicine that could be ameliorating the treatment of ALI/ARDS is imminent.

**Materials and methods**

**Plant materials**

The herbs of *Sarcandra glabra* (Thunb.) Nakai were purchased from pharmacy.
**Preparation of SGE**

Whole herbs of *Sarcandra glabra* (Thunb.) Nakai (2.0 kg) were powdered and then decocted with 10 times amount of water 2 times, and every time 2 h. The aqueous extracts were combined, evaporated to relative density 1.3 under 70°C and then precipitated with 80% ethanol for 2 times, refrigerating 48 h every time. Reclaimed of ethanol and concentrated to obtain SGE 10 g each 1 mL. The aqueous extracts were dissolved in injection water, and then refrigerated 24 h. The solution pH was adjusted to 9.0 with sodium hydroxide solution. The aqueous extracts were added activated carbon to the mass fraction of 0.05%, boiled 30 mins, filtered, then diluted with physiological saline for injection to 1000 mL, filtrated with membrane aperture as 0.46 μm for sterilization.

**Reagents**

Myeloperoxidase (MPO) detection kit was obtained from Jiancheng Bioengineering. Enzyme-linked immune sorbent assay (ELISA) kits for TNF-α and IL-6 were supplied by KeyGEN Biotech (Nanjing, China). All antibodies were from Cell Signaling Technology, Inc. (Beverly, MA).

**Animal preparation**

Adult male Sprague-Dawley rats, which weighed about 180-220 g, were provided by Experimental Animal Center of Yangzhou University (Yangzhou, China). Mice were raised in a temperature-controlled house and were fed free diet and water.

**Model and grouping**

Rats were randomly divided into six groups: 1) Negative control group (n=10): rats were respectively pretreated with 0.9% normal saline 8 h after the first normal saline administration; 2) LPS control group (n=10): rats were respectively pretreated with LPS (6 mg/kg) by intraperitoneal injection 8 h after normal saline administration; 3) LPS/SGE groups (i.g. n=20): rats were respectively pretreated with LPS (6 mg/kg) by intraperitoneal injection 8 h after different doses of SGE (5 g crude drug/kg (n=10), 10 g crude drug/kg (n=10)) by intramuscular administration; 4) LPS/SGE groups (i.m. n=20): rats were respectively pretreated with LPS (6 mg/kg) by intraperitoneal injection 8 h after different doses of SGE (5 g crude drug/kg (n=10), 10 g crude drug/kg (n=10)) by intravenous administration; 5) LPS/SGE groups (i.v. n=20): rats were respectively pretreated with LPS (6 mg/kg) by intraperitoneal injection 8 h after given different doses of SGE (5 g crude drug/kg (n=10), 10 g crude drug/kg (n=10)) by intravenous administration; 6) LPS/DXM groups (i.v. n=15): rats were respectively pretreated with LPS (6 mg/kg) by intraperitoneal injection 8 h after given dexamethasone (1 mg/kg) by intragastric (n=5), intramuscular (n=5) and intravenous (n=5) administration. In all groups, all administration measurements were made 8 h before LPS administration. The current dose and timing of SGE administration were based on our previous experiments. Rats were executed half by exsanguination from the carotid artery at 14 h and 24 h after LPS intraperitoneal injection. At the end of the experiments, blood samples were obtained or blood gas analysis in each animal group. The oxygenation index (\(\text{PaO}_2/\text{FiO}_2\)), wet-to-dry (W/D) weight ratio, myeloperoxidase (MPO) activity, and histopathologic changes were measured. To investigate the mechanisms of SGE improvement in LPS-induced ALI, the inflammatory cytokines (TNF-α, IL-6, NF-κB and TLR4) were detected.

