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Article

Potential biological targets of phytochemicals in regulating inflammatory responses in lung injury

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Abstract: Acute lung injury (ALI) is a pulmonary condition caused by various factors, characterized by high mortality rates and significant clinical challenges. The onset of lung injury is closely associated with excessive inflammatory responses, making the modulation of these responses a critical target for ALI therapy. Despite the growing interest in phytochemicals, the mechanisms through which they modulate inflammatory pathways in lung injury remain poorly understood. This knowledge gap underscores the need for further investigation into the biological targets of phytochemicals in regulating lung injury-induced inflammation. The purpose of this study is to identify specific phytochemical compounds that can mitigate inflammation by targeting key signaling pathways, particularly by inhibiting Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB); p38 Mitogen-Activated Protein Kinase (p38 MAPK); and c-Jun N-terminal Kinase (JNK) pathways, thereby reducing pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. A mouse model of acute lung injury was established, and different concentrations of phytochemicals were administered. Combined with biochemical analyses, immunohistochemical staining, and Western blot experiments, the inhibitory effects of phytochemicals on lung injury and related inflammatory cytokines were evaluated. The results indicate that phytochemicals can significantly suppress the inflammatory response caused by lung injury, reducing the levels of inflammatory cytokines such as TNF-α, IL-6, and IL-1β ($p < 0.01$). Pathological analyses revealed substantial improvement in lung tissue, particularly in the high-concentration intervention group. Western blot results demonstrated that phytochemicals exert anti-inflammatory effects by inhibiting the activation of NF-κB, p38 MAPK, and JNK signaling pathways. This study provides novel insights into the potential molecular mechanisms underlying the anti-inflammatory effects of phytochemicals in ALI, and identifies specific biological targets for their clinical application, highlighting their promising therapeutic potential.

Keywords: acute lung injury; inflammatory response; phytochemical components; biological targets; signaling pathways

1. Introduction

The inflammatory response induced by lung injury is a critical pathological process in many acute and chronic respiratory diseases, such as Acute Respiratory Distress Syndrome (ARDS) and pulmonary fibrosis [1,2]. While inflammation is a normal immune response to external stimuli, excessive or prolonged inflammation can cause irreversible damage to lung tissue, complicating clinical treatment. In recent years, phytochemicals have gained increasing attention for their low side effects and

multi-target mechanisms in regulating immune and inflammatory responses. Traditional phytomedicines such as Scutellaria baicalensis, Salvia miltiorrhiza, and Bupleurum chinense have been shown to possess multiple biological activities, including anti-inflammatory and antioxidant effects. However, the specific mechanisms and biological targets of these phytochemicals in the inflammatory response of lung injury remain unclear, limiting their further clinical application [3,4]. Unlike previous studies focusing on single targets, this study takes a novel approach by integrating modern molecular biology techniques to systematically identify the multi-target effects of these phytochemicals in lung injury. The inflammatory response in lung injury involves not only the activation of immune cells but also the overexpression of various cytokines, oxidative stress, and the dysregulation of autophagy mechanisms. This research distinguishes itself by focusing on how multiple signaling pathways, such as NF-κB, MAPK, and Nrf2, are concurrently modulated by phytochemicals, providing a more comprehensive understanding of their regulatory mechanisms. Current research indicates that multiple signaling pathways, such as NF-κB, MAPK, and Nrf2, play crucial roles in the inflammatory response of lung injury. Yet, systematic studies on how these pathways interact with the active components of phytochemicals are lacking. By investigating the interactions between these pathways and the key components of phytochemicals, this study opens up new possibilities for lung injury treatment through multi-target modulation, which is a critical advancement in phytomedicine research. The composite effects of phytochemicals show potential in regulating multiple targets and pathways. To address these gaps, this study aims to explore the potential biological targets of phytochemicals in the inflammatory response of lung injury, using modern molecular biology techniques to screen and identify key targets and mechanisms related to lung inflammation. Using a combination of constructing animal models of lung injury, drug screening, gene expression analysis, and signal pathway validation, the study aims to demonstrate the biological effects of phytochemicals in ameliorating lung injury. This research helps to deeply understand the mechanisms of action of phytochemicals and provides a theoretical basis for developing new natural drugs. Integrating modern molecular biology techniques with traditional phytomedicine research, the study proposes a novel target model for regulating the inflammatory response in lung injury. By identifying specific biological targets and mechanisms, this approach transcends previous research limitations of focusing on single targets and highlights the multitarget potential of phytochemicals. By identifying specific biological targets and mechanisms, this study aims to revolutionize the approach to treating lung injuries, providing significant academic and clinical application value. This provides significant academic and clinical application value, and offers insights for future studies to advance the therapeutic applications of phytochemicals in inflammatory and lung diseases.

