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ORIGINAL ARTICLE

Evaluation of Lipopolysaccharide-Induced Acute Lung Injury Attenuation in Mice by *Glycyrrhiza glabra*

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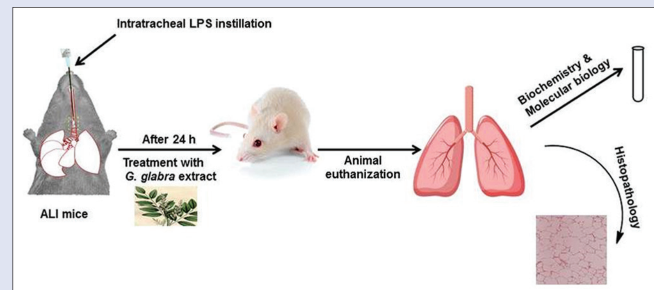
ABSTRACT

Objective: The medicinal importance of *Glycyrrhiza glabra* is well reported in Asian folk core medicine. In the current study, we have investigated the treatment effects of ethanolic extract of *G. glabra* in acute lung injury (ALI) mice models. **Materials and Methods:** ALI murine models were established by intratracheal instillation of bacterial lipopolysaccharide (LPS). Bronchoalveolar lavage fluid (BALF) was prepared for measuring cell migration and cell count, protein content, and superoxidase dismutase (SOD) activity. Lung tissues were collected for evaluating the wet-to-dry weight ratio, messenger ribonucleic acid (mRNA) expression of pro-inflammatory cytokine, and histological change. **Results:** It was observed that the treatment of ALI mice significantly decreased the total cell count and exudation of protein into BALF. Further, SOD activity in BALF of LPS-induced ALI mice was greatly improved when treated with *G. glabra* extract at 200 or 400 mg/kg of body weight, and the SOD activity increased in a dose-response manner. Evaluation of lung wet-to-dry weight ratio, pro-inflammatory mRNA expression levels, and histologic examination of lung tissues indicates that plant extract has significantly attenuated the tissue injury. **Conclusions:** The results showed that *G. glabra* had a protective effect on LPS-induced ALI in mice which might be contributed due to diverse phytoconstituents of the plant extract. To fulfill the unmet needs of treating ALI, the active principles in *G. glabra* could be promising lead-alternative therapy to develop high-potent anti-inflammation agents for ALI.

Key words: Acute lung injury, bronchoalveolar lavage fluid, cell migration, *Glycyrrhiza glabra*, lipopolysaccharide

SUMMARY

- This is the first study of its kind in which protective effects of ethanolic extract of *Glycyrrhiza glabra* in acute lung injury (ALI) murine models that were generated by intratracheal lipopolysaccharide (LPS) administration.



Abbreviations used: ALI: Acute lung injury; BALF: Bronchoalveolar lavage fluid; LPS: Lipopolysaccharide; SOD: Superoxide dismutase; mRNA: Messenger ribonucleic acid.

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INTRODUCTION

Acute lung injury (ALI) is a syndrome of acute and persistent lung inflammation with enhanced vascular permeability. Acute respiratory distress syndrome (ARDS) was first described in 1967 as a complex and cascading process evolving from ALI.[1] ALI is characterized by rapid onset of acute respiratory failure with significant illness and death of humans. Reports suggest many patients who survive the ALI, their quality of life is severely affected.[2,3] Recent studies have been made in the understanding of the epidemiology, pathogenesis, and treatment of this disease. However, more advancement is needed to further reduce both ALI-associated death and morbidity.

Lipopolysaccharide (LPS), the major components of the outer cell wall of Gram-negative bacteria, is the principal inducer of inflammatory responses to this class of bacteria.[4] LPS, when intratracheally administered to mice, generates an acute inflammation, which is characterized by enhanced permeability of pulmonary capillaries, interstitial and alveolar edema, and an influx of circulating inflammatory cells.[5-7] Earlier reports have demonstrated that lung injury due to bacterial endotoxin, i.e., LPS administration, is initiated by a rapid influx of neutrophils into the air spaces during the first 24 h and then resulted in excessive inflammation.[8,9] This inflammatory cascade played a crucial role in the development of ALI, and the main pathological change of ALI was the manifestation of acute leakage inflammatory response with leakage of protein into the alveolar space, accumulation of inflammatory cell, interstitial edema, and disruption of epithelial integrity.[10]