**Histopathology study**

The right middle lobes of the lungs of all groups (n=5, respectively) were collected at 24 h after LPS intraperitoneal injection. The tissue blocks size of 0.2 cm ×0.2 cm ×0.2 cm approximately were fixed with 10% neutral formalin, dehydrated by dimethylbenzene and absolute ethyl alcohol, embedded in paraffin, and sectioned into 4 μm thickness. After xylol deparaffinization and rehydration, the pathological sections were stained with hematoxylin and eosin (H&E) and observed under light microscope. Histopathological sections of the lung were scored for pulmonary edema, infiltration of neutrophils, pulmonary alveolar capillary hemorrhage, and alveolar epithelial microvilli desquamation in a blinded fashion.

**Oxygenation index (\(\text{PaO}_2/\text{FiO}_2\)) analysis**

At 14 h and 24 h after LPS intraperitoneal injection, carotid arterial blood sample was collected from carotid artery and measured immedi-
Sarcandra glabra and acute lung injury

Analytically using blood gas analyzer. At the same time, respiratory rates were measured.

Analysis of lung index and wet-to-dry weight ratio (W/D)

At 14 h and 24 h after LPS intraperitoneal injection, the whole lung was weighed immediately after its excision. Lung index was calculated via dividing the wet weight of whole lung by body weight. Furthermore, the superior lobe of right lung was weighed to get wet weight after removed from the whole lungs. Then, it was placed in an incubator for 72 h at 80°C and weighed again when it was constant weight. The ratio of W/D was finally calculated by the following formula. W/D ratio = Wet weight/Dry weight ×100%.

Myeloperoxidase activity assay

At 14 h and 24 h after LPS intraperitoneal injection, the left lungs was dissected from rats, rinsed and homogenized. The homogenate was centrifuged at 10,000 g for 5 min and the supernatant was collected and used to measure MPO activity using the myeloperoxidase (MPO) activity colorimetric assay kit.

Assay of IL-6 and TNF-α concentration

Lung homogenate was prepared according to method described for myeloperoxidase assay. IL-6 and TNF-α concentration in lung was evaluated using specific rat ELISA as the manufacturer’s instructions.

Western blot analysis

Protein levels of NF-κB p65, phospho-NF-κB p65, and TLR4 were analyzed by Western blotting. Total proteins were recovered from lung tissue of each group and instantly homogenized in buffer with Tissue protein extraction reagent (TPPER) under the condition of ice-bath. The homogenate was centrifuged at 12,000 g for 5 min and the supernatant was collected. Protein quantification was determined by the bicinchoninic acid protein assay kit (BCA) using bovine

![Figure 1](image-url)
Sarcandra glabra and acute lung injury

serum albumin (BSA) as standard. Fifty micrograms of proteins were loaded into a 4% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and separated in a 10% SDS-PAGE according to electrophoresis. Separated proteins were transferred onto polyvinylidene fluoride (PVDF) membrane. Blots were detected by an enhanced chemiluminescent detection kit according to the manufacturer’s instructions and specific antibodies. The blotting bands were analyzed with Image J software (National Institutes of Health). Expression of interest proteins was normalized to GAPDH expression.

Statistical analysis

All results are presented as mean ± SD from 3 independent experiments. Statistical differences between groups were determined by the one-way analysis of variance and Student’s t-test. Values of \( P < 0.05 \) were considered statistically significant differences.

Results

Oxygenation index (\( \text{PaO}_2/\text{FiO}_2 \)) analysis

Compared with the negative control group, hypoxemia was much more marked in the LPS control group, as Figure 1 described. \( \text{PaO}_2 \) was significantly reduced in the LPS control group compared to the negative control group (58.15 ± 6.13 mm Hg versus 108.30 ± 8.27 mm Hg, \( P < 0.01 \)). The \( \text{PaO}_2/\text{FiO}_2 \) was also significantly different contrasted the LPS control group with the negative control group (167.14 ± 17.33 versus 482.67±23.47, \( P < 0.01 \)). Respiratory rate was substantially increased between the LPS control group and the negative control group (152.48 ± 6.27 beat/min versus 78.25 ± 4.13 beat/min, \( P < 0.01 \)). These results suggest that the model of ALI induced by LPS (i.e., 6 mg/kg) in rats was viable. Compared to LPS control group, LPS/SGE groups administration by different ways diversely increased \( \text{PaO}_2 \), \( \text{PaO}_2/\text{FiO}_2 \) and reduced respiratory rate, indicating that SGE dose dependently ameliorated LPS-induced ALI, in addition, the trend of improvement by different ways is that intravenous > intramuscular > intragastric administration (Figure 1).