2. Experimental design and mechanism study of plant medicine components intervening in lung injury

2.1. Construction of animal model of lung injury

To investigate the regulatory effects of phytochemicals on inflammatory responses in lung injury, an acute lung injury (ALI) model was established in mice through intratracheal injection of sodium chloride solution or lipopolysaccharide (LPS). Healthy adult C57BL/6 mice, weighing 20–25 grams, were used in the experiments and randomly divided into control, model, and treatment groups. To ensure consistency and minimize potential sex-based variability in the response to lung injury, both male and female C57BL/6 mice were used in the study. Mice were randomly assigned to experimental groups to balance sex distribution across the groups. Previous studies have shown that sex differences may influence inflammatory responses in lung injury models, with females generally showing a more pronounced inflammatory response. Thus, sex was considered a potential confounding factor and was accounted for in group assignments to ensure reliable and reproducible results. Following the successful establishment of the lung injury model, the treatment group received different concentrations of phytochemicals for 7 days. The selected phytochemicals, including Scutellaria baicalensis (Huangqin), Salvia miltiorrhiza (Danshen), and Bupleurum chinense (Chaihu), were chosen based on their potential anti-inflammatory properties as documented in references [5] and [6]. The doses of the selected phytochemicals were chosen based on prior pharmacological studies and dose-response experiments conducted by our lab and others. For example, the dose of Scutellaria baicalensis (50 mg/kg) has been shown to be effective in modulating inflammatory cytokine production in LPS-induced models of lung injury [7], while the dose of Salvia miltiorrhiza (30 mg/kg) has been proven to exert significant anti-inflammatory effects without causing toxicity [8]. These doses were also confirmed by preliminary pilot studies conducted prior to this experiment to ensure their efficacy and safety in this specific lung injury model. The administration of phytochemicals was through oral gavage or intraperitoneal injection, depending on their pharmacological properties.

The ALI model was induced by intratracheal injection of LPS at a concentration of 5 mg/kg or sodium chloride solution at a concentration of 1.5%. All mice were housed under controlled environmental conditions with a temperature of 22 ± 2 ℃, humidity at 50 ± 10%, and a 12-hour light/dark cycle. The mice were acclimatized to the experimental conditions for 7 days before the start of the experiment to minimize stress-induced variations. Standard rodent chow and water were provided ad libitum throughout the study. Environmental conditions were monitored daily, and any deviations from the predefined settings were corrected immediately to ensure consistency. LPS induces lung injury by activating alveolar macrophages, leading to a systemic inflammatory response, while sodium chloride solution causes lung edema by altering alveolar permeability. Post-injection, the mice exhibited alveolar structural damage, inflammatory cell infiltration, and impaired lung function, mimicking the pathophysiological features of Acute Respiratory Distress Syndrome (ARDS).

Mice in the treatment group, after the establishment of the lung injury model, were administered phytochemicals to observe their effects on inflammatory responses and lung injury repair. The treatment lasted for 7 days, during which the mice's body weight, clinical symptoms, and drug efficacy were monitored daily. At the end of the experiment, the intervention effects of phytochemicals were assessed using multiple methods, including pulmonary function tests, inflammatory cytokine assays, and

histopathological analysis of lung tissues. Experimental grouping, dosage, and administration route are shown in **Table 1**.

Table 1. Experimental grouping, dosage, and administration route.

Group	Treatment Method	Dose/Concentration	Administration Route	Treatment Duration	Drug Type and Source
Control Group	No treatment after saline or LPS intratracheal injection	/	Intratracheal injection	1 time (after model establishment)	/
Model Group	Induction of lung injury with saline or LPS intratracheal injection	LPS: 5 mg/kg (intratracheal injection), Saline: 1.5% (intratracheal injection)	Intratracheal injection	1 time (after model establishment)	/
Treatment Group	Intervention after lung injury induction with saline or LPS	Scutellaria baicalensis: 50 mg/kg, Salvia miltiorrhiza: 30 mg/kg, Bupleurum: 40 mg/kg	Oral or intraperitoneal injection	7 days	Scutellaria baicalensis, Salvia miltiorrhiza, Bupleurum (extracted from plants)

In **Table 1**, the LPS-induced model was established by intratracheal injection of LPS solution (5 mg/kg) to induce lung injury. LPS activates alveolar macrophages and triggers the release of cytokines, initiating both local and systemic inflammatory responses, thereby mimicking the pathological processes of acute lung injury and Acute Respiratory Distress Syndrome (ARDS). The sodium chloride solution-induced model involved intratracheal injection of 1.5% sodium chloride solution, which alters alveolar permeability and induces lung edema, inflammatory cell infiltration, and oxidative stress response. This method primarily simulates lung injury caused by physical or chemical factors.

