

Original Research Article

Inula racemosa ameliorates acute lung injury through integrated modulation of innate immune signalling, inflammation activation and redox homeostasis

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ABSTRACT

Background: Chronic respiratory diseases are associated with persistent inflammation and dysregulated immune responses. Although corticosteroids are widely used, prolonged therapy may cause immunosuppression and increase susceptibility to secondary infections. *Inula racemosa* (*IR*) Hook. F. (*Pushkarmool*), an Ayurvedic medicinal plant traditionally used for respiratory and inflammatory disorders, contains bioactive sesquiterpene lactones, particularly alantolactone and isoalantolactone, with reported anti-inflammatory and immunomodulatory activities.

Methods: The study employed human lung epithelial NCI-H460 cells and an lipopolysaccharide (LPS) induced pulmonary inflammation model in BALB/c mice. Cytotoxicity and inflammatory responses were evaluated following treatment with *IR* extract. Gene expression of key inflammatory and signalling markers was analysed by RT-qPCR, while protein levels of inflammatory cytokines were quantified using ELISA. Cytokine profiling, BALF cellular analysis, and lung histopathological examination were performed to assess the anti-inflammatory effects of the extract. The expression of genes associated with the TLR4/MyD88/NF- κ B, MAPK, NLRP3 inflammasome, and Nrf2/HO-1 pathways was investigated to elucidate the potential molecular mechanisms underlying observed effects.

Results: *IR* extract attenuated LPS-induced inflammatory responses in NCI-H460 cells, with reduced production of TNF- α , IL-6, IL-1 β , IL-8, IL-18, IL-4, and CCL2. In LPS-challenged BALB/c mice, treatment reduced inflammatory cell infiltration, including neutrophils, in BAL fluid and improved inflammation-associated histopathological alterations in lung tissue.

Conclusions: The findings demonstrate that whole *IR* extract possesses significant anti-inflammatory activity in cellular and experimental pulmonary inflammation models. Its effects may involve modulation of multiple inflammatory and antioxidant signalling pathways. These findings provide experimental support for the traditional use of *IR* in respiratory inflammatory conditions and warrant further mechanistic and translational investigations.

Keywords: *Inula racemosa*, Cytokines, Lung inflammation, LPS, COVID-19

INTRODUCTION

A properly functioning immune system is essential for preserving physiological homeostasis and enabling effective tissue repair and recovery. Individuals with compromised immune function are at an increased risk of

developing bacterial, viral, fungal, and parasitic infections as well as chronic inflammatory disorders such as inflammatory bowel disease, rheumatoid arthritis, and cardiovascular diseases.¹⁻⁴ The importance of an intact immune response became particularly evident during the COVID-19 pandemic, where immunocompromised

individuals exhibited a higher incidence of severe complications, including acute respiratory distress syndrome (ARDS), prolonged recovery, multiple organ dysfunction, and increased mortality.⁵⁻¹⁰ The clinical use of corticosteroids for controlling excessive inflammation during COVID-19 demonstrated both significant therapeutic benefits and important limitations. Although corticosteroids effectively suppressed hyper inflammatory responses, administration of higher doses was associated with an increased risk of mortality among patients with COVID-19-associated hypoxia.^{11,12} Furthermore, prolonged or high-dose corticosteroid therapy can impair immune competence, predisposing patients to secondary infections and other serious adverse effects.

In several Asian countries, particularly India and China, traditional systems of medicine, including Ayurveda, have been extensively utilized for centuries in the prevention and management of inflammatory and infectious disorders.¹³ Among the medicinal plants described in Ayurvedic literature, *Inula racemosa* (*IR*) Hook. f., commonly known as *Pushkarmool*, is an important therapeutic herb belonging to the family *Asteraceae*.²⁶ The plant is predominantly distributed in the temperate and alpine regions of the western Himalayas, especially in Kashmir and Himachal Pradesh.^{8,12,14,25} The roots of *IR* have been traditionally employed as an expectorant and are also used as a tonic in ethnoveterinary medicine. The rhizome possesses a broad spectrum of pharmacological activities, including antiseptic, antibacterial, antifungal, anti-inflammatory, analgesic, and mild diuretic properties.²⁴ Traditionally, it has been used for the treatment of ischemic heart disease, angina pectoris, infectious fevers, bronchial asthma, hiccups, dyspepsia, and flatulence.¹⁵ Owing to its antimicrobial activity, root paste is also applied topically for the management of wounds and ulcers. In addition, the roots are traditionally prescribed for liver disorders, rejuvenation therapy, anti-aging applications, and the management of rheumatic conditions. Due to the abundance of bioactive substances found in *IR* including sesquiterpene lactones, alantolactone, isovalantolactone, and other phytochemicals, it has been studied for its potential as a hepatoprotectant.¹⁴ Studies on *IR* have shown that it has antioxidant and anti-inflammatory properties. These properties are essential for scavenging harmful free radicals and reducing oxidative stress and inflammation that contributes to liver damage. Alantolactone and isovalantolactone are two important sesquiterpene lactones eudesmanolides with a 4:6 ratio in the essential oil of *IR*.¹⁶ Recent pharmacological investigations have demonstrated that alantolactone possesses potent anti-inflammatory, antioxidant, immunomodulatory, and cytoprotective properties. Mechanistic studies conducted in various experimental systems have suggested that alantolactone can suppress the activation of the NF- κ B signaling pathway, inhibit phosphorylation of MAPKs (ERK, JNK, and p38), reduce the production of pro-inflammatory mediators such as

