Original Research Article

Tribuloside: Mechanisms and Efficacy in Treating Acute Lung Injury Revealed by Network Pharmacology and Experimental Validation

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Abstract

Background: Acute lung injury (ALI) is a serious illness that has few treatment options available. Tribuloside, a natural flavonoid extracted from the Tribulus Terrestris plant in China, is potent in addressing many health issues such as headaches, dizziness, itching, and vitiligo.

Objective: This study intends to explore the mechanisms of action of Tribuloside in treating ALI through a combination of network pharmacology and experimental validation.

Methods: We obtained the 2D structure and SMILES number of Tribuloside from the PubChem database. We used the SwissTargetPrediction database to identify pharmacological targets. We found 1215 targets linked to ALI by examining the GeneCards database. We used the String database and Cytoscape software to create the "drug or disease-target" network as well as the protein-protein interactions (PPI). Key targets were identified by evaluating associated biological processes and pathway enrichment. A Venny Diagram showed 49 intersection points between Tribuloside and ALI. Molecular docking with AutoDockTools found that Tribuloside had a high affinity for IL6, BCL2, TNF, STAT3, IL1B, and MAPK3, the top 6 targets in the PPI network by Degree values. To test Tribuloside's therapeutic efficacy in ALI, an acute lung damage model in mice was constructed using lipopolysaccharide. Tribuloside treatment reduced inflammatory cell infiltration, decreased fibrotic area, repaired damaged alveoli, and suppressed inflammatory factors IL-6, TNF-α, and IL-1β in the lungs through many pathways and targets.

Conclusion: This study reveals that Tribuloside has the potential to treat ALI by targeting various pathways and targets, according to network pharmacology predictions and experimental confirmation.

Keywords

acute lung injury, tribuloside, network pharmacology, experimental validation, inflammatory factors, targets

Introduction

Acute lung injury (ALI) is a common clinical disease with high morbidity and fatality rates.[1] It is characterized by damage to the endothelium of lung capillaries and alveolar epithelium, which results in increased capillary permeability and the production of pulmonary edema.[2] Damaged cells emit inflammatory mediators such as cytokines (TNF-α, IL-1, IL-6), chemotactic factors, and leukocytes, which draw

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neutrophils to the lungs and cause more inflammation and tissue damage.[3] Neutrophils can release reactive oxygen species, further damaging lung tissue.[4] ALI can also stimulate the coagulation and fibrinolytic systems, causing the production of microthrombi and decreased oxygenation in the lungs.[5] Damage to the alveolar epithelium can cause lower lung compliance and alveolar collapse.[6] Chronic inflammation and tissue damage may cause pulmonary fibrosis and limit lung function.[7] Early detection and management of the underlying cause, as well as regulating the inflammatory response and supporting lung function, are crucial in the prevention and treatment of acute lung injury. However, conventional treatments have limited efficacy, necessitating the development of novel therapeutic techniques. Tribulus Terrestris, a perennial plant, includes saponin components that have exhibited varied biological effects, such as decreasing inflammation and having antioxidant capabilities.[8] Tribuloside, a chemical compound identified in Tribulus Terrestris, is thought to have pharmacological properties such as diuretic, hypotensive, anti-inflammatory, antioxidant, anticancer, and sexual function enhancing effects.[9-12] Tribulosides have antioxidant capabilities, which assist battle oxidative stress and cellular damage produced by free radicals.[13] Some studies suggest that Tribulosides may influence the immune system, although greater research in this area is needed.[14] To summarize, tribuloside is a possibly pharmacologically active molecule. However, the particular methods and targets for treating ALI are not entirely understood, necessitating additional scientific research. Network pharmacology, which combines computer science, bioinformatics, and pharmacology, can give a systematic and complete assessment of the links between medications, targets, and disorders.[15,16] This technique has proven promising in investigating the pharmacological mechanisms of traditional Chinese medications.[17] This study used network pharmacology analysis to identify the major biological processes and signaling pathways connected with Tribuloside, allowing for a more comprehensive knowledge of its physiological effects. Furthermore, molecular docking tests examined the binding affinity of important targets and Tribuloside's chemical structure.[18,19] Furthermore, Tribuloside's therapeutic efficacy in ALI was established using an experimental model. The flowchart of the study is depicted in Figure 1. Overall, the study's findings add to the current understanding of Tribuloside's potential therapeutic implications in the treatment of ALI. Future research should concentrate on further understanding its mechanisms of action and running clinical trials to assess its efficacy and safety in humans.