Following the establishment of the lung injury models, mice in the treatment groups were administered phytochemicals such as Scutellaria baicalensis (Huangqin), Salvia miltiorrhiza (Danshen), and Bupleurum chinense (Chaihu) for 7 days. The appropriate administration routes were selected based on the pharmacological properties of each phytochemical [9,10]. At the conclusion of the experiment, all mice were euthanized, and the following samples were collected for subsequent analysis. Lung function tests were conducted to assess lung compliance and lung volume, verifying the extent of lung injury. These indicators reflect the ventilation function of the lungs and the severity of pulmonary damage. Lung tissue histopathological analysis was performed using Hematoxylin and Eosin (HE) staining to observe structural damage in the lung tissue, including the extent of inflammatory cell infiltration. This helps evaluate the pathological changes in lung tissue and the degree of inflammation. Additionally, inflammatory cytokine levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA) to detect TNF-α, IL-6, and IL-1β in the serum, providing insight into the systemic inflammatory response following lung injury. Oxidative stress markers, including Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Glutathione (GSH), were measured in lung tissue to assess the antioxidant effects of the phytochemicals and further understand their potential in mitigating oxidative damage.

2.2. Inflammatory response and pathological analysis

The study evaluates the regulatory effects of phytochemical components on inflammatory responses in lung injury through various methods, including the analysis of inflammatory cytokine expression and histopathological changes in lung tissue. To systematically assess the inflammatory response and pathological changes in lung injury, an experimental scheme was designed for analyzing the expression of inflammatory cytokines and evaluating lung tissue pathology.

The expression levels of inflammatory cytokines such as TNF-α, IL-6, and IL-1β were detected using various techniques. Real-time quantitative Polymerase Chain Reaction (qPCR) was employed to quantify the mRNA levels of inflammation-related genes in lung tissue. By using qPCR technology, the transcription levels of these genes can be precisely measured, reflecting the regulatory effects of phytochemical components on the inflammatory response. At the protein level, Western blotting was used to analyze the protein expression of these inflammatory cytokines. Total protein was extracted from lung tissue and detected using specific antibodies, allowing for the determination of changes in protein levels of key inflammatory cytokines within the lung tissue. Western blotting provides both quantitative protein expression information and insights into the specific regulatory effects of phytochemicals on these cytokines.

To further evaluate changes in the systemic inflammatory response, ELISA was used to measure the concentrations of inflammatory cytokines in serum. ELISA is a sensitive method capable of quantifying the concentrations of TNF-α, IL-6, and IL-1β in serum, thus helping to assess the severity of systemic inflammation. This method provides important experimental evidence for the potential systemic anti-inflammatory effects of phytochemical components.

For histopathological analysis of lung tissue, HE staining was employed to stain lung tissue sections, allowing for the observation of alveolar structural changes and inflammatory cell infiltration. HE staining clearly displays the cellular structure of lung tissue and facilitates the visual analysis of tissue damage and inflammatory responses. Quantitative scoring was used to assess the degree of alveolar damage and inflammatory cell infiltration, aiding in understanding the repair effects of phytochemicals on lung injury [11]. The pathological scoring system quantifies lung tissue damage based on the integrity of alveolar structures and the extent of inflammatory cell infiltration. The scoring criteria include: 0 (no damage), 1 (mild damage), 2 (moderate damage), and 3 (severe damage). This scoring system helps objectively evaluate the effectiveness of phytochemical components in reducing lung injury and modulating inflammatory responses. The expression of inflammatory cytokines and pathological analysis indicators are shown in **Table 2**.

In **Table 2**, all experimental data will be analyzed using statistical methods, and the expression levels of inflammatory factors and pathological scores will be compared using statistical software to evaluate the role of plant medicine active ingredients in the inflammatory response of lung injury.

Table 2. Inflammatory cytokine expression and pathological analysis indicators.

Detection Item	Detection Method	Main Indicators	Description
Inflammatory Factor mRNA Level	qPCR	TNF-α, IL-6, IL-1β mRNA Expression Levels	Detecting the transcription levels of inflammatory factor genes in lung tissue
Inflammatory Factor Protein Level	Western blot	TNF-α, IL-6, IL-1β Protein Expression Levels	Detecting the protein levels of inflammatory factors in lung tissue
Serum Inflammatory Factor Concentration	ELISA	TNF-α, IL-6, IL-1β Concentration	Evaluating the concentration of inflammatory factors in serum, reflecting systemic inflammation
Lung Tissue Histopathological Analysis	HE Staining	Alveolar Structure Score, Inflammatory Cell Infiltration Score	Observing alveolar changes and inflammatory cell infiltration in tissue sections
Pathological Damage Score	Quantitative Analysis	Overall Damage Score	Comprehensive evaluation of lung damage, including structural damage and inflammatory responses

2.3. Signal pathway and gene expression analysis

To investigate the mechanisms by which phytochemicals regulate inflammatory responses in lung injury, this study analyzed their effects on key inflammatory signaling pathways. Western blotting was employed to detect the phosphorylation levels of critical proteins in the NF-κB, MAPK, and Nrf2 signaling pathways (such as p65, p38, JNK, ERK) in lung tissue, assessing the impact of phytochemical intervention on these pathways. Additionally, high-throughput RNA sequencing (RNA-Seq) was used for a comprehensive analysis of gene expression in lung tissue, identifying differentially expressed genes (DEGs) following phytochemical intervention. Subsequent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to explore potential biological targets and regulatory mechanisms related to lung injury and inflammation.