TNF- α , IL-1 β , IL-6, COX-2, and iNOS, and attenuate intracellular reactive oxygen species (ROS) generation.¹⁷ Furthermore, alantolactone has been reported to activate the Nrf2/HO-1 antioxidant pathway, thereby enhancing endogenous antioxidant defences and protecting cells against oxidative injury. Emerging evidence also indicates that alantolactone may inhibit activation of the NLRP3 inflammasome, leading to reduced maturation and secretion of IL-1 β and IL-18.¹⁸ Similarly, isovalantolactone has been shown to exhibit anti-inflammatory and antioxidant effects through modulation of multiple signaling cascades, including PI3K/Akt, STAT3, and NF- κ B, while simultaneously reducing oxidative stress-induced cellular damage and apoptosis. Collectively, these molecular mechanisms suggest that *IR* may exert therapeutic effects by simultaneously regulating inflammatory signaling, oxidative stress, immune responses, and cell survival pathways.¹⁹⁻²² Despite these encouraging findings, the available literature is primarily limited to isolated compound-based studies, cancer cell models, macrophage-based assays, or investigations in unrelated disease models. Very limited information is available regarding the molecular mechanism of the whole extract of *IR* in chronic inflammatory lung diseases. More importantly, no comprehensive study has systematically evaluated the molecular mechanism of *IR* in human lung epithelial cell lines (NCI-H460), with validation in BALB/c experimental models, to delineate the involvement of the TLR4/MyD88/NF- κ B, MAPK, NLRP3 inflammasome, and Nrf2/HO1 signalling pathways. Therefore, the present investigation is designed to provide the first comprehensive mechanistic evaluation of *IR* in this experimental setting by integrating *in vitro*, *in vivo*, histopathological, inflammatory, and molecular analyses. Elucidation of these signaling pathways will not only establish the scientific basis for the traditional use of *IR* but will also facilitate the development of evidence-based phytotherapeutics for chronic inflammatory respiratory disorders.

METHODS

Experimental animals

All the experiments were performed as per the guidelines of the Institutional Animal Ethics Committee (NIA/IAEC/2025/94) of the National Institute of Ayurveda (NIA), Jaipur, India, and all the experiments were carried out following the CPCSEA guidelines (Govt. of India).

The 8-12 weeks old female BALB/c mice (18-25 g) were procured and housed in individually ventilated cages with controlled air flow as per the institute's experimental animal guidelines. Access to normal chow and water was provided to them *ad libitum*. The animals were acclimatized for one week in the above conditions prior to initiating the experiments.

Cell culture

NCI-H460 and A549 cell line (human lung carcinoma cells) was procured from the National Centre of Cell Science, Pune, India, and was routinely maintained in RPMI-1640 and DMEM medium containing 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen, USA). Cells were maintained in a humidified incubator with 5% CO₂ at 37 °C. Aqueous extract of the *IR* was prepared in the RRDR lab facility at National Institute of Ayurveda, Jaipur Rajasthan.

In vitro anti-inflammatory activity of IR and cell viability assay

NCI-H460/A549 cells were seeded at a density of 1×10^4 cells/well in RPMI/ DMEM medium with 10% FBS in a 96-well plate and incubated at 37°C in a CO₂ incubator for 24 h followed by treatment with WS at 10, 3, 1, 0.3, 0.1, 0.003 mg/mL. LPS (1 µg/mL) was added 30 minutes after the treatment with *IR* and the plate was incubated further in the CO₂ incubator for 24 h. Dexamethasone was used as a positive control (10 µM). The cell culture supernatants were used to measure the levels of cytokines by ELISA as per the manufacturer's instructions. For gene expression analysis, cells were grown at a density of 4×10^5 cells/well in a 6 well plate. After 24 h, cells were treated with various concentrations of *IR* (10, 3, 1 mg/mL) in RPMI medium with 10% FBS followed by stimulation with LPS (1 µg/mL), and the plate was incubated in the CO₂ incubator for another 24 h. Cells

were lysed using the Trizol reagent (Thermo Fisher Scientific) and total RNA was isolated as per the manufacturer's protocol. To determine the cytotoxicity of *IR*, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. Cells treated as above in the inflammatory profiling setup were treated with MTT (100 µL of 0.5 mg/mL MTT stock in PBS) in an independent experiment, and the plate was incubated for 4 h at 37°C in a CO₂ incubator. The MTT mixture was aspirated and 100 µL of DMSO was added to each well to dissolve the formazan particles. The plate was shaken for 30 min on a plate shaker at 37°C to ensure complete dissolution of formazan crystals and read at 570 nm to measure the probable cytotoxicity of test substances if any.

Cell viability (%) = $\frac{\text{Total viable cells (unstained)}}{\text{Total cells (unstained) + blue stained}} \times 100$.