ALI/ARDS, an acute respiratory failure, is now more common both in the developing countries and in the developed world; it would be fair to say that ALI/ARDS is an urgent unmet medical need. In other words, novel therapies need to be developed to further improve clinical outcomes. Although significant contributions have been made

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in understanding the mechanism underlying the pathogenesis of this syndrome, little progress has been made in the development of specific therapies to combat ALI/ARDS-associated injury and inflammation. Over the recent past, several natural products have been investigated in experimental models and have been shown to inhibit multiple inflammatory pathways associated with ALI and ARDS at a molecular level.[11] *G. glabra* L. is found in the subtropical and temperate regions of the world.[12] It is ethnopharmacologically a high-value medicinal plant used in the traditional medicine for the treating antibacterial, anti-inflammatory, antifungal, antidiabetic, antiviral, antiulcer, antioxidant, skin whitening, and antidiuretic agent. In the current study, we investigated the role of *G. glabra* L. in the attenuation LPS-induced ALI in murine models.

MATERIALS AND METHODS

Reagents

LPS (*Escherichia coli* 055:B5) was obtained from Sigma Chemical Co. (St. Louis, MO). TRIzol reagent was purchased from Invitrogen Co. (Carlsbad, CA). Dexamethasone (DEX) was purchased from Changle Pharmaceutical Co. (Xinxiang, Henan, China). Bio two-step real-time polymerase chain reaction (PCR) kit was purchased from Bioer Co. (Hangzhou, Zhejiang, China). Interleukin-6 (IL-6), IL-1β, and IL-10 ELISA kits and mouse tumor necrosis factor-α (TNF-α) were purchased from Biolegend, Inc. (San Diego, CA, USA). Mouse myeloperoxidase and mouse superoxide dismutase (SOD) ELISA kits were purchased from USCN Life Co. (Missouri City, TX, USA).

Plant material

G. glabra L. plant was procured from Xiansheng DrugStore (Nanjing, China) and assigned a batch number: 140304X. Fresh plant was washed under the running tap water, air-dried under the shade for 48 h in well-ventilated rooms, chopped, and then blended to a fine powder. Plant powder was stored in airtight containers under refrigeration for further use.

Preparation of *Glycyrrhiza glabra* extract

The air-dried *G. glabra* plant powder weighing 500 g was extracted exhaustively by percolation with 95% ethanolic using Soxhlet extractor. The extract was concentrated under vacuum at 50°C using a rotary evaporation and then left over solvent was removed by water bath, air-dried for 24 h, and the lyophilized until the solvent was completely removed, yielding an ethyl acetate extract weighing 165 g. Normal saline (NS), a vehicle, was used as diluent for the *G. glabra* extract (GGE) for treatment of animals.

Animal

Two-month-old female mice weighing 23 ± 28 g were provided by Shanghai Jingke Industrial Co., Ltd., China, and used in the study. Animals were maintained in the Animal Holding Facility of the institute under pathogen-free conditions. Mice were given free access to animal diet and water. Animals were kept in well-ventilated rooms with controlled setting (12 h) of light and dark cycle, temperature of 25 ± 2, and humidity of 45%–63%. The protocols for the animal study were approved by the institutional animal ethical committee, and efforts were made to minimize the animal sufferings. The animal studies were conducted in agreement with the guide for the care and use of laboratory animals published by the US National Institutes of Health. Animals were kept in the animal holding facility for about a week before the start of experimentation to allow the animals to acclimatize to the local environment.

Establishment of the animal model and treatment regimens

Sixty animals were randomly divided into six study groups with 10 mice/group [Table 1]: NS group consisted of animals that were orally gavaged with NaCl only. LPS group consisted of animals that were intranasally instilled with LPS only at the dosage of 0.5 mg/kg dissolved in NS. *G. glabra* extract control (GGEC) group is the plant extract control group and consisted of animals that were orally gavaged plant extract only at the highest tested dose. Treatment groups that were treated with GGE that was suspended into NS and orally administered to mice at the dosage of 200 mg/kg (GGE 200 group) or 300 mg/kg (GGE 400 group) body weight. DEX group consisted of animals that were administered through intraperitoneal (IP) route with DEX at the dosage of 5 mg/kg body weight. Treatment with the plant extract (GGE 200 group or GGE 400 group) or DEX began 2 days before the application of LPS and continued for 2 days after challenge with the LPS. After fasting for 24 h, animals were dosed through IP injection with pentobarbital sodium (90 mg/kg body weight) to induce anesthesia.

