

Short Communication

Lipopolysaccharide-Induced Acute Lung Injury Attenuated by *Ocimum gratissimum* Aqueous Extract in Mice

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Abstract

Background: Acute lung injury (ALI) is characterized by diffuse inflammatory damage to the alveolar–capillary barrier, and effective pharmacological options remain limited. *Ocimum gratissimum* contains bioactive constituents with anti-inflammatory and antioxidant properties, but its effects in experimental ALI have not been sufficiently characterized. **Methods:** Male BALB/c mice were assigned to control, lipopolysaccharide (LPS), or LPS plus *O. gratissimum* aqueous extract (OGE) groups (n = 4 per group). OGE was administered at 2.4 mg/mL in drinking water for three days before the intratracheal LPS challenge. Since individual consumption was not recorded, 300 mg/kg/day is reported as an estimated exposure only. Mice were euthanized 30 h after LPS administration. Lung histopathology was assessed by hematoxylin and eosin staining and blinded semiquantitative injury scoring. Serum interleukin-6 (IL-6) levels were measured by enzyme-linked immunosorbent assay. **Results:** LPS induced inflammatory-cell infiltration, alveolar septal thickening, and focal hemorrhage, along with increased lung-injury score and serum IL-6 levels. OGE prophylactic pretreatment reduced these histopathological abnormalities, lowered the injury score, and suppressed the LPS-associated increase in circulating IL-6. These findings are preliminary because each group contained four animals and only systemic IL-6 and histology were assessed. **Conclusion:** In this preliminary prophylactic study, short-term oral pretreatment with OGE attenuated LPS-induced ALI in mice. Larger, dose-ranging studies using standardized extract, measured dosing, bronchoalveolar lavage endpoints, and post-injury administration are required before therapeutic conclusions can be drawn.

Keywords: acute lung injury; interleukin-6; lipopolysaccharides; *Ocimum gratissimum*; inflammation

1. Introduction

Acute lung injury (ALI) and its severe clinical manifestation, acute respiratory distress syndrome (ARDS), involve disruption of the alveolar-capillary barrier, diffuse inflammatory injury, impaired gas exchange, and hypoxemia. Despite advances in supportive care, ARDS continues to cause substantial morbidity and mortality, and specific pharmacological therapies remain limited [1,2,3].

Lipopolysaccharide (LPS) activates Toll-like receptor 4 (TLR4) and downstream inflammatory pathways, including nuclear factor-kappa B (NF-κB), resulting in neutrophil recruitment and release of cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α. The intratracheal LPS model therefore reproduces important features of inflammation-driven ALI and is widely used for preclinical evaluation of anti-inflammatory interventions [4,5].

Ocimum gratissimum L. is an aromatic medicinal plant used traditionally in several regions and contains polyphenols and other constituents with anti-inflammatory

and antioxidant properties [6]. Extracts or flavonoid-rich fractions of *O. gratissimum* have reduced inflammatory mediators, oxidative stress, and leukocyte accumulation in experimental systems [7,8,9,10]. However, evidence in acute pulmonary injury remains limited. This study examined whether an aqueous extract of *O. gratissimum* (OGE) could reduce LPS-induced lung damage and systemic IL-6 elevation in mice.

2. Materials and Methods

2.1 Animals and Experimental Design

Male BALB/c mice (18–20 g; approximately 7 weeks old) were obtained from the National Laboratory Animal Center, Taipei, Taiwan. Mice were maintained under a 12 h light/dark cycle with free access to standard chow and water and were acclimatized for 5–6 days. All procedures were approved by the Institutional Animal Care and Use Committee of China Medical University/China Medical University Hospital Animal Center (CMUIACUC-2023-015).



At 8 weeks of age, 12 mice were randomly allocated to three groups ($n = 4$ each): control, LPS, and LPS plus OGE. The OGE group received extract in drinking water at 2.4 mg/mL (0.24% w/v) for three consecutive days before LPS challenge. The nominal exposure of approximately 300 mg/kg/day was estimated from an assumed intake of 2.5 mL/mouse/day and a body weight of 20 g. Individual water consumption was not recorded; therefore, 2.4 mg/mL is the experimentally controlled dose and the mg/kg/day value should be interpreted only as an estimate. Control and LPS-only animals received regular drinking water.