Quantitative real-time PCR (qPCR)

Total RNA was isolated from cells treated with different conc. of *IR* RNA was used for the cDNA synthesis. cDNA was synthesized with an iScript cDNA synthesis kit (Biorad) as per the manufacturer's protocol. The gene-specific primers (Table 1) were designed using NCBI primer blast tool, and qPCR was performed on the real-time PCR systems (Biorad). Cycling program used was: 7 min at 95 °C and then 40 cycles with 10 s at 95 °C and 20 s at 60 °C. The specificity of the amplicons was analyzed by a thermal dissociation curve. Data were normalized against the housekeeping gene, GAPDH.

Table 1: Gene-specific primers.

Gene	Forward primer	Reverse primer
TNF-α	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGCTACTC
IL-6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
IL-1beta	CCACAGACCTTCCAGGAGAATG	GTGCAGTTCAGTGATCGTACAGG
IL-8	GAGAGTGATTGAGAGTGGACCAC	CACAACCCTCTGCACCCAGTTT
IL-18	GATAGCCAGCCTAGAGGTATGG	CCTTGATGTTATCAGGAGGATTCA
IL-4	CCGTAACAGACATCTTTGCTGCC	GAGTGTCTTCTCATGGTGGCT
CCL2	AGAATCACCAGCAGCAAGTGTC	TCCTGAACCCACTTCTGCTTGG
GAPDH	ACCATCTTCCAGGAGCGAGA	CAAATGAGCCCCAGCCTTCT

LPS-induced neutrophilia in BALB/c mice

Healthy mice were randomized based on their body weights and divided into five different groups: (1) Control (2) LPS (25µg/mice) (3) Dexamethasone (1 mg/kg) (4) *IR* 100 mg/kg. All the groups except the control group were injected intranasally with LPS in 200 µl PBS (pH 7.4). Mice in groups 4 were treated orally with (*IR*) mixed in saline and challenged with LPS after 30 min of drug treatment. Mice in control group were challenged with saline only and animals were euthanized 4 days after the (*IR*) or saline challenge. Bronchoalveolar lavage (BAL) fluid was collected from the lungs of mice in ice-cold Hank's balanced salt solution (HBSS) or

1×Phosphate buffer saline (PBS) and processed for the quantification of total and differential leukocyte counts.

BAL fluid analysis

Smears were prepared using 50-100 µl of the freshly isolated BAL fluid on a frosted glass slide. Slides were dried and fixed with 100% ethanol, further stained with Giemsa stain, and cell counts were taken by scientists who were blinded to the treatment groups.

Histological evaluation

Healthy mice were randomized based on their body weights and divided into seven different groups: (1)

Control (2) LPS (25 µg/mice) (3) Dexamethasone (1 mg/kg) (4) IR, (100 mg/kg). All the groups except the control group were injected intranasally with LPS in 200 µl PBS (pH 7.4). Mice were treated orally with IR, mixed in saline after 30 min of LPS challenge. Mice in the control group were challenged with saline only and animals were euthanized 4 days after the IR, or saline challenge.

Histological evaluation was done using the lung tissues of different groups of mice. Mice were sacrificed 4 days after the LPS administration and lower lobes from the left lung were dissected and fixed in 10% formalin, dehydrated with ethanol followed by embedding in paraffin and cutting into 5 µm sections. After deparaffinization, the tissues were stained with Hematoxylin and Eosin (H and E) as reported previously. Pathological changes among different groups were evaluated under a light microscope and lung injury scores were assessed using a semi-quantitative method.

Statistical analysis

Each experiment was performed three or more times independently with different cell passages. For animal work, each group consisted of n=5 mice. Statistical analysis was performed on GraphPad PRISM 7 software (GraphPad, La Jolla, CA, USA) using one-way ANOVA. The p<0.05 were considered statistically significant. The results were calculated as mean±SD, and data are shown as mean±SD.

RESULTS

Cytotoxicity assessment of IR in human lung cell lines

The cytotoxicity of IR extract was evaluated in A549 and NCI-H460 human lung epithelial cell lines using a cell

viability assay following LPS stimulation (1 µg/mL). LPS treatment alone resulted in a modest but significant increase in cell viability compared with the untreated control in both cell lines.

Dexamethasone, used as the positive control, significantly restored and enhanced cell viability. In A549 cells, treatment with IR at concentrations of 10, 3, 1, and 0.3 mg/mL significantly increased cell viability compared with the LPS-treated group, with the 10 mg/mL concentration producing the greatest protective effect.

Cell viability remained above 100% at these concentrations, indicating the absence of cytotoxicity and suggesting a cytoprotective effect. Lower concentrations (0.1 and 0.03 mg/mL) maintained cell viability close to that of the untreated control, with no evidence of marked cytotoxicity.

Similarly, in NCI-H460 cells, IR treatment did not produce any concentration-dependent cytotoxic effect. Cell viability remained approximately 95-110% across all tested concentrations. The 10 mg/mL concentration significantly improved cell viability, whereas the remaining concentrations-maintained viability comparable to the untreated control. These findings indicate that the extract is well tolerated by NCI-H460 cells within the tested concentration range.