Bronchoalveolar lavage and cell counting

Twenty-four after LPS instillation or NS administration of bronchoalveolar lavage fluid (BALF) extraction was done thrice through a tracheal cannula with 0.5 ml of sterile NS, instilled up to a total volume of 1.2 ml. Afterward, BALF was immediately harvested by centrifuged at 5000 rpm for 10 min at 4°C. The supernatants of BALF were stored at −70°C till further use for other assays such as protein quantification, determination of cytokine levels, and SOD activity. The cell pellets were resuspended in NS, and the total cell number was counted using a standard hemocytometer. Differences in cell numbers were examined by counting at least 200 cells on a smear prepared by Wright–Giemsa staining.

Effect of *Glycyrrhiza glabra* treatment on wet-to-dry lung weight ratio

After the 24 h of the intratracheal LPS instillation and animal euthanization, lung tissues from the animals were aseptically removed, and the left side of the lungs was excised out. Lung tissues were washed thrice in NS, blotted dry in air to remove the moisture, weighed, kept in an oven at 70°C for 48 h, and then weighed again. The ratio of the wet to dry weight of the lung was calculated to measure the tissue edema.

Protein assay

The protein content in the BALF was measured using bicinchoninic acid method. Protein levels were expressed in mg protein/mL BALF.

Table 1: Experimental design adopted for animal grouping

Group	Treatment	Remarks
NS	Animals that were orally gavaged with NaCl	Normal control mice
GGEC	Animals that were orally gavaged Glycyrrhiza glabra extract only at the highest tested dose	Normal control mice
LPS	Animals that were intranasally instilled with LPS only at the dosage of 0.5 mg/kg dissolved in normal saline	Acute lung injury mice
DEX	Animals that were administered via intraperitoneal (i.p) route with dexamethasone at the dosage of 5 mg/kg body weight two days before LPS instillation	Acute lung injury mice
GGE200	Animals that were orally gavaged with Glycyrrhiza glabra extract at the dosage of 200mg/kg body weight two days before LPS instillation	Acute lung injury mice
GGE400	Animals that were orally gavaged with Glycyrrhiza glabra extract at the dosage of 400mg/kg body weight two days before LPS instillation	Acute lung injury mice

Where NS: Normal saline group; LPS: Lipopolysaccharide group; DEX: Dexamethasone group; GGEC: *Glycyrrhiza glabra* extract control group; GGE200: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 200mg/kg body weight; GGE400: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 400mg/kg body weight.

Assays for cytokines and SOD

The levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1 β in the BALF were measured by a sandwich ELISA kit. Cytokine levels were measured using commercially available kits (Jiancheng Bioengineering Institute, China) carefully according to the manufacturer's written instructions. SOD activity in the BALF was quantified using a mouse SOD ELISA kit.

Quantitative real-time polymerase chain reaction assay for tumor necrosis factor-α, interleukin-6, and interleukin-1β messenger ribonucleic acid expression

The mice from the aforementioned groups were euthanized, and left lung tissues, weighing approximately 100 mg, were collected with aseptic techniques and frozen in liquid nitrogen and stored at −80°C. Tissues were thawed and homogenized, and total RNA was extracted from lung tissues with TRIzol reagent. Impurities of DNA, if any, were removed by subjecting the isolated RNA samples to DNase I digestion. RNA integrity was confirmed by agarose gel electrophoresis and quantified by spectrophotometric analysis. Afterward, reverse transcription to complementary DNA was performed using 2.5 µg of the total RNA. The PCR mixture was prepared according to the supplier's instruction manual and the following mouse primers were used for cytokine messenger ribonucleic acid (mRNA) quantification [Table 2], and PCR products were resolved by 1% TAE agarose gel electrophoresis.

Assessment of histological analysis

Mice under pentobarbital anesthesia were euthanized 24 h after LPS instillation; right lungs were aseptically removed, inflated, and then fixed with 10% paraformaldehyde; samples were embedded in paraffin, and hematoxylin and eosin staining was performed using 5 µM tissue slides. Evaluations of lung injury and inflammatory cell infiltration were conducted using the modified scoring system described elsewhere.[13] Briefly, 10 fields from 3 lobes of the right lung tissue for each mouse were randomly selected by two histopathology experts with good experience. Fields were carefully examined using a light microscope at 200 × magnification to investigate and score the level of damage in these sections. The level of the damage was scored at the scale of 10 to monitor the presence and extent of interstitial cellular infiltration, alveolar protein exudation, and tissue hemorrhage. The sum of the scores from each category from 10 different microscopic fields was recorded as the final damaged score for mice.