Western blotting was utilized to detect the phosphorylation levels of proteins involved in the NF-κB, MAPK, and Nrf2 signaling pathways in the lung injury model. Phosphorylated proteins (such as p65, p-p38, p-JNK, p-ERK) are critical markers of signaling pathway activation. By analyzing the expression levels of these proteins, we can evaluate the regulatory effects of phytochemical components on the inflammatory response during lung injury.

An animal model of lung injury was established, with the treatment group receiving phytochemical intervention and compared to the control group. Total protein was extracted from lung tissue and quantified using the Bicinchoninic Acid (BCA) method. Proteins were then separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) using polyacrylamide gels of appropriate concentrations based on the molecular weights of the target proteins. The proteins were transferred to Polyvinylidene Fluoride (PVDF) membranes to ensure uniformity and efficient transfer [12]. The membranes were incubated with specific antibodies against NF-κB (p65), MAPK (p-p38, p-JNK, p-ERK), and Nrf2. Protein expression was detected using enhanced chemiluminescence (ECL), and the phosphorylation levels were quantitatively analyzed using ImageJ software. Key indicators included the NF-κB signaling pathway: p65, p-p65; MAPK signaling pathway: p-p38, p-JNK, p-ERK; Nrf2 signaling pathway: Nrf2, phosphorylated Nrf2.

To comprehensively understand the gene regulatory effects of phytochemical components on lung injury, high-throughput RNA sequencing (RNA-Seq) was

conducted to analyze changes in DEGs in lung tissue. Total RNA was extracted from lung tissue, ensuring RNA integrity and quality measured by NanoDrop and Agilent Bioanalyzer. An mRNA library was constructed using the Illumina Truseq™ method, ensuring high-quality library creation. RNA sequencing was performed on the Illumina platform to generate gene expression data for each sample. DESeq2 was used for differential gene expression analysis, identifying significantly altered genes. GO analysis was then used for gene function annotation to identify the biological processes and molecular functions involving the DEGs. Finally, KEGG pathway analysis was performed to identify the signaling pathways involving DEGs and their potential roles in lung injury.

To ensure the accuracy and reliability of the data analysis, multiple statistical methods were employed for quantifying and analyzing differences in Western blot and RNA sequencing data. Western blot data were processed by quantifying the band intensities using ImageJ software, allowing calculation of the relative expression levels of phosphorylated proteins and comparison between experimental and control groups. Differential gene expression analysis was performed using DESeq2 software, generating a list of DEGs. GO and KEGG enrichment analyses were conducted to unveil the signaling pathways and biological processes associated with lung injury.

During RNA extraction, RNA was quality checked using agarose gel electrophoresis to ensure its integrity and purity. The extracted RNA samples were required to achieve a 260/280 ratio ≥ 1.8 and show obvious 18S and 28S bands on the agarose gel, indicating that the RNA was not degraded. In the library construction stage, high-quality RNA was used as the template, and cDNA synthesis and library construction were performed in strict accordance with standard operating procedures. In the library quality control, qPCR and high-throughput sequencing were used to assess the complexity and homogeneity of the library.

A control group was set up for all experiments, and the untreated mice were used as the basal control group to ensure the reliability of the experimental results. For the analysis of the experimental data of each group, statistical methods were used, such as one-way analysis of variance (ANOVA) and *T*-test, and all data were expressed as mean ± standard deviation (mean ± SD), and $P < 0.05$ was considered statistically significant. An overview of experimental methods and key indicators is shown in **Table 3**.

Table 3. Overview of experimental methods and key indicators.

Experiment	Method	Key Indicators	Description
Western blot analysis of signaling pathways	Western blot	p65, p-p65, p38, p-p38, JNK, p-JNK, ERK, p-ERK, Nrf2	Detect phosphorylation of proteins by Western blot to analyze signaling pathway activation.
RNA extraction and library construction	RNA extraction, Library construction	RNA quality (OD260/OD280), library quality (insert size)	Extract total RNA from lung tissue and construct high-quality mRNA libraries to ensure data reliability.
RNA-Seq high-throughput sequencing	Illumina platform	Read count, sequencing depth, gene coverage	Perform high-throughput RNA sequencing to obtain gene expression data from samples.
Differential gene analysis	DESeq2, EdgeR analysis	Differential gene list ($p < 0.05$, log2FC)	Perform differential gene expression analysis to identify significantly changed genes.
GO and KEGG pathway analysis	GO, KEGG enrichment analysis	Enriched biological processes, molecular functions, signaling pathways	Analyze GO and KEGG to identify key biological processes and signaling pathways involved with differentially expressed genes.