Overall, the results demonstrate that IR is non-cytotoxic to both A549 and NCI-H460 human lung cell lines at concentrations ranging from 0.03 to 10 mg/mL. The observed maintenance or enhancement of cell viability further suggests that the extract possesses cytoprotective properties, supporting its suitability for subsequent in vitro anti-inflammatory and mechanistic investigations (Figure 1).

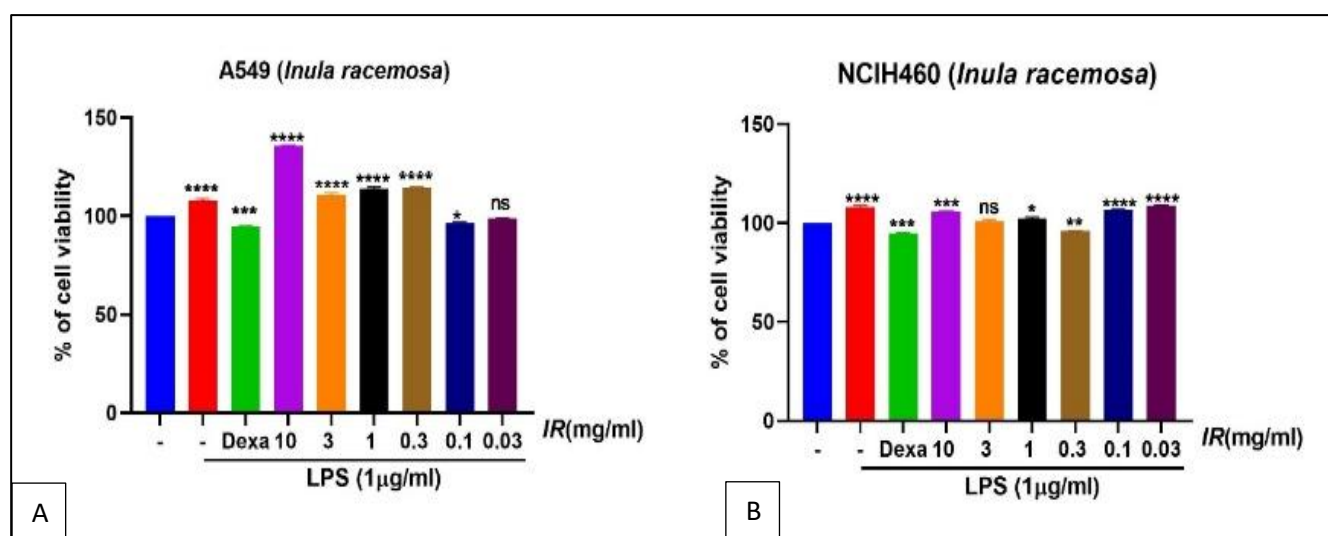


Figure 1 (A and B): Cytotoxicity of IR on NCI-H460 and A549 cells, expressed as percentage cell viability.

*In A549 and NCIH40 cells, treatment with IR at concentrations of 10, 3, 1, and 0.3 mg/mL significantly increased cell viability compared with the LPS-treated group, with the 10 mg/mL concentration producing the greatest protective effect.