Statistical analysis

All values of the *in vivo* study ($n = 5$) are expressed as mean ± standard deviation difference between the mean values of normally distributed data were assessed by one-way ANOVA (Dunnett's *t*-test) and the two-tailed Student's *t*-test for multiple comparisons. $P < 0.05$ was considered as statistically significant.

RESULTS

Cell infiltration and lung edema index

We examined the cell migration in the BALF of five mice from each study group. An increased cell migration was observed in the BALF of mice that were challenged with LPS [Table 3]. BALF of the mice in the LPS group contained a significantly higher number of leukocytes compared to the animals in the control groups (NS and GGEC), DEX group, and GGE group ($P < 0.05$). Cell migration was significantly reduced in the NS, DEX, GGE 200, and GGE 400 groups compared with the LPS group ($P < 0.05$). In addition, no prominent changes were seen in the total number of leukocytes in the GGEC group when matched with the NS group, DEX group, and GGE 200 or GGE 400 group. The assessment of the differential cell count revealed that the number of neutrophils was significantly higher in the LPS group when compared with all other study groups ($P < 0.05$) excepting that of the GGE 200 group. In addition, the percentage of neutrophils in the LPS group was much higher than in the NS, DEX, GGE 200, or GGE 400 group ($P < 0.05$). The amount of alveolar macrophages was substantially lower in the LPS group ($P < 0.05$) compared with other experimental groups excepting that of the GGE 200 group. Moreover, the percentage of macrophages in the GGE 400 group was much reduced when compared to the NS, DEX, and GGE 400 groups ($P < 0.05$). No considerable variation in the number of lymphocytes between the LPS, DEX, GGE 200, and GGE 400 groups was observed. Moreover, the percentage of lymphocytes in these groups was significantly lower in relation to the NS, DEX, and GGE 400 groups ($P < 0.05$). There was no statistically significant difference in the number of total leukocytes, alveolar macrophages, lymphocytes, and neutrophils between the GGE 200 and GGE 400 groups.

Table 2: Primer sequences used for cDNA preparation of the tissue cytokines

Cytokine	Primer direction	Primer sequence
TNF-α	forward	5-AAATGGGCTCCCTCTATCAGTTC-3′
	reverse	5-TCTGCTTGGTGGTTTGCTACGAC-3′
IL-6	forward	5-TCCTACCCCAACTTCCAATGCTC-3′
	reverse	5-TTGGATGGTCTTGGTCCTTAGCC-3′
IL-1β	forward	5′-GCTTCAGGCAGGCAGTAT3′
	reverse	5′-ACAAACCGCTTTTCCATCT-3′

Table 3: Effects of treatment on the total and differential cell count in the bronchoalveolar lavage fluid of normal control mice or acute lung injury mice models that were treated with dexamethasone or plant extract at the dosage of 200 (*Glycyrrhiza glabra* extract 200) or 400 (*Glycyrrhiza glabra* extract 400) mg/kg body weight

Group	Total (x10^6/ml	Neutrophils (%)	Macrophages (%)	Lymphocytes (%)
NS	0.8 ± 04	2.24 ± 1.34	66.67 ± 11.10	11.78 ± 1.04
LPS	3.00 ± 1.03	25.08 ± 7.44	60.00 ± 7.66	6.22 ± 1.11
DEX	0. 9 ± 0.55	12.34 ± 6.52	77.23 ± 9.66	11.53 ± 1.22
GGEC	1.11 ± 0.22	2.34 ± 1.04	75.03 ± 4.15	14.08 ± 1.15
GGE200	2.42 ± 1.52	18.11 ± 7.87	68.33 ± 9.12	11.13 ± 1.33
GGE400	1.64 ± 1.42	14.34 ± 6.52	72.66 ± 7.76	8.96 ± 1.42

The values shown in the table are the average values ± SD (n=5). Where NS: Normal saline group; LPS: Lipopolysaccharide group; DEX: Dexamethasone group; GGEC: *Glycyrrhiza glabra* extract control group; GGE200: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 200mg/kg body weight; GGE400: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 400mg/kg body weight.