Analysis of lung index and wet-to-dry weight ratio (W/D)

The pulmonary edema is deemed to be a distinguishing feature of ALI. Lung index and wet-to-dry lung weight ratio (W/D) were determined in various groups. Compared with the negative
Sarcandra glabra and acute lung injury

Figure 3. Effects of SGE on neutrophil recruitment in LPS-induced rats. Myeloperoxidase activity (U/gm tissue) was detected. Values are expressed as mean ± SD (n=10 in each group). **P<0.01 vs. negative control group, *P<0.05, **P<0.01 vs. LPS control group.

Figure 4. Effects of SGE on inflammatory cytokines in LPS-induced rats. A. IL-6; B. TNF-α. Values are expressed as mean ± SD (n=10 in each group). **P<0.01 vs. negative control group, *P<0.05, **P<0.01 vs. LPS control group.

control group, Lung index (1.24% ± 0.12% versus 0.49% ± 0.08%, P<0.01) and W/D ratio (7.12 ± 0.59 versus 4.29 ± 0.48, P<0.01) obviously increased after administration of LPS. While, Compared to LPS control group, LPS/SGE groups administration by different ways diversely decreased lung index and W/D ratio (Figure 2).

Myeloperoxidase activity assay

Compared with the negative control group, MPO activity in lung tissue were significantly increased in the LPS control group (7.95 ± 0.20 units/g tissue versus 2.04 ± 0.16 units/g tissue, P<0.01). However, Compared to the LPS control group, LPS/SGE group administration by different ways evidently decreased MPO activity in lung tissue (Figure 3).

Levels of cytokines and chemokines assay

Levels of IL-6 (320.27 ± 20.20 pg/ml versus 73.26 ± 9.16 pg/ml, P<0.01) and TNF-α (620.27 ± 20.20 pg/ml versus 53.26 ± 9.16 pg/ml, P<0.01) in the peripheral blood of LPS-induced rats were significantly increased compared to the negative control group. The increased levels of TNF-α and IL-6 were markedly attenuated by SGE treatment (Figure 4). These results suggest that SGE
The expression of NF-κB p65, phospho-NF-κB p65, and TLR4 proteins in lung tissue were detected by Western blot. As shown in Figure 5, the protein expression of NF-κB p65/phospho-NF-κB p65 (0.50 ± 0.005 versus 3.02 ± 0.07, P<0.01) and TLR4 (0.05 ± 0.005 versus 0.38 ± 0.007, P<0.01) in lung tissue in the LPS control group declined obviously compared with those of the negative control group. Compared to the LPS control group, dexamethasone stimulated obviously the protein expression of Compared to LPS control group, LPS/SGE group administration by different ways diversely stimulated the protein expression of NF-κB p65/phospho-NF-κB p65 in lung tissue (Figure 5).