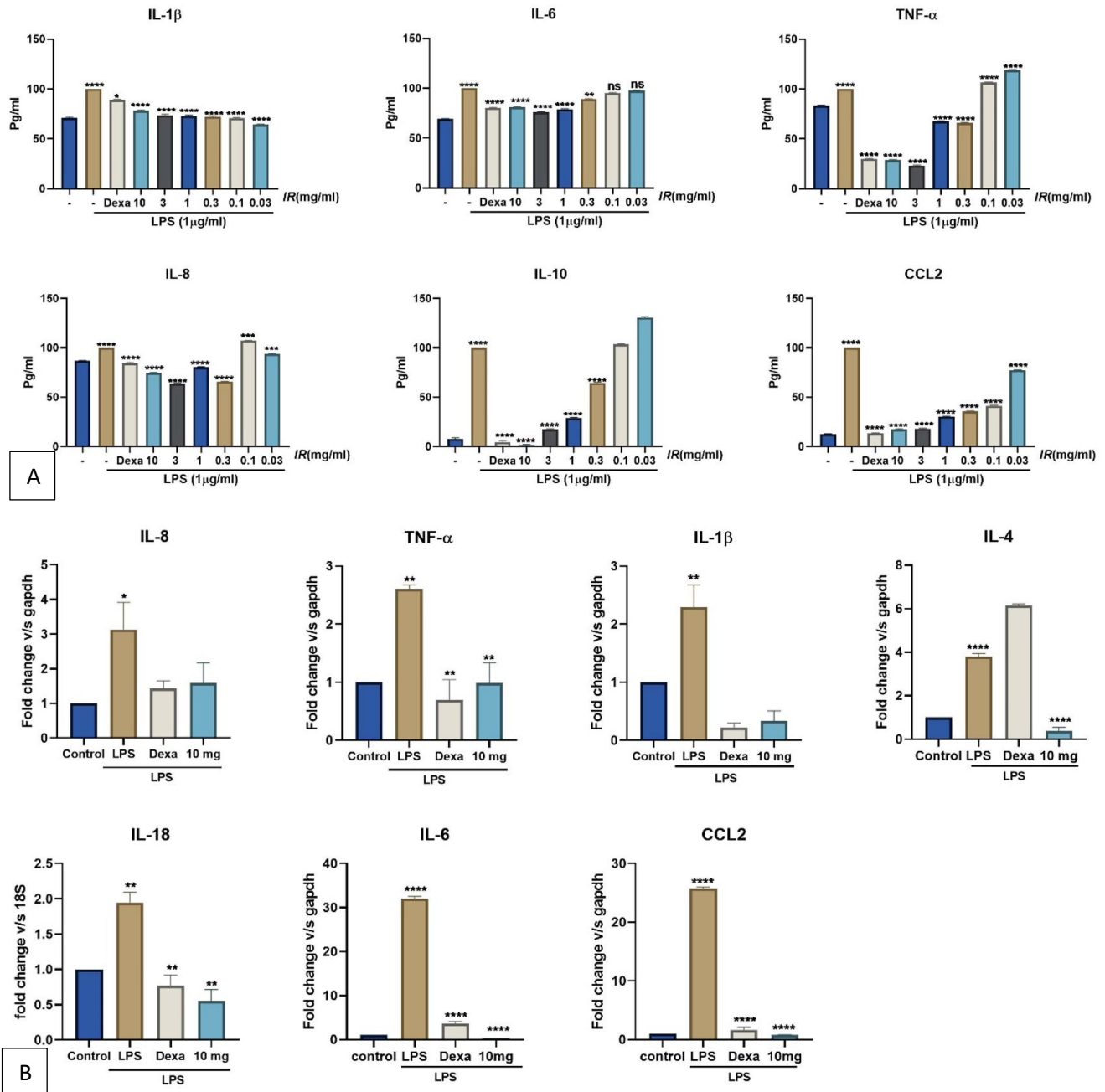


Figure 2 (A and B): IR suppresses the levels of LPS induced increase in the mRNA expression of inflammatory cytokines and chemokine(s) *in vitro* in NCI-H460 cell line. A) Cell culture supernatants were collected and analysed for the levels of inflammatory cytokines by ELISA. a) IL1β, b) IL-6, c) TNF-α, d) IL-8, e) IL-10, and f) CCL-2. B) Cells were lysed and expression levels of various inflammatory markers were analysed by genes by semi-quantitative real-time PCR analysis. a) IL-8 b) TNF-α, c) IL-1β, d) IL4, e) IL-18, f) IL-6, and g) CCL2.

*Cells were pre-treated with IR for 30 min, followed by LPS challenge for 4 h. Dexamethasone was used as a positive control. Data is presented as the mean±SEM (n=3). **, ###p<0.001 and *p<0.05 by one-way ANOVA.

IR extract reduces the levels of LPS-induced increase in the levels of pro-inflammatory cytokines in NCI-H460 cells

To recapitulate the anti-inflammatory properties of IR *in vitro*, cell-based assays were conducted using a lung carcinoma cell line, NCI-H460.

Treatment with LPS led to a significant increase in the secretion of a) IL1β, b) IL-6, c) TNF-α, d) IL-8, e) IL-10, and f) CCL-2 while pre-treatment of the cells with different concentrations of IR (10-0.03 mg/mL) showed a dose-dependent inhibition of pro-inflammatory cytokines by ELISA and 10 mg/mL by RTPCR and (Figure 2 A and B) suggesting the anti-inflammatory properties of IR.

Treatment with dexamethasone resulted in a decrease in the levels of pro-inflammatory cytokines.

IR attenuates the LPS-induced increase in the neutrophil counts in the BAL fluid of BALB/c mice

To explore the beneficial effects of *IR* on endotoxin-induced airway inflammation *in vivo*, BALB/c mice were treated with dose of *IR* (10 mg/kg,) for 30 minutes, followed by the LPS challenge by the intranasal route, and BAL fluid was collected after 4 h. Total number of leukocytes and differential neutrophil counts were analyzed in the BALF (Figure 3 A and B). LPS challenge in mice resulted in a significant increase in the leukocytes influx compared to the unchallenged mice. Treatment with different doses of *IR* attenuated the number of total leukocytes as well as neutrophil counts in a dose-dependent manner. Dexamethasone was used as a positive control and led to reduction in neutrophil counts.

IR ameliorates the LPS-induced lung damage in BALB/c mice in a 4 day study

Intranasal administration of LPS has been reported to cause lung tissue damage in mice. Histological analysis of the lung tissues was carried out to assess the LPS-induced lung damage in a 4 day LPS challenge model using BALB/c mice (Figure 4). Lungs of mice in the control group, showed normal pulmonary architecture whereas the lungs of mice challenged with LPS showed notable inflammatory cell infiltration and alveolar haemorrhage. Treatment with *IR* significantly attenuated such pathological changes induced by LPS. The positive control dexamethasone also improved the histopathological conditions in LPS-induced mice. For semi-quantitative evaluation, the changes were also evaluated by calculating a lung injury score. Our data suggests that pre-treatment *IR* alleviates LPS-induced pathological changes in the mouse model.