Effects of *Glycyrrhiza glabra* treatment on lung wet/dry weight ratio and protein content in lipopolysaccharide-induced lipopolysaccharide mice

The effect of GGE treatment on the lung wet/dry weight ratio and the total protein in the BALF of mice were quantified 24 h after LPS instillation. A significant difference in lung wet/dry weight ratio was observed between the LPS, GGEC, and NS groups ($P < 0.01$ and $P < 0.01$, respectively) [Figure 1a]. Lung wet/dry weight ratio was significantly reduced in the animals that were treated with DEX or GGE at 200 or 400 mg/kg body weight after 24 h of LPS

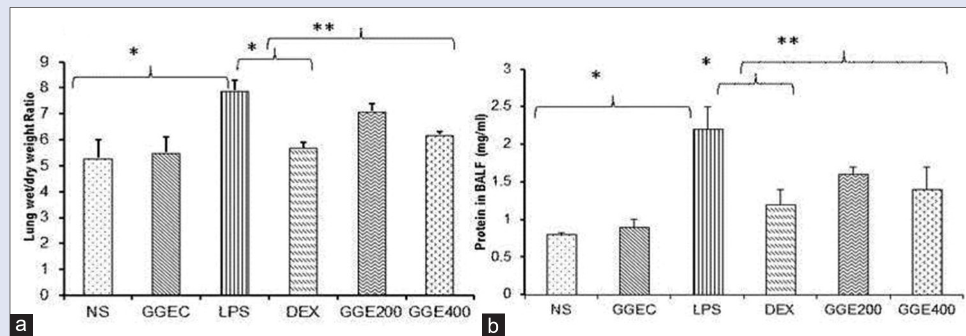

Figure 1: Treatment effects of *Glycyrrhiza glabra* extract on the lung wet/dry weight *Glycyrrhizaio* (a) and levels of total protein in the bronchoalveolar lavage fluid of lipopolysaccharide-induced acute lung injury mice (b). The values shown in the figures are the average values ± standard deviation ($n = 5$). *$P < 0.01$ versus control group; **$P < 0.05$ versus LPS group. Where NS: Normal saline group; LPS: Lipopolysaccharide group; DEX: Dexamethasone group; GGEC: *Glycyrrhiza glabra* extract control group; GGE200: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 200mg/kg body weight; GGE400: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 400mg/kg body weight

challenge ($P < 0.05$). A substantial increase in BALF protein levels was observed in the LPS mice 24 h after LPS instillation [Figure 1b] when compared with NS mice ($P < 0.01$). The protein content in the GGEC control group, GGE-treated groups, and DEX group was much lower than in the LPS group ($P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively).

Effect of *Glycyrrhiza glabra* extract treatment on SOD activity

The effect of GGE on the oxidative stress in the BALF of LPS-induced ALI mice was determined by measurements of superoxide dismutase (SOD) activity [Figure 2]. A significant reduction in SOD activity was observed in the animals of the LPS group when compared with the animals in the NS or GGEC group ($P < 0.005$). In the animals of the GGEC group, there was also a significant increase in SOD activity when compared with the animals in the NS group ($P < 0.005$), showing that *G. glabra* possesses excellent antioxidant potential. A significant increase in SOD activity was observed in the BALF of ALI mice that were treated with DEX or GGE at the doses of either 200 or 300 mg/kg body weight when compared with the animals in the LPS group. Moreover, an increase in SOD activity in ALI mice treated with *G. glabra* showed a dose-dependent response.

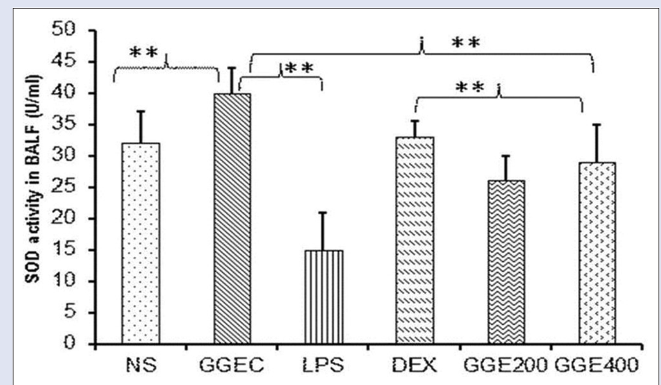

Figure 2: Effects of *Glycyrrhiza glabra* extract on SOD activity in bronchoalveolar lavage fluid of lipopolysaccharide-induced acute lung injury mice. SOD activity was determined by SOD-specific enzyme-linked immunosorbent assay kits. Data are presented in the figure is an average ± standard deviation ($n = 5$). *$P < 0.01$ versus control group; **$P < 0.01$ versus LPG group NS: Normal saline group; LPS: Lipopolysaccharide group; DEX: Dexamethasone group; GGEC: *Glycyrrhiza glabra* extract control group; GGE200: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 200mg/kg body weight; GGE400: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 400mg/kg body weight. SOD; Superoxide dismutase