Materials and Methods

Identifying Potential Drug Targets for Tribuloside via Computational Analysis

The monomers of Tribuloside compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), along with their matching 2D structures and SMILES numbers.[20] To find possible therapeutic targets for Tribuloside, the SMILES number was entered into the SwissTargetPrediction database (https://swisstargetprediction.ch/), and the species "*Homo sapiens*" was selected.[21] Targets with Probability >0 were deemed possible drug targets. The UniProt database (https://www.uniprot.org/) was used to find the protein names of the indicated targets.[22] For standardized gene name change, the species "*Homo sapiens*" was chosen.

Identification of Potential Targets for ALI Treatment with Tribuloside

Targets related with ALI were gathered from the GeneCards database (https://www.genecards.org/)[23] using the keyword "Acute lung injury" and filtered based on a Relevance score >15. The acquired disease-related targets were standardized as gene names using the UniProt database, with "*Homo sapiens*" as the species. A Venny diagram developed using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) revealed overlapping targets between acute lung injury-related and Tribuloside-related targets. This overlap shows that Tribuloside may be useful in treating acute lung damage.

Protein-Protein Interaction Network Analysis of Tribuloside and ALI Targets

The STRING database (https://string-db.org/) is a useful resource for discovering and evaluating direct and indirect protein interactions.[24] For this investigation, we used the STRING 12.0 database to create a PPI network based on Tribuloside and ALI's shared targets. The analysis was limited to the species "*Homo sapiens*". Furthermore, we chose hub targets with higher levels of betweenness and closeness centrality than the median. These hub targets were then selected for additional investigation, which included molecular docking and experimental confirmation.

Functional Enrichment Analysis of Tribuloside in ALI Treatment

To understand the underlying biological processes and pathways linked to the application of Tribuloside in ALI treatment, we used the Database for Annotation Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) for gene ontology (GO) function enrichment analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.[25] This analysis sought to discover enhanced entries with significant GO and route terms ($P < .05$).[26]

Network Analysis of Interactions Between Medicines and Diseases

In this investigation, we used Cytoscape 3.7.1 software (version 3.7.1) to create networks that show and analyze the