**Histological examination**

To investigate the effective treatment of SGE on ALI induced by LPS, first of all, we examined the pathologic changes in the lung by light microscope. As shown in Figure 6, according to the lung tissue thickness stained by HE, there is no obvious neutrophil infiltration in the pulmonary alveolar cavity in the negative control group, and the structure of alveolar epithelium is absolutely holonomic. By contraries, in the LPS control group, the membrane of alveolar capillary epithelium appeared swelling, broaden and alveolar space became small, neutrophil infiltration, even mitochondrial vacuolization. Compared to the LPS control group, SGE group significantly alleviated the condition of lung in the LPS-induced ALI (Figure 6).
Figure 6. SGE attenuates histopathological changes in lung (200× magnification). A. Negative control group. B. LPS control group. C. LPS/SGE groups (i.g. 5 g crude drug/kg). D. LPS/SGE groups (i.g. 5 g crude drug/kg). E. LPS/SGE groups (i.m. 5 g crude drug/kg). F. LPS/SGE groups (i.m. 10 g crude drug/kg). G. LPS/SGE groups (i.v. 5 g crude drug/kg). H. LPS/SGE groups (i.v. 10 g crude drug/kg). I. LPS+DXE group (i.g. 1 mg/kg). J. LPS+DXE group (i.m. 1 mg/kg). K. LPS+DXE group (i.v. 1 mg/kg).

Discussion

Acute lung injury (ALI) is a severe systemic inflammatorome in the lung with high rates of morbidity and mortality in clinics, which clinical manifestations are respiratory distress and intractable hypoxemia [23]. ALI has high rates of morbidity and mortality in clinics on account of its equivocal pathogenic mechanism and scarce effective treatment [24]. However, it is
generally accepted that LPS, an exogenous toxin, play an important role on the pathogenesis of ALI [25-27]. Therefore, we established the model of ALI induced by LPS administered intraperitoneally [28]. ALI induced by LPS is characterized by neutrophil infiltration, release of inflammatory mediators in lung, pulmonary edema [29]. Systemic inflammation in lung produced by LPS is nearly similar with many of typical clinical presentation of ALI [30].

Sarcandra glabra, which alternate name is zhongjiefeng, whole herbs of Sarcandra glabra (Thunb.) Nakai, has been used clinically to treat diseases [31], such as rheumatism, pneumonia at al. Previous study has showed that SGE can ameliorate ALI induced by LPS such as inflammatory infiltration, pulmonary edema, and pulmonary permeability index in mice. However, whether SGE can alleviate ALI induced by LPS has not yet been reported.

In the present study, we found the effect of SGE on ALI induced by LPS in rats. Previous research has shown that pulmonary lesions achieve peaks at 6-8 h and 12-24 h after given LPS intraperitoneally. So experimental datum were detected at the time point 24 h after LPS administered [32].

Results showed that SGE attenuated LPS-induced lung injury in rats, which was manifested by the improvement of histological characteristic in lung, neutrophil infiltration into the lungs which was assessed by MPO activity in lung homogenates, pulmonary edema which was assessed by lung index and W/D weight ratio, and respiratory disorder which was assessed by oxygenation index. All of these results suggested that SGE may ameliorate ALI induced by LPS.

To further explore the mechanism that SGE performed its protective effect on acute lung injury (ALI), we detected the expression of pro-inflammatory cytokines, such as TNF-α and IL-6, which are key mediators and related to the development of ALI. Studies demonstrated that SGE significantly reduced the release of TNF-α and IL-6 in lung. Moreover, results suggested that the expression of NF-κB and TLR4 was inhibited by SGE. These results indicated that SGE may alleviate inflammatory response in rat to LPS-induced ALI.

In addition, previous studies have shown that the effects of SGE are correspondingly dependent on dose within a certain range, and the way of administration has an important role on pesticide effectiveness. According to pesticide effectiveness, its sequencing is intravenous > intramuscular > intragastric administration.

In conclusion, SGE can alleviate LPS-induced ALI in rats, and the mechanism of SGE protective effect on acute lung injury (ALI) are associated with suppressing the inflammatory response.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shibiao Chen, Department of Anesthesiology, The First Affiliated Hospital of Nanchang University, 17 Yongwaizheng Avenue, Nanchang 330006, Jiangxi Province, China. E-mail: csb196811@163.com

References

Sarcandra glabra and acute lung injury