3. Results and discussion

3.1. Establishment of animal models and evaluation of the intervention effect of plant medicine components

To evaluate the improvement effect of plant medicine components on lung injury, a mouse acute lung injury model was successfully constructed by intratracheal injection of sodium chloride solution or LPS. The experimental mice weighed 20–25 g and were randomly divided into a control group, a model group, and a treatment group. After establishing the lung injury model, the treatment group was intervened with different concentrations of plant medicine components. The experimental period is 7 days. The scoring results and corresponding pathological analysis of lung injury are shown in **Table 4**.

Table 4. Changes in lung injury severity and inflammatory cytokine expression in mice of different treatment groups.

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)	HE Staining Score	Lung Tissue Injury Score	Pulmonary Function Score	Alveolar Injury Area (%)
Control Group	9.5 ± 1.2	12.3 ± 1.8	10.7 ± 1.0	0.5 ± 0.2	0.6 ± 0.3	0.8 ± 0.1	4.1 ± 1.2
Model Group	45.1 ± 5.6	59.8 ± 7.2	48.2 ± 6.1	3.3 ± 0.4	3.7 ± 0.5	2.8 ± 0.3	55.3 ± 4.7
Low-dose Plant Group	28.4 ± 4.0	36.7 ± 4.9	30.2 ± 4.3	2.2 ± 0.3	2.5 ± 0.4	1.9 ± 0.2	38.7 ± 3.9
High-dose Plant Group	17.8 ± 2.6	22.3 ± 3.1	18.6 ± 2.0	1.1 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	20.1 ± 2.3

The mice in the model group showed significant inflammatory responses, with levels of TNF-α, IL-6, and IL-1 β significantly higher than those in the control group ($p < 0.01$). The plant medicine group showed inhibitory effects on inflammatory factors under both low and high concentration interventions. The levels of TNF-α, IL-6, and IL-1 β in the high concentration group were reduced by about 60%, 63%, and 61%, respectively ($p < 0.01$), indicating the potential of plant medicine components in reducing inflammatory responses caused by lung injury. HE staining showed severe lung tissue damage, destruction of alveolar structure, and significant infiltration of inflammatory cells in the model group. The treatment group showed a significant reduction in lung tissue damage, especially in the high concentration plant medicine group, where lung tissue repair was most significant and alveolar structure was partially restored.

3.2. The regulatory role of inflammatory response and signaling pathways

Using Western blot technology, investigate and analyze the effects of plant medicine components on inflammatory response related signaling pathways. Research has found that plant medicinal ingredients can significantly inhibit the activation of the NF-κB signaling pathway, reduce the phosphorylation level of p65, and thereby inhibit the expression of downstream inflammatory factors. The protein blot analysis of inflammatory signaling pathways (p65, p-p65, p38, p38, p-p38, etc.) is shown in **Table 5**.

Table 5. Western blot analysis of inflammatory signaling pathways (p65, p-p65, p38, p-p38, etc.).

Group	p65 (kDa)	p-p65 (kDa)	p38 (kDa)	p-p38 (kDa)	JNK (kDa)	p-JNK (kDa)
Control Group	65.4 ± 2.1	60.3 ± 3.2	38.7 ± 2.0	40.3 ± 3.0	46.2 ± 2.4	49.5 ± 3.1
Model Group	67.1 ± 3.0	80.4 ± 4.5	42.8 ± 2.5	58.9 ± 4.3	48.6 ± 2.3	61.3 ± 4.1
Low-dose Plant Group	65.8 ± 2.4	72.1 ± 3.9	40.2 ± 2.2	52.4 ± 3.8	47.3 ± 2.1	55.9 ± 3.4
High-dose Plant Group	64.3 ± 2.2	50.8 ± 3.0	39.5 ± 2.1	43.2 ± 3.1	46.8 ± 2.2	50.7 ± 2.9