Ocimum gratissimum L. was cultivated from plant material that met the qualification requirements established by Taiwan's Ministry of Agriculture, Executive Yuan. The propagation of its seedlings for agricultural use was officially authorized under Letter No. 0158875A, dated December 6, 1980. Fresh leaves were collected from a privately operated farm in Mingjian Township, Nantou County, Taiwan (120°42' E, 23°50' N). No voucher specimen was prepared or deposited for this study; therefore, no voucher specimen number or repository is available.

The *O. gratissimum* aqueous extract (OGE) was prepared using a standardized procedure, with its polyphenol content verified before experimental use. Briefly, freshly harvested leaves were thoroughly washed and homogenized using a Polytron homogenizer. The resulting homogenate was boiled, filtered, and centrifuged at 20,000 $\times$ g for 15 min at 4 °C. The collected supernatant was freeze-dried and stored at -70 °C until further use. Before each experiment, the lyophilized OGE was reconstituted to the required concentration. OGE was prepared as a food-grade botanical ingredient, and its chemical composition was characterized using liquid chromatography–mass spectrometry (LC–MS), as previously reported [11].

2.2 Induction of Acute Lung Injury

Mice were anesthetized with Zoletil (Virbac, Carros, France) at 25 mg/kg and xylazine (Rompun TS, Bayer, Leverkusen, Germany) at 7.5 mg/kg. ALI was induced by intratracheal instillation of 25 μ g LPS (Sigma-Aldrich L2630) from *Escherichia coli* O111:B4 in 20 μ L sterile saline. After visualization of the tracheal opening, the instillate was delivered centrally into the trachea rather than into a mainstem bronchus; animals were then maintained in a near-vertical position briefly to promote bilateral distribution. Control mice received saline alone. All mice were euthanized 30 h after LPS challenge for subsequent analyses.

2.3 Histopathology and IL-6 Measurement

At 30 h after the LPS challenge, mice were euthanized by gradual-fill inhalation of 100% carbon dioxide (CO₂) at a displacement rate of 30% of the chamber volume per minute. Death was confirmed by cessation of respiration followed by a secondary physical method (cervical dislocation) before blood and lung tissues were collected. Lung tis-

sues were fixed in 4% paraformaldehyde for 48 h, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Histological assessment was performed independently by two investigators blinded to the treatment-group assignments. Four features— inflammatory-cell infiltration, interstitial edema, alveolar-wall/septal thickening, and focal hemorrhage—were evaluated across representative non-overlapping fields. An overall semiquantitative grade was assigned as follows: 0, no detectable injury; 1, minimal focal change (<10% of the field); 2, mild involvement (10–25%); 3, moderate involvement (26–50%); 4, severe involvement (51–75%); and 5, very severe/diffuse involvement (>75%).

Serum IL-6 concentrations were determined using an enzyme-linked immunosorbent assay (Sigma-Aldrich RAB0308) according to the manufacturer's instructions. Samples were analyzed in triplicate against a standard curve, and results were expressed as pg/mL.

2.4 Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean, with each mouse as the experimental unit. Lung-injury scores (0–5) were treated as ordinal data and are presented as individual values and median (interquartile range). The three groups were compared using an exact permutation-based Kruskal–Wallis test. Pairwise comparisons were performed using two-sided exact permutation tests on ranks. Serum IL-6 concentrations were performed by one-way analysis of variance followed by Tukey's post hoc test. A two-sided p value < 0.05 was considered statistically significant.

3. Results

3.1 OGE Reduced LPS-Induced Lung Injury

Lungs from control mice showed preserved alveolar architecture with minimal inflammatory infiltration (Fig. 1A). In contrast, LPS challenge caused extensive inflammatory-cell accumulation, alveolar septal thickening, and focal hemorrhagic changes (Fig. 1B). Pretreatment with OGE visibly attenuated these pathological findings (Fig. 1C). Consistent with the microscopic observations, the lung-injury score increased significantly after LPS administration and was significantly reduced in the OGE-pretreated group (Fig. 1D).

3.2 OGE Suppressed the Increase in Serum IL-6

LPS challenge markedly increased serum IL-6 compared with the control group. OGE pretreatment significantly reduced the LPS-associated elevation in circulating IL-6 (Fig. 1E), supporting an anti-inflammatory effect that accompanied the improvement in lung histopathology.