Effect of *Glycyrrhiza glabra* extracts on pro-inflammatory cytokines in the lung tissues of lipopolysaccharide-induced acute lung injury mice

The levels of pro-inflammatory cytokines in the lung tissue of LPS-induced ALI mice were significantly upregulated compared to the NS and GGEC groups ($P < 0.005$). A significant difference in TNF-α [Figure 3a], IL-1β [Figure 3b], and IL-6 [Figure 3c] was observed when the NS and GGEC groups were compared with the LPS group. Treatment with GGE significantly blocks this rise in these cytokine levels in the GGE 200 and GGE 400 groups ($P < 0.005$). The levels of these cytokines fairly remain constant in the DEX- and GGE-treated groups with GGE treatment showing dose-dependent activity. These results indicate that *G. glabra* induces the favorable effect to attenuate the lung injury in mice.

Effect of *Glycyrrhiza glabra* on expression of tumor necrosis factor-α, interleukin-6, and interleukin-1β messenger ribonucleic acid

The levels of the cytokines such as TNF-α and IL-6 in the lung tissues of LPS-induced ALI mice increased to a significant level compared to the NS and GGEC groups ($P < 0.005$)). Treatment with DEX or GGE significantly increased the levels of pro-inflammatory cytokine such as TNF-α [Figure 4a], IL-1β [Figure 4b], and IL-6 [Figure 4c] mRNA expression in the lung tissues of ALI mice measured after 24 h of treatment compared with the animals in the LPS group ($P < 0.005$). The increase in these cytokine levels in lung tissues showed a dose-response manner. A significant downregulation of mRNA cytokine levels was observed in the LPS group when compared with mice in the NS or GGEC group ($P < 0.01$). Moreover, the upregulation in pro-inflammatory cytokine levels was found to be significantly higher for the animals that were treated with DEX or plant extract (GGE 200 or GGE 400 groups) compared to the animals of the LPS group ($P < 0.05$).

Effects of *Glycyrrhiza glabra* on lung histology

The histological changes in the lung tissues of normal or LPS-induced ALI mice models were examination after 24 h of NS or intratracheal LPS administration. The lung tissues harvested after 24 h in all the study groups were subjected to H and E staining. In the NS and GGEC