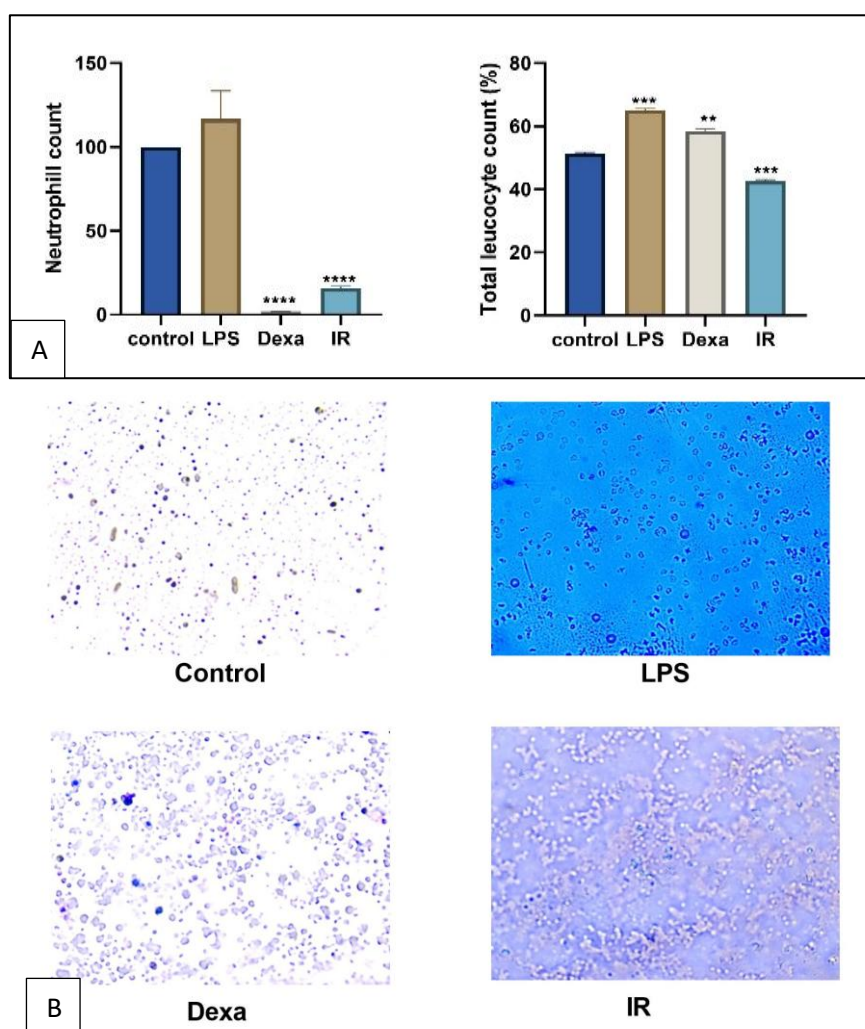


Figure 3 (A and B): *IR* reduced the infiltration of LPS induced increase in the infiltration of total leukocytes and neutrophils in the BAL fluid of BALB/c mice.

*Mice were acclimatized, randomized, and prophylactically treated with *IR* for 30 min followed LPS challenge for 4 h. Mice were anesthetized after 4 h and BAL fluid was isolated and analysed for a) total neutrophil counts b) total leukocyte counts c) microscopic studies showing the inhibition of neutrophil counts by *IR*.