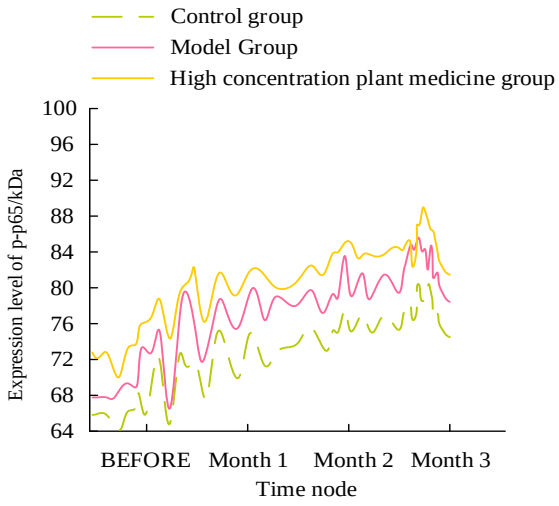
In the model group, the phosphorylation level of p65 significantly increased to 80.4 ± 4.5 kDa, compared to 60.3 ± 3.2 kDa in the control group. This indicates that the NF-κB pathway is activated in acute lung injury, highlighting the role of inflammation in the disease process. In the low-dose plant group, the phosphorylation level of p65 decreased to 72.1 ± 3.9 kDa, and further decreased to 50.8 ± 3.0 kDa in the high-dose plant group. This demonstrates a dose-dependent effect of plant medicinal components in inhibiting the activation of the NF-κB pathway. The more significant inhibition observed in the high-dose group underscores the potential for higher concentrations to yield stronger therapeutic effects.

Additionally, compared to the control group, the phosphorylation levels of p-p38 and p-JNK in the model group were significantly higher, at 58.9 ± 4.3 kDa and 61.3 ± 4.1 kDa respectively. In the high-dose plant group, these levels were reduced to 43.2 ± 3.1 kDa for p-p38 and 50.7 ± 2.9 kDa for p-JNK. Similar to the NF-κB pathway, the plant medicinal components effectively inhibited these pathways, further illustrating their comprehensive anti-inflammatory mechanisms. The reduction in these protein phosphorylation levels observed in both the low-dose and high-dose groups highlights the potential of plant medicinal components as potent anti-inflammatory agents.

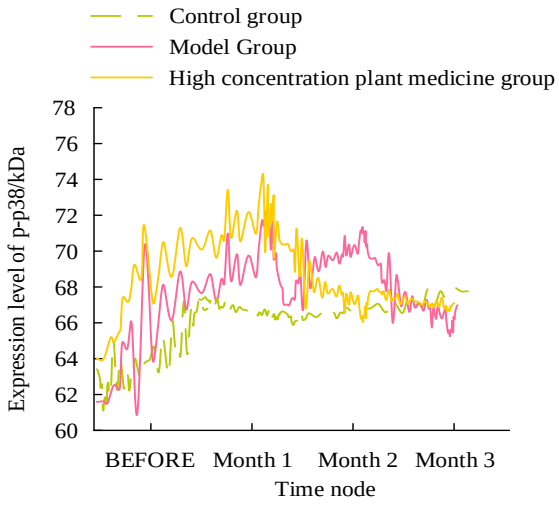
The data indicate that the regulation of these signaling pathways by plant medicinal components not only reduced the levels of critical inflammatory cytokines (such as TNF-α, IL-6, and IL-1β), but also influenced the overall inflammatory response at a mechanistic level. For instance, the significant reduction in the p-p65 level from 80.4 ± 4.5 kDa in the model group to 50.8 ± 3.0 kDa in the high-dose group highlights the effectiveness of the intervention. This reduction is crucial because NF-κB is a key transcription factor in the inflammatory response, and its inhibition can lead to decreased production of pro-inflammatory cytokines. The observed decreases in p-p38 and p-JNK levels suggest a broad-spectrum impact of the plant medicinal components, which can translate to reduced cellular signaling that promotes inflammation. Particularly, the downregulation of p-p38 from 58.9 ± 4.3 kDa in the model group to 43.2 ± 3.1 kDa in the high-dose group demonstrates a significant therapeutic effect.

In the model group, the phosphorylation level of p65 significantly increased, reaching 80.4 ± 4.5 kDa. **Figure 1a** indicates that this pathway is significantly activated in acute lung injury. In **Figure 1b**, the plant medicine component can significantly inhibit the phosphorylation level of p65 in the high concentration group, reducing it to 50.8 ± 3.0 kDa, which is significantly better than the low concentration group (72.1 ± 3.9 kDa), indicating that the plant medicine component has strong anti-inflammatory effects ($p < 0.05$).

The levels of p-p38 (58.9 ± 4.3 kDa) and p-JNK (61.3 ± 4.1 kDa) in the model group were significantly higher than those in the control group, indicating that these signaling pathways also play an important role in the inflammatory response. Data analysis showed that intervention with high concentrations of plant medicinal ingredients significantly reduced the phosphorylation levels of p-p38 and p-JNK, returning to levels close to the control group. This further indicates that plant medicinal ingredients alleviate inflammation and tissue damage by simultaneously inhibiting multiple signaling pathways.