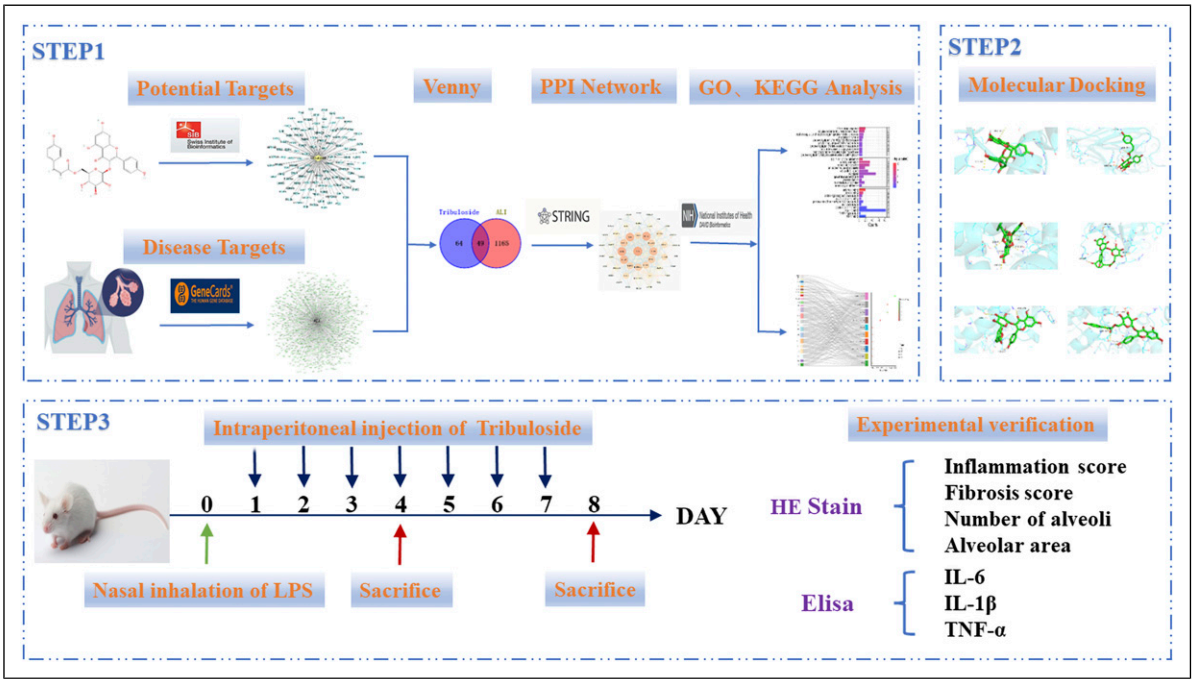

Figure 1. Strategy for investigating Tribuloside's therapeutic effects on ALI using network pharmacology analysis and experimental models.

complicated linkages between medicine, targets, and diseases (https://cytoscape.org/).[27] Cytoscape software was used to create Medicine-Target (M-T) and Disease-Target (D-T) networks, which represented distinct potential targets or signaling pathways with nodes of varied colors and sizes. Edges reflected the connections that existed between nodes. Figure 2 illustrates this representation.

Docking Simulations for Tribuloside with Key Target Proteins

To validate the results of network pharmacology screening, the AutoDockTools program (https://autodocksuite.scripps.edu/adt/) and vina were used to perform docking simulations of important targets with Tribuloside's three-dimensional structures. AutoDock is a widely used method for predicting the binding of small-molecule medicines to proteins or other macromolecular targets.[28] To perform docking simulations, 6 distinct targets were chosen from the PPI network, each of which exceeded 33°. Tribuloside's three-dimensional structures were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the key proteins' structures were retrieved from the Protein Data Bank database (https://www.pdb.org/).[29] The procedure of selecting and importing data into PyMOL 2.5 software (https://pymol) was based on its higher resolution, robustness, biological relevance, and adaptability. The three-dimensional configurations obtained from the PDB were modified using AutoDock Tools software (https://autodock.scripps.edu/) to remove water molecules, nonspecific ligands, and ions, before the amino acids were optimized and combined. The structures were hydrogenated and protonated, with non-polar hydrogens linked to the rotational changes of the relevant carbon atoms, and torsion angles automatically adjusted. Chem3D software was used to simplify the 3D structure of tribuloside compounds for the purpose of estimating energy force fields, whereas receptor and ligand structures were maintained in PDBQT files. These were imported into the AutoDock Tools 1.5.6 program. The software created a docking mechanism focused on the macromolecule, assuring comprehensive protein coverage by calculating the number of points on the x, y, and *z* axes. The molecular docking process was carried out using AutoDock Tools Vina software, with a docking degree (exhaustiveness) of 20, modes (num_modes) of 20, and an energy range of 5. The binding affinity (measured in kcal/mol) of a ligand and a receptor shows their capacity to bind, with lower values indicating more stable binding. The docking results were shown using the PyMOL2.5 software for the final analysis (https://pymol.org/2).[30]

Animal Experiments and Conditions for Mice

Male mice, 6 weeks old, with specific pathogen-free (SPF) status and a body weight of 30-40g were procured from SPF Biotechnology Ltd. (Beijing, China, Certificate No. SCXK20230006. Mice were housed in a pathogen-free (SPF) room with a regulated temperature of 25 ± 2°C, humidity of 50 ± 10%, and a 12-hour light and dark cycle. The use of animals in this study was approved by Baotou Medical College's Ethics Committee.