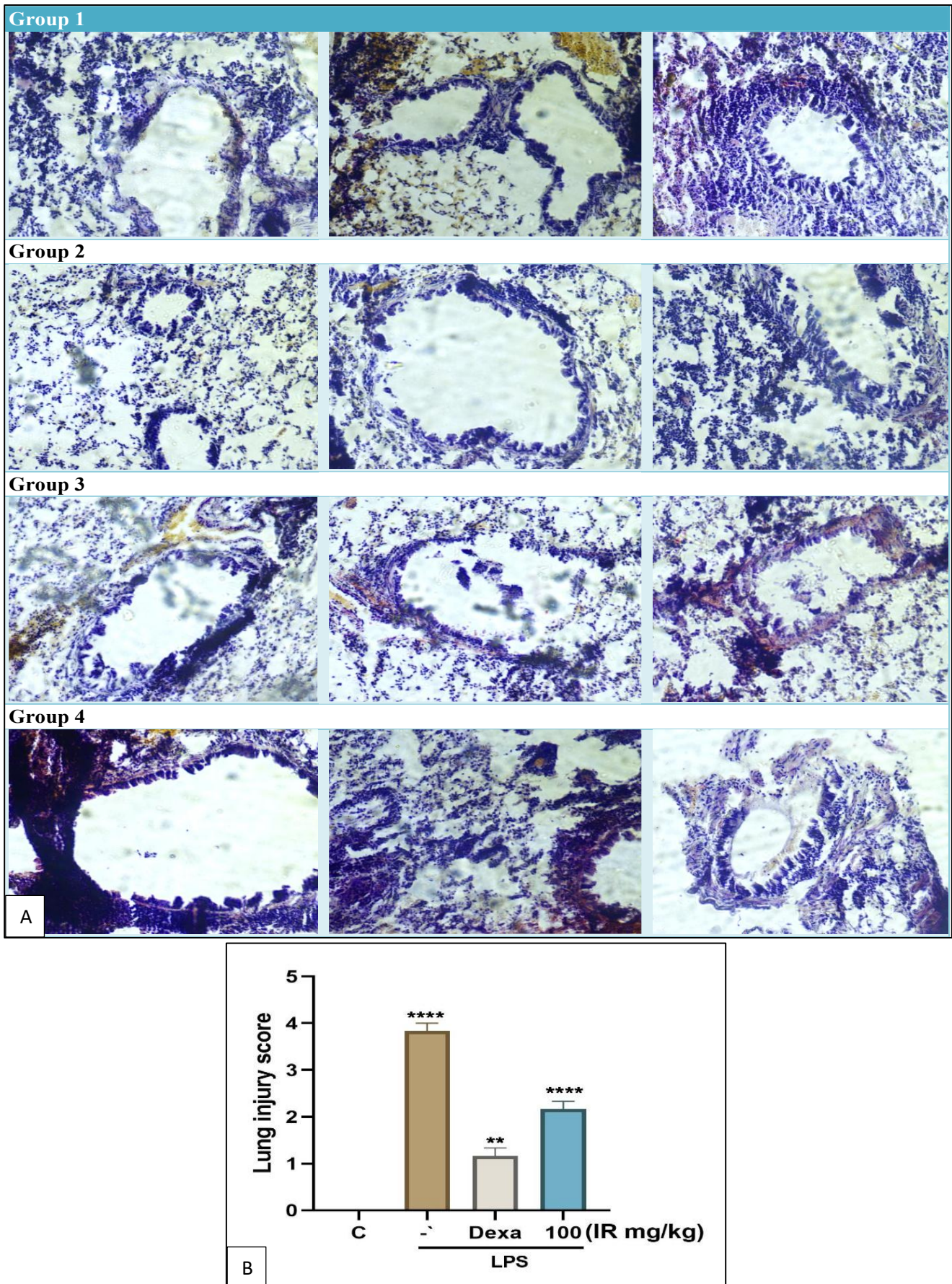


Figure 4 (A and B): IR ameliorates the LPS-induced lung damage in BALB/c mice in a 4-day study.

Effect of *IR* on LPS-induced lung injury was studied in a 4 day LPS challenge model using BALB/c mice. Lungs from each experimental group were obtained 4 days after LPS challenge as described in the methodology section. Tissue sections were stained with H and E and sections from each group are represented as control (group 1), LPS (1 mg/kg, group 2), Dexamethasone (1 mg/kg, group 3), LPS+*IR* (100 mg/kg, group 4), H and E stained lung sections are shown in original magnification×200. b) Assessment of lung injury was done based on the scores obtained as a mark of lung damage (bar diagram). Scores such as 0, 1, 2, 3, and 4 represented no damage, mild damage, moderate damage, severe damage, and very severe damage, respectively. Data have been expressed as means±SEM.

DISCUSSION

The present study provides compelling evidence that *IR* exerts significant anti-inflammatory and pulmonary protective effects against LPS-induced lung inflammation, as demonstrated through complementary *in vitro* and *in vivo* experimental models. The findings demonstrate that *IR* exhibits a favourable safety profile in human lung epithelial cells while suppressing inflammatory cytokine production, reducing neutrophilic infiltration, and preserving pulmonary architecture following LPS challenge. These results support its traditional use in respiratory disorders and highlight its potential as a multi-target therapeutic agent for inflammatory lung diseases.^{14-16,26,27}

Assessment of cytotoxicity represents a critical prerequisite for evaluating the biological activity of phytopharmaceuticals. In the present study, *IR* exhibited negligible cytotoxicity toward NCI-H460 human lung epithelial cells over the investigated concentration range, indicating that the observed anti-inflammatory effects were independent of nonspecific cellular toxicity. Preservation of cellular viability suggests that the extract selectively modulates inflammatory responses without compromising epithelial cell integrity, thereby providing a reliable foundation for mechanistic interpretation of its pharmacological effects.^{5,14,16,28} LPS is a well-established inducer of acute pulmonary inflammation through activation of the TLR4/MyD88 signaling axis, which subsequently stimulates the NF-κB and MAPK (ERK, JNK, and p38) pathways, leading to transcriptional activation of multiple pro-inflammatory genes.^{1,2,17-22,29,30} Consistent with this mechanism, LPS markedly increased the production of TNF-α, IL-6, IL-1β, IL-8, IL-18, and CCL2 in NCI-H460 cells, reflecting activation of a robust inflammatory response.

Treatment with *IR* significantly reduced TNF-α, IL-6, IL-1β, IL-8, IL-18, and CCL2 while modulating IL-4, indicating broad-spectrum immunomodulatory activity. TNF-α, IL-1β, and IL-6 are key mediators of inflammatory responses.^{1,2,19} Whereas IL-8, IL-18, and CCL2 regulate neutrophil, monocyte, and macrophage

recruitment during inflammation.^{2,7,8,29,30} Their simultaneous suppression suggests inhibition of upstream inflammatory signalling pathways controlling cytokine production and immune cell recruitment.