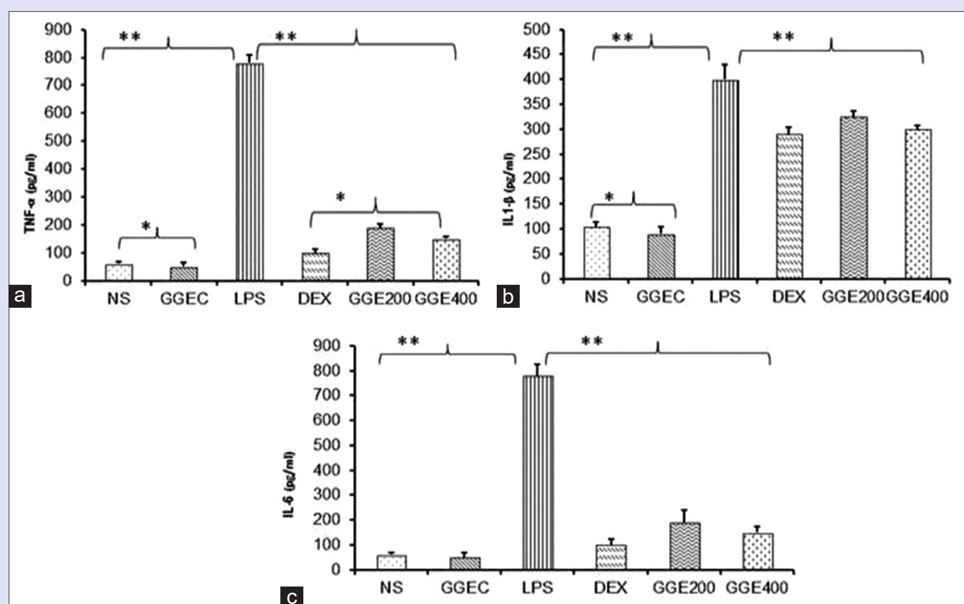

Figure 3: Effect *Glycyrrhiza glabra* extracts on pro-inflammatory cytokines in the lung tissues of lipopolysaccharide-induced acute lung injury mice were detected by quantifying the expression of messenger ribonucleic acid. Levels of pro-inflammatory cytokines such as tumor necrosis factor-α (a), interleukin-1β (b), and interleukin-6 (c) were determined after 24 h of treatment with dexamethasone or *Glycyrrhiza glabra* extract. The values shown in the figures are the average values ± standard deviation (n = 5). *P < 0.01 versus control group; **P < 0.05 versus LPG group. Where NS: Normal saline group; LPS: Lipopolysaccharide group; DEX: Dexamethasone group; GGEC: *Glycyrrhiza glabra* extract control group; GGE200: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 200mg/kg body weight; GGE400: *Glycyrrhiza glabra* extract group treated with plant extract at the dosage of 400 mg/kg body weight; TNF-α: Tumour necrosis factor-alpha; IL-1β: Interleukin 1 beta; IL 6: Interleukin 6

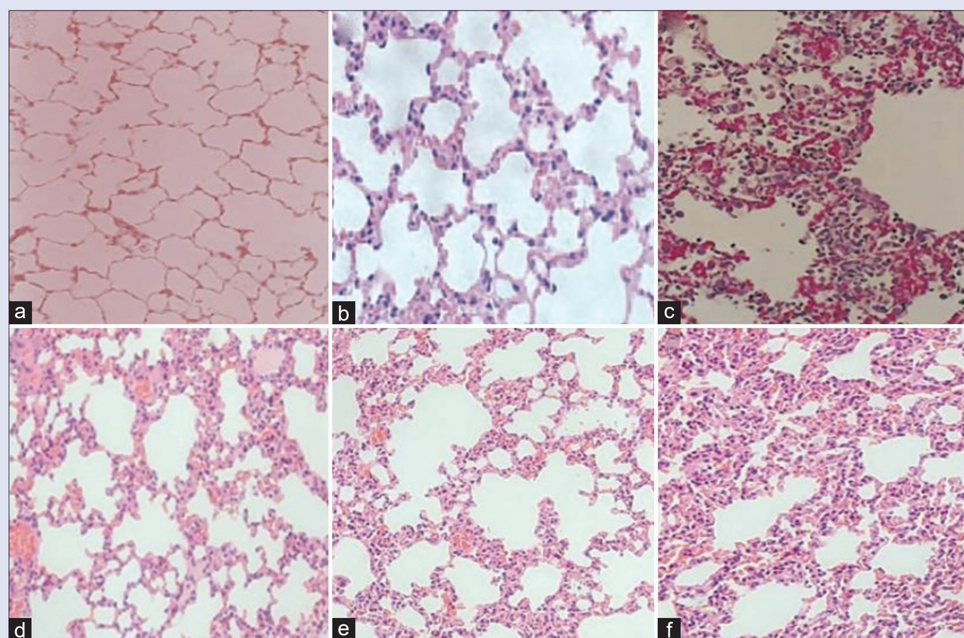

Figure 4: Histological analysis of the effect of *Glycyrrhiza glabra* extracts on lipopolysaccharide-induced acute lung injury mice after 24 h of lipopolysaccharide instillation. Lung tissue specimens were stained with H and E and observed under microscope at ×400. Where specimen A is for normal saline group, B is for *Glycyrrhiza glabra* extract control group, C is for lipopolysaccharide group, D is for dexamethasone group, E is for *Glycyrrhiza glabra* extract 200 group, and R is for *Glycyrrhiza glabra* extract 400 group

groups, no prominent histological change was witnessed in prepared lung specimens [Figures 4a]. Contrary to this, the lung specimens from the LPS group alone showed pathological changes which were evidenced by significant cell infiltration and cell clumping, dense deposits of fibrin along the alveolar septae and lumen of small vessels, lung edema, and alveolar hemorrhage. These changes in lung tissues were significantly reduced in LPS-induced ALI mice that were treated with DEX or plat extract (at 200 or 300 mg/kg body weight). These results imply that *G. glabra* exhibits an excellent potential to attenuate the LPS-induced lung inflammation, decrease the level of pathological

changes, and lower the lung tissues and alveolar hemorrhage during ALI.