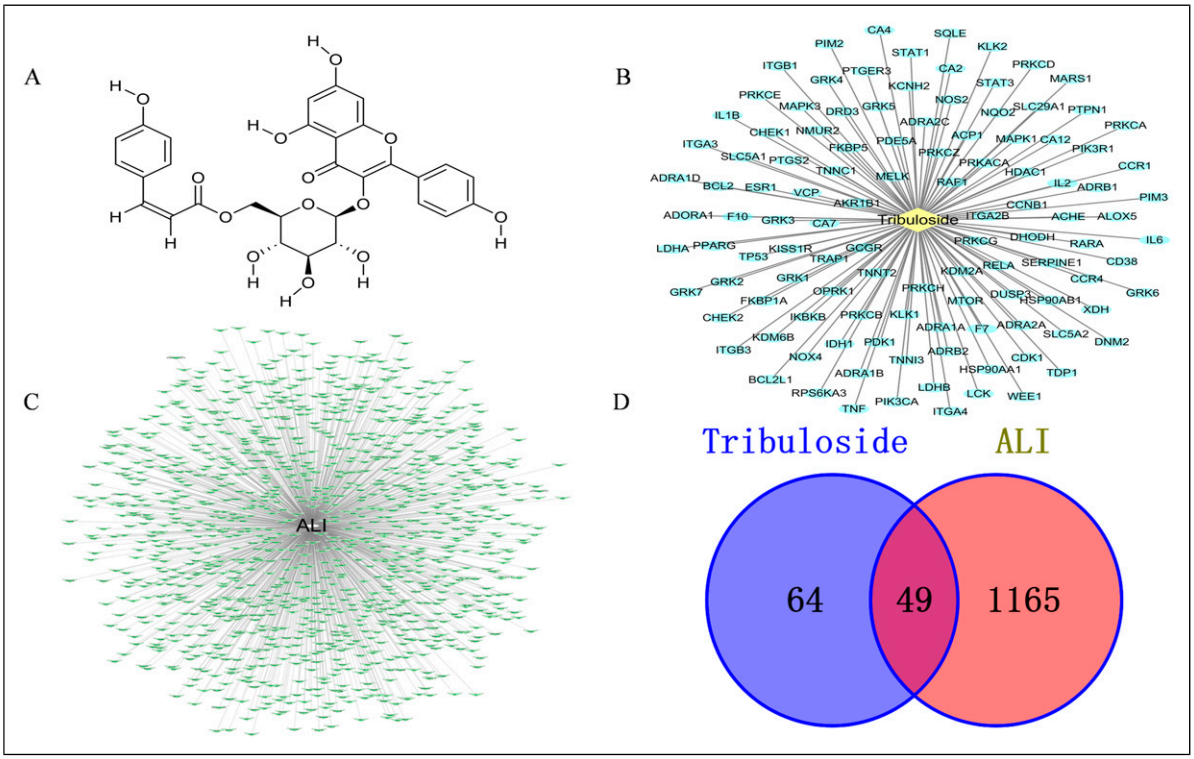

Figure 2. Integrated Analysis and Visualization of Medicine-target and Disease-target Interconnections. (A) 2D Structure of Tribuloside Obtained from PubChem Database. (B) Medicine-Target (M-T) network Constructed Using 113 Drug Targets Predicted by the SwissTargetPrediction Database. (C) Disease-Target (D-T) Network Generated from 1214 ALI-Related Targets Identified from the GeneCards Database. (D) Venny Diagram Illustrating 49 Intersecting Genes among the Potential Targets.

Experimental Design and Drug Administration in Mice

The National Institutes for Food and Drug Control in Beijing, China, furnished us with Tribuloside (batch number: C15302803; CAS number: 22 153-44-2; molecular formula: C30H26O13). We prepared it for delivery to mice by dissolving it in saline at a dosage of 10 mg/mL. The LPS used in this study (lot 120222230327) was acquired from Umicore in Brussels, Belgium. It was suspended in saline at a concentration of 3.5 mg/mL for testing purposes. Following a 1-week adaption period, we randomly assigned 48 male mice to 3 groups: control, LPS, and LPS + Tribuloside, each with 16 animals. The mice were sedated with an intraperitoneal dose of 1% pentobarbital sodium (10 mL/kg body weight). The Control group was exposed to room air and received an intraperitoneal injection of .2 µl/kg saline every day. In the LPS and LPS + Tribuloside groups, mice were intranasally injected with a single dose of LPS (3.5 mg/kg) following anesthesia.[31] In the LPS + Tribuloside group, Tribuloside was delivered intraperitoneally at a dosage of 10 mg/kg immediately after the mice recovered consciousness after the LPS intranasal injection.[32] For 3 or 7 days, the body's weight was continuously checked. We slaughtered 12 mice in each group on day 3 following LPS intranasal injection, with the remaining 6 sacrificed on day 7.