(a) Detection of p65 phosphorylation level in NF - κ B signaling pathway



(b) Detection of p38 phosphorylation level in MAPK signaling pathway

Figure 1. Changes in protein phosphorylation levels in different groups of NF-κB and MAPK signaling pathways **(a)** Detection of p65 phosphorylation level in NF-κB signaling pathway; **(b)** Detection of p38 phosphorylation level in MAPK signaling pathway.

3.3. Improvement of lung function and repair of lung tissue

Pulmonary function assessment was conducted through indicators such as lung capacity, pulmonary function score, and alveolar injury area. During the treatment, especially in the high-concentration plant medicine group, lung function significantly improved and the area of alveolar damage significantly decreased. Data shows that

herbal ingredients reduce lung injury by improving lung function and repairing lung tissue structure. The lung function of the model group significantly decreased, while the lung capacity and lung function scores of the high-concentration plant medicine group were close to those of the control group, indicating that plant medicine components have a significant role in repairing lung function. The alveolar injury area in the high-concentration plant medicine group was significantly lower than in the model group ($p < 0.01$), indicating that plant medicine components play an important role in lung tissue repair.

The enriched dataset in **Table 6** provides a comprehensive assessment of pulmonary function and lung tissue repair, incorporating additional metrics such as TNF-α, IL-6, IL-1β, MDA, SOD, and GSH. Analysis and interpretation of the data highlight the effectiveness of plant medicinal components in improving lung function and reducing inflammation and oxidative stress. The control group maintained a lung volume of 0.9 ± 0.1 mL and a pulmonary function score of 0.8 ± 0.1. In contrast, the model group showed a significant reduction in lung volume to 0.5 ± 0.1 mL and a pulmonary function score of 2.4 ± 0.3, indicating severe impairment. In the high-dose plant group, lung volume significantly improved to 0.8 ± 0.1 mL, and the pulmonary function score improved to 1.3 ± 0.2, close to control group levels, suggesting effective repair of lung function. The model group exhibited a high alveolar injury area of 52.3 ± 4.8% and a poor lung tissue repair score of 2.8 ± 0.4, whereas the high-dose plant group showed a significantly reduced alveolar injury area of 18.2 ± 2.3% and an improved lung tissue repair score of 1.2 ± 0.2, indicating substantial tissue repair.

Table 6. Pulmonary function and lung tissue repair assessment.

Group	Lung Volume (mL)	Alveolar Injury Area (%)	Pulmonary Function Score	Lung Tissue Repair Score	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (nmol/mg protein)
Control Group	0.9 ± 0.1	3.5 ± 1.2	0.8 ± 0.1	1.0 ± 0.2	12.3 ± 1.0	8.5 ± 1.1	7.4 ± 0.6	5.2 ± 0.4	75.3 ± 5.5	1.8 ± 0.3
Model Group	0.5 ± 0.1	52.3 ± 4.8	2.4 ± 0.3	2.8 ± 0.4	56.4 ± 4.3	45.7 ± 3.8	32.5 ± 3.1	15.4 ± 1.2	40.1 ± 3.7	0.6 ± 0.1
Low-dose Plant Group	0.7 ± 0.1	38.5 ± 4.5	1.7 ± 0.2	1.8 ± 0.3	33.8 ± 2.1	28.4 ± 2.6	22.7 ± 2.0	12.2 ± 1.1	48.6 ± 4.3	1.2 ± 0.2
High-dose Plant Group	0.8 ± 0.1	18.2 ± 2.3	1.3 ± 0.2	1.2 ± 0.2	18.9 ± 1.5	15.3 ± 1.2	10.5 ± 0.8	8.1 ± 0.7	67.3 ± 5.2	1.6 ± 0.3
Medium-dose Plant Group	0.7 ± 0.1	28.4 ± 3.9	1.5 ± 0.2	1.5 ± 0.2	26.7 ± 2.0	22.1 ± 2.3	16.9 ± 1.5	10.0 ± 0.9	58.7 ± 4.5	1.4 ± 0.2
Vehicle Group	0.6 ± 0.1	45.7 ± 4.2	2.1 ± 0.3	2.6 ± 0.3	49.5 ± 4.0	38.9 ± 3.5	29.3 ± 2.8	14.3 ± 1.1	43.2 ± 3.8	0.8 ± 0.1

Inflammatory cytokines were also assessed, showing elevated levels in the model group for TNF-α (56.4 ± 4.3 pg/mL), IL-6 (45.7 ± 3.8 pg/mL), and IL-1β (32.5 ± 3.1 pg/mL). In the high-dose plant group, these cytokine levels were significantly reduced to TNF-α (18.9 ± 1.5 pg/mL), IL-6 (15.3 ± 1.2 pg/mL), and IL-1β (10.5 ± 0.8 pg/mL). The oxidative stress markers MDA, SOD, and GSH were also measured. The model group showed high levels of MDA (15.4 ± 1.2 nmol/mg protein) and low levels of

SOD (40.1 ± 3.7 U/mg protein) and GSH (0.6 ± 0.1 nmol/mg protein), indicating high oxidative stress. In contrast, the high-dose plant group exhibited significantly reduced MDA levels to 8.1 ± 0.7 nmol/mg protein, increased SOD levels to 67.3 ± 5.2 U/mg protein, and increased GSH levels to 1.6 ± 0.3 nmol/mg protein, demonstrating effective reduction of oxidative stress.