DISCUSSION

Several reports are available describing the ethnopharmacological significance of *G. glabra* Linn. as an important herb in traditional medicine. The roots of *G. glabra* have been used as a prophylactic agent for the treatment of stomach and intestinal ulcers. Its use has also been recommended for dyspepsia as an anti-inflammatory agent during allergenic reactions.[14] *Glycyrrhiza* roots are useful for treating cough because of its demulcent and expectorant property.[15] These pharmacological activities of *G. glabra* are attributed to its diverse phytoconstituents with the major constituents being glabrin A and B, glycyrrhizin, glycyrrhizinic acid, and isoflavones.

The role of one of the isolated phytoconstituents of *G. glabra* to attenuate the lung injury in carrageenan-induced lung injury in mice models has been previously investigated.[16] This study is, to the best of our knowledge, the first to investigate the effect of ethanolic extract of the roots of *G. glabra* on LPS-induced ALI in mice. ALI mice models were established by administration of single dose of LPS (0.5 mg/kg body weight) intratracheally, and effects on lung injury were evaluated by measuring the several parameters characteristic of ALI such as cell migration into the BALF, lung edema index, ratio of lung wet/dry weight, protein content in the BALF, SOD activity, pro-inflammatory cytokines in the lung tissues, and mRNA expression levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β.

In the present study, we found that plant extract exerted potent anti-inflammatory effects on LPS-induced ALI mice. The total cell count including neutrophils and lymphocytes significantly increased after LPS challenge in the BALF of ALI mice. However, treatment with *G. glabra* significantly inhibited LPS-induced inflammatory cell infiltration in BALF of mice that were treated with DEX or plant extracts [Table 3].

Morphologically, the damage to lung tissues of ALI mice resulting from the LPS instillation is predominated by infiltration of inflammatory cells and pulmonary edema.[17] Edema in lung tissues is a typical symptom of inflammation both in local inflammation and in systemic inflammation.[18] Pulmonary edema was evaluated by measuring the lung wet/dry weight ratio. It was found that plant extract could decrease the LPS-induced lung wet/dry weight ratio in a dose-response manner. These results indicate that *G. glabra* has a protective effect on LPS-induced ALI [Figure 1a].

A number of proteins are secreted into the BALF during ALI. Proteins such as nuclear nonhistone protein are implicated as endogenous danger signaling molecule[19] as well as a late mediator of systemic inflammation in septic shock.[20,21] These protein molecules are considered to have a putative role in the pathogenesis of ALI.[22,23] LPS instillation induces the significant protein exudation into the BALF of ALI mice. *G. glabra* was found to be much effective in reducing the protein exudation into the BALF of ALI mice that were treated with DEX or plant extract [Figure 1b]. Treatment with DEX or plant extract was more or less equally effective in preventing protein exudation and lung tissue damage.

High levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, have a central role in the initiation and progression of the inflammatory cascade in LPS-induced ALI.[24,25] Cytokines such as IL-6, IL-8, IL-10, IL-1β, and TNF-α are secreted by alveolar macrophages and stimulate more chemotaxis and attract more neutrophils to injured lungs. The mRNA expression of TNF-α, IL-1β, and IL-6 increased significantly in LPS mice. The treatment of ALI mice with DEX or *G. glabra* plant extract after 24 h of LPS challenge significantly reduced the expression levels of these pro-inflammatory cytokines. This downregulation of these cytokines may have contributed to the reduced lung tissue damage in ALI mice that were treated with plant extract [Figure 3].

The histological results showed that *G. glabra* treatment substantially protected the animals from LPS-induced ALI such as lung tissue edema, hemorrhage, and alveolar structural and blood vessel damage [Figure 4]. The protective effects by plant extract could be contributed to its ability in preventing the inflammatory cell migration into the alveolar spaces.

CONCLUSIONS

The data showed that treatment with the ethanolic extract of *G. glabra* markedly prevented inflammatory cell migration and total cell count, attenuated the lung edema, reduced the total protein content and improved the SOD activity in the BALF, reduced the expression of pro-inflammatory cytokines in lung tissues, and significantly protected the lung injury in LPS-induced ALI mice. Histological studies demonstrated that *G. glabra* substantially inhibited the infiltration of neutrophils in the lung tissue of ALI mice. The results indicated that *G. glabra* had a protective effect on LPS-induced ALI in mice. Further, pharmacological evaluations of the pure phytoconstituents of *G. glabra* will be essential to elucidate the detailed mechanism of action of this extract, and these isolates might relate to a high potential for the prevention and treatment of ALI.

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Conflicts of interest

There are no conflicts of interest.

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