Sample Collection and Analysis for the Experimental Study

To obtain blood-free lung samples, we performed cardiac perfusion after inducing anesthesia. After that, the left lung was ligated, and the right lung underwent bronchoalveolar lavage (BAL) by flushing 2 mL of saline via the bronchus.[33] This approach enabled us to get bronchoalveolar lavage fluid (BALF). After collecting BALF samples, they were centrifuged at 3000 r/min for 10 minutes. The cell-free supernatants were kept at −80°C. These supernatants were then submitted to enzyme-linked immunosorbent assay (ELISA) to determine the expression levels of certain biomarkers. Furthermore, the left lungs were promptly removed and preserved in 4% paraformaldehyde for histological analysis.

Histological Examination

We processed the left lungs according to known techniques. After preparation, the paraffin slices were stained with hematoxylin and eosin (H&E) to evaluate inflammation and fibrosis. The stained sections were inspected using a light microscope (Olympus, Tokyo, Japan). Inflammation[34] and tissue fibrosis[35] were assessed and scored by an unbiased pathologist based on defined criteria. Lung tissue samples

were magnified ×1020 times under a microscope and examined using Image J software (https://imagej.net/) to determine the number of alveoli and % alveolar area.[36]

Tissue inflammation[34]

0: absence of inflammation;

1: random distribution of inflammatory cells;

2: bronchi and veins mainly surrounded by a sparse layer of inflammatory cells (1-5 cells thick);

3: bronchi and veins predominantly enveloped by a dense layer of inflammatory cells (>5 cells thick);

4: extensive pulmonary inflammation surrounding all veins and bronchi.

Tissue fibrosis[35]

0: normal pulmonary tissue;

1: minimal thickness of alveoli and bronchiole walls;

2-3: moderate thickness of alveoli and bronchiole walls with no significant lung structure damage;

4-5: increased fibrosis with notable damage to lung structure and the formation of small fibrotic clusters;

6-7: severe destruction of lung structure, extensive areas of fibrosis, and presence of "honeycomb lung";

8: complete fibrosis of the lung.

Cytokine Levels in BALF Were Measured Using ELISA

The quantities of IL-1β, IL-6, and TNF-α in BALF were measured using an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's specifications. The experiments were run in duplicate wells, and each sample's average absorbance at 450 nm was evaluated using a microplate reader (Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis of Data

The data were reported as mean ± SD. Statistical analysis was performed using IBM SPSS 21.0 software (IBM SPSS, Chicago, IL, USA). Group differences were assessed using one-way analysis of variance (ANOVA), with statistical significance defined at $P < .05$.

Results

Identifying Potential Targets for ALI Treatment Using Network Analysis

The 2D structure of tribuloside was determined by scanning the PubChem database. (Figure 2A). Using the SwissTargetPrediction database, 113 drug targets with a probability >0 were predicted, leading to the establishment of a Medicine-Target (M-T) network (Figure 2B) that demonstrates the connections between medicines and targets. In addition, 1214 targets related with ALI were discovered from the GeneCards database. These targets were shown in Cytoscape 3.7.1, demonstrating the interconnectivity of illnesses and targets. (Figure 2C). Next, a Venny diagram was generated, resulting in the identification of 49 intersecting genes (Figure 2D). These targets hold potential for the treatment of ALI.

Building a PPI Network for Tribuloside-based ALI Treatment

We built a PPI network to acquire a better understanding of the mechanisms underpinning Tribuloside treatment for ALI. Initially, we identified 49 common targets and then imported them into the STRING database to build the PPI network. The resulting network (Figure 3A) consisted of 51 nodes and 261 edges, with an average node degree of 10.2 and an average local clustering coefficient of .66. IL-6, TNF, IL-1β, MAPK3, BCL2, and STAT3 were selected as hub targets for experimental validation based on their network degrees.