The anti-inflammatory activity observed *in vitro* was further validated in the LPS-induced BALB/c mouse model of acute lung injury. Neutrophilic infiltration into the alveolar compartment is a defining pathological hallmark of acute lung inflammation, contributing to tissue injury through excessive release of reactive oxygen species, proteolytic enzymes, myeloperoxidase, and pro-inflammatory mediators.^{1,2,11,31} In agreement with the cytokine findings, administration of *IR* significantly reduced neutrophil counts in BAL fluid compared with LPS-treated animals, indicating effective suppression of inflammatory cell recruitment into pulmonary tissue. This reduction is likely attributable to decreased production of neutrophil chemoattractants such as IL-8 and CCL2 together with attenuation of endothelial activation, thereby limiting leukocyte transmigration across the pulmonary vasculature.^{2,7,29,31} Histopathological analysis further substantiated the protective effects of *IR*. Treatment with *IR* markedly ameliorated these pathological alterations, preserving alveolar structure and significantly reducing inflammatory damage.^{11,14-16,31} The observed anti-inflammatory effects of *IR* are likely attributable to its bioactive phytoconstituents, including sesquiterpene lactones (alantolactone and isoalantolactone), flavonoids, and phenolic compounds.¹⁴⁻¹⁶ Previous pharmacological studies have demonstrated that alantolactone and isoalantolactone inhibit activation of NF-κB and MAPK signaling pathways, suppress NLRP3 inflammasome activation, activate the Nrf2/HO-1 antioxidant pathway, and reduce oxidative stress and inflammatory cytokine production.^{17-22,32-34} Although the underlying molecular mechanisms were not directly investigated in the present study, the marked reduction in pro-inflammatory cytokines, neutrophil infiltration, and lung tissue damage strongly suggests that *IR* exerts its pulmonary protective effects through coordinated modulation of the TLR4/MyD88/NF-κB, MAPK, NLRP3 inflammasome, and Nrf2/HO-1 signalling pathways, thereby attenuating inflammatory responses and oxidative stress during LPS-induced pulmonary inflammation.^{18-23,32-34}

Our data suggest that TLR-4 agonist, LPS increases the levels of pro-inflammatory cytokines such as IL-6, IL-8, TNF-α, and IL-1β in a lung cancer cell line which was significantly reduced in the cells pre-treated with *IR*. In the *in vivo* set also BALB/c mice dosed prophylactically with *IR* showed a reduction in LPS-induced increase in the neutrophil influx by *IR* in BAL fluid of LPS-treated mice. The results obtained in the present study clearly suggest the therapeutic potential of *IR* aqueous extract in mitigating LPS-induced inflammation in lung tissue.

Despite the promising protective effects of *IR* observed in the present study, several limitations should be

considered when interpreting these findings. The experimental investigation was predominantly focused on acute lung injury induced by LPS, and therefore the observed effects cannot be directly extrapolated to chronic inflammatory respiratory disorders such as asthma, COPD, and bronchiectasis, which involve more complex and persistent pathological mechanisms. Although the study demonstrated attenuation of inflammatory responses and modulation of signalling pathways associated with TLR4/NF- κ B, MAPK, NLRP3 inflammasome activation, and redox homeostasis, the precise molecular targets and causal relationships among these pathways remain to be established. Future studies employing pathway-specific pharmacological inhibitors, gene-silencing or knockout approaches, and target-specific molecular assays would help confirm whether these pathways represent direct molecular targets of *IR* or secondary consequences of its anti-inflammatory and antioxidant effects.

CONCLUSION

The present study demonstrates that *IR* Hook. f. (*Pushkarmool*) possesses significant anti-inflammatory and immunomodulatory potential against LPS-induced pulmonary inflammation. The extract attenuated inflammatory responses in NCI-H460 lung epithelial cells and reduced the levels of key pro-inflammatory mediators, including TNF- α , IL-6, IL-1 β , IL-8, IL-18, IL-4, and CCL2, as assessed through gene and protein expression analyses. In the LPS-induced BALB/c mouse model, treatment with *IR* reduced inflammatory cell infiltration in BAL fluid, particularly neutrophil accumulation, and ameliorated inflammation-associated histopathological alterations in lung tissue. The observed effects were associated with modulation of inflammatory signalling pathways involving TLR4/MyD88/NF- κ B, MAPK, NLRP3 inflammasome, and Nrf2/HO-1.

Collectively, these findings provide experimental evidence supporting the traditional use of *IR* in respiratory inflammatory conditions and suggest that its whole extract may exert multi-target anti-inflammatory effects. The findings also highlight the potential of *IR* as a source of bioactive phytoconstituents for the development of evidence-based phytotherapeutic interventions for pulmonary inflammation. However, further studies involving detailed molecular validation, pharmacokinetic evaluation, dose optimization, chronic disease models, and clinical investigation are warranted to establish its therapeutic efficacy, safety, and translational potential.

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