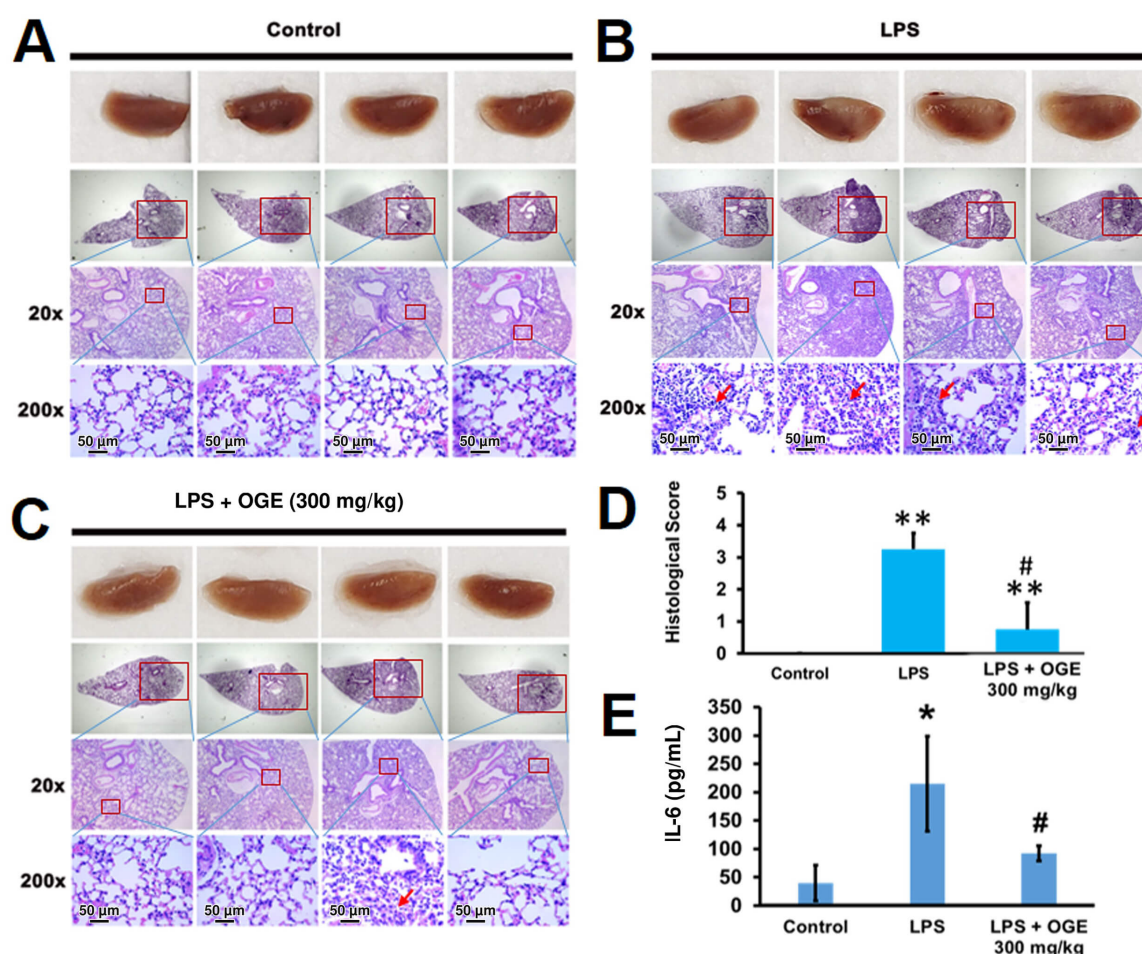


Fig. 1. Effects of *O. gratissimum* aqueous extract (OGE) on lipopolysaccharide (LPS)-induced acute lung injury in BALB/c mice. (A) Control group: gross lungs and representative hematoxylin and eosin (H&E) sections at 20× and 200× magnification, scale bar: 50 μm. (B) LPS group: gross lungs and H&E sections showing inflammatory foci, septal thickening, and focal hemorrhagic injury (red arrows). (C) LPS + OGE group: gross lungs and H&E sections after prophylactic OGE exposure (2.4 mg/mL in drinking water; nominal estimate 300 mg/kg/day), and focal hemorrhagic injury (red arrows). (D) Semiquantitative lung-injury score. (E) Serum interleukin-6 (IL-6) concentration measured by ELISA. Bars show mean ± SEM; n = 4 biologically independent mice per group. * $p < 0.05$ and ** $p < 0.01$ versus control group; # $p < 0.05$ versus LPS group.