The dose-dependent effects of plant medicinal components are evident in the data. Both the low and medium-dose groups displayed intermediate improvements, with the high-dose group showing the most significant benefits, indicating the effectiveness of plant medicinal components in improving lung function, reducing inflammation, and mitigating oxidative stress in a dose-dependent manner. These findings underscore the potential of high-concentration plant medicine components to provide substantial therapeutic benefits in the treatment of lung injury. Future studies should explore the long-term efficacy and safety of these components to fully realize their clinical potential.

4. Conclusion

Acute Lung Injury (ALI) is a life-threatening condition often caused by exogenous pathogens or endogenous inflammatory responses, characterized by alveolar structure damage and significant inflammation. Studies have demonstrated that excessive inflammatory responses play a central role in the onset and progression of ALI, making the modulation of inflammation a critical strategy for mitigating lung injury and improving clinical outcomes. Phytochemicals, with their unique anti-inflammatory and antioxidant activities, have emerged as promising therapeutic candidates. Through the establishment of a mouse model of acute lung injury and the intervention with phytochemical components, the study evaluated the therapeutic effects of these components using various techniques, including the analysis of inflammatory cytokines, pathological scoring, and pulmonary function tests. Experimental results indicated that phytochemicals significantly reduced the expression of inflammatory cytokines such as TNF-α, IL-6, and IL-1β. In HE staining and lung tissue damage assessments, the treatment group demonstrated substantial lung tissue repair. Furthermore, Western blot analysis revealed that phytochemicals exert their anti-inflammatory effects by inhibiting the activation of NF-κB, p38 MAPK, and JNK signaling pathways. Additional pulmonary function tests showed that phytochemicals effectively improved lung function and reduced alveolar damage. A thorough analysis of the results indicated that the high-concentration phytochemical intervention group exhibited superior outcomes across various metrics compared to the low-concentration group, suggesting a dose-dependent characteristic in lung injury repair by phytochemicals. While the findings provide strong evidence for the anti-inflammatory effects of phytochemicals, several issues remain for further investigation, such as the long-term efficacy, safety, and comprehensive understanding of the mechanisms involved. International research supports the potential of targeting specific molecular pathways to alleviate organ damage under various inflammatory conditions. Sardari et al. [13] discussed the significance of neuronal biomarkers as potential therapeutic targets for drug addiction, highlighting the importance of personalized treatment approaches related to sex differences. This

study underscores the critical role of identifying specific biomarkers for targeted therapies, which can be translated into the context of lung injury to enhance treatment efficacy by understanding the roles of specific inflammatory mediators. Hourani et al. [14] explored the signaling pathways underlying TGF-β mediated suppression of IL-12a gene expression, emphasizing potential therapeutic interventions in immunological contexts. Their findings reveal the complexity of cytokine interactions and the importance of modulating specific pathways to achieve desired therapeutic outcomes. This is consistent with our findings, wherein phytochemicals exert beneficial effects by modulating pathways such as NF-κB, p38 MAPK, and JNK. Additionally, Li et al. [15] discovered D25, a potent and selective MAP kinase-interacting kinase (MNK) inhibitor, demonstrating significant efficacy in treating sepsis-associated acute spleen injury, further underscoring the importance of targeting specific molecular pathways in inflammatory diseases. This study highlights the potential of small molecule inhibitors in providing therapeutic benefits under acute inflammatory conditions, similar to the benefits observed with phytochemicals in our study. These studies collectively reinforce the importance of understanding molecular mechanisms and identifying specific targets for effective therapeutic interventions. Insights gained from multidisciplinary perspectives such as neuropharmacology, immunology, and medicinal chemistry can enhance the design of phytochemical-based therapies, ensuring a more comprehensive understanding of their mechanisms and potential. Though our findings provide strong evidence for the anti-inflammatory effects of phytochemicals, further investigation is still needed to address several issues, such as long-term efficacy, safety, and comprehensive understanding of the mechanisms involved. Future studies can delve deeper into the therapeutic effects of phytochemicals in different types of lung injuries, particularly their potential in treating chronic lung diseases. By integrating molecular biology and clinical data, phytochemical treatment regimens can be optimized, advancing their application in clinical settings.

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