Functional Analysis and Pathway Enrichment of Overlapped Targets

To determine the functional importance of the 49 overlapped targets, we performed GO functional analyses on the DAVID database. The study identified 283 biological processes (BP), 29 cell components (CC), and 59 molecular functions (MF) with significant thresholds of $P < .05$. To facilitate the understanding of the main findings from the GO analysis, Figure 3B presents a summarized representation of the top 10 results. Significant processes identified by the BP analysis include gene expression upregulation, response to external substances, promotion of smooth muscle cell proliferation, cytokine-mediated signaling pathways, inflammatory response, and facilitation of interleukin-6 production. The CC analysis indicated the cytosol, macromolecular complex, mitochondrion, and transcription factor complex as the primary biological components involved with the overlapped targets. In terms of MF results, functions such as protein binding, protein phosphatase binding, enzyme binding, and protein kinase binding were significantly enhanced. The KEGG enrichment analysis identified 162 highly enriched pathways at a threshold of $P < .05$. The T-P network in Figure 4, presents the top 10 pathways along with the overlapping targets. Tribuloside's efficacy against ALI was linked to KEGG pathways including PI3K-AKT, TNF, TLR, MAPK, and NF-κB.

Molecular Docking Analysis of Tribuloside and Target Proteins

Six putative target proteins were docked against Tribuloside's three-dimensional structure. The data were shown using the PyMOL2.5 program. As depicted in Figure 5A, Tribuloside's structure interacted with ARG-179, LYS-66, and GLN-175 via 1 hydrogen bond each in IL6. In Figure 5B, Tribuloside interacted with ARG-26, ARG-66, SER-64, and ARG-68, as

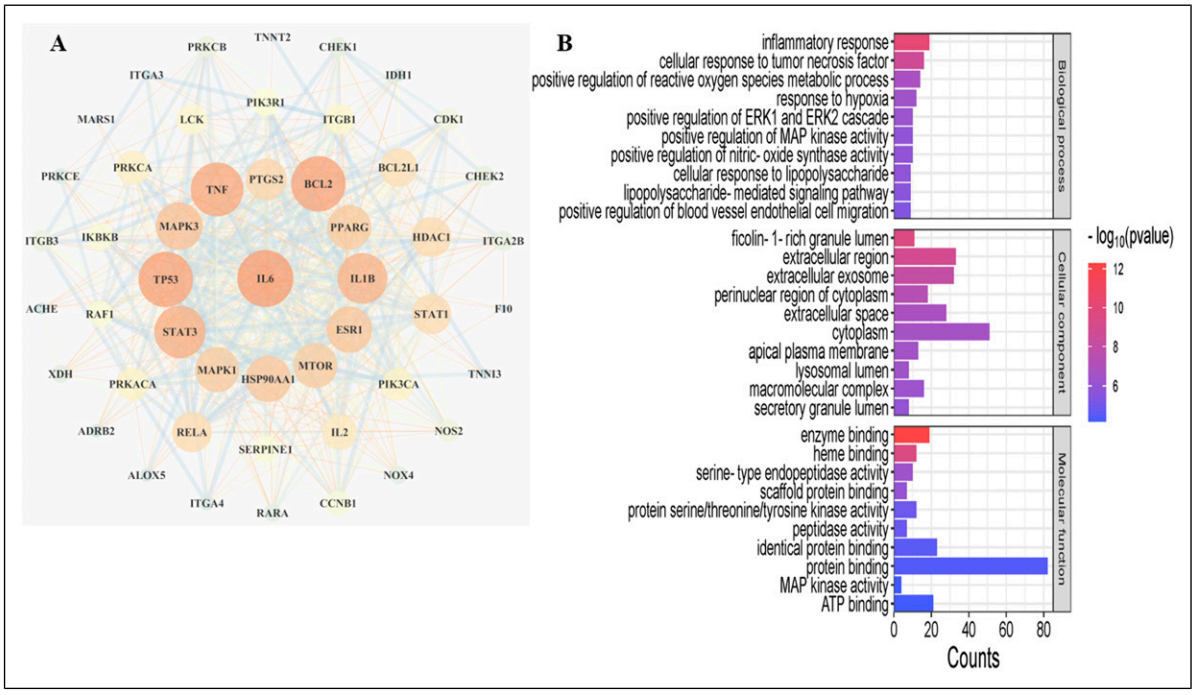

Figure 3. Construction and Analysis of PPI Network to Elucidate Tribuloside's Mechanisms in ALI Treatment. (A) The PPI Network Established Using 49 Overlapping Targets via the STRING database. The network Comprised 51 Nodes and 261 Edges, with an Average Node Degree of 10.2 and an Average Local Clustering Coefficient of .66. (B) Top 10 GO analysis Results Highlighting Key Biological Processes, Cell Components, and Molecular Functions Associated with the Overlapping Targets.