4. Discussion

The present study provides preliminary *in vivo* evidence that prophylactic oral OGE exposure attenuates LPS-induced ALI. A three-day exposure to OGE at 2.4 mg/mL in drinking water reduced inflammatory-cell infiltration, alveolar-wall injury, and hemorrhagic changes and lowered serum IL-6. Because water intake was not individually measured, the nominal 300 mg/kg/day value cannot be regarded as a precise administered dose.

IL-6 is an important mediator of inflammatory amplification and neutrophil recruitment in ALI. Excessive cytokine signaling contributes to endothelial and epithelial injury, increased vascular permeability, and deterioration of gas exchange. The reduction in serum IL-6 observed here is consistent with previous studies in which *O. gratissimum* preparations suppressed LPS-induced cytokine production

in airway epithelial cells, macrophages, and mouse inflammatory models [7,8,12]. OGE has also been reported to reduce oxidative stress and myeloperoxidase activity, mechanisms that may be relevant because neutrophil-derived oxidants contribute substantially to pulmonary tissue injury [9,13].

Other plant-derived preparations—including polyphenol-, flavonoid-, and terpenoid-rich extracts—have also reduced cytokine production, neutrophilic inflammation, oxidative stress, or edema in experimental LPS-induced ALI [14,15,16]. These reports support inflammation and oxidative stress as plausible botanical targets but also underscore the need for direct comparison using standardized preparations and common endpoints. The present work is distinct in evaluating an aqueous *O. gratissimum* preparation, but it does not establish compara-

tive superiority or an optimal dose. OGE was administered before LPS; therefore, the experiment demonstrates a prophylactic association rather than treatment of established ALI. Future studies should include dose-response and post-injury treatment designs, oral gavage or measured individual consumption, bronchoalveolar-lavage analyses, pulmonary permeability measurements, and evaluation of TLR4/NF- κ B, NOD-like receptor protein 3 (NLRP3), oxidative-stress, and neutrophil-associated pathways. Chemical standardization of the extract will also be necessary for reproducibility and pharmaceutical development.

5. Limitations

This exploratory study has several limitations. First, the sample size was small ($n = 4/\text{group}$), no a priori power calculation was performed, and the findings require confirmation in a larger independent cohort. Second, administration in group drinking water did not provide individual consumption or exact mg/kg exposure; the concentration of 2.4 mg/mL is therefore more reliable than the nominal dose estimate. Third, the extract batch was not chemically standardized and extraction yield and marker-compound concentrations were not available, limiting reproducibility and cross-study comparison. Fourth, an OGE-only control was not included, so an effect of OGE on baseline lung histology or IL-6 cannot be excluded. Fifth, the endpoints were limited to histology and serum IL-6; bronchoalveolar-lavage cellularity, local cytokines, protein leakage, lung wet/dry ratio, and mechanistic markers were not measured. Sixth, only one prophylactic concentration and one post-challenge time point were tested, precluding conclusions about dose response, durability, or efficacy after injury onset. Finally, the semiquantitative histological assessment may be observer-dependent despite blinding. Collectively, these limitations restrict the conclusions to a preliminary prophylactic signal and do not establish clinical or therapeutic efficacy.

6. Conclusion

OGE pretreatment reduced histological lung injury and serum IL-6 in an LPS-induced mouse model. These preliminary findings justify confirmatory studies using a standardized extract, precise dosing, additional pulmonary endpoints, larger cohorts, an OGE-only control, and post-injury treatment.

Availability of Data and Materials

The datasets generated and analyzed during the study are available from the corresponding author upon reasonable request.

Author Contributions

CCH, WTY, JYL, YLC, and JCY contributed to study conception and design. CCH, WTY, and YLC performed the animal experiments and sample analyses. JYL and JCY interpreted the data and prepared the manuscript. All authors reviewed and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal procedures were approved by the Institutional Animal Care and Use Committee of China Medical University/China Medical University Hospital Animal Center (CMUIACUC-2023-015) and were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by Taiwan's Council of Agriculture (now the Ministry of Agriculture).

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Conflicts of Interest

Jer-Yuh Liu is a member of the Society of Adaptive Science in Taiwan. The authors declare that this affiliation does not constitute a conflict of interest. The authors declare no other conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/Pharmazie55633>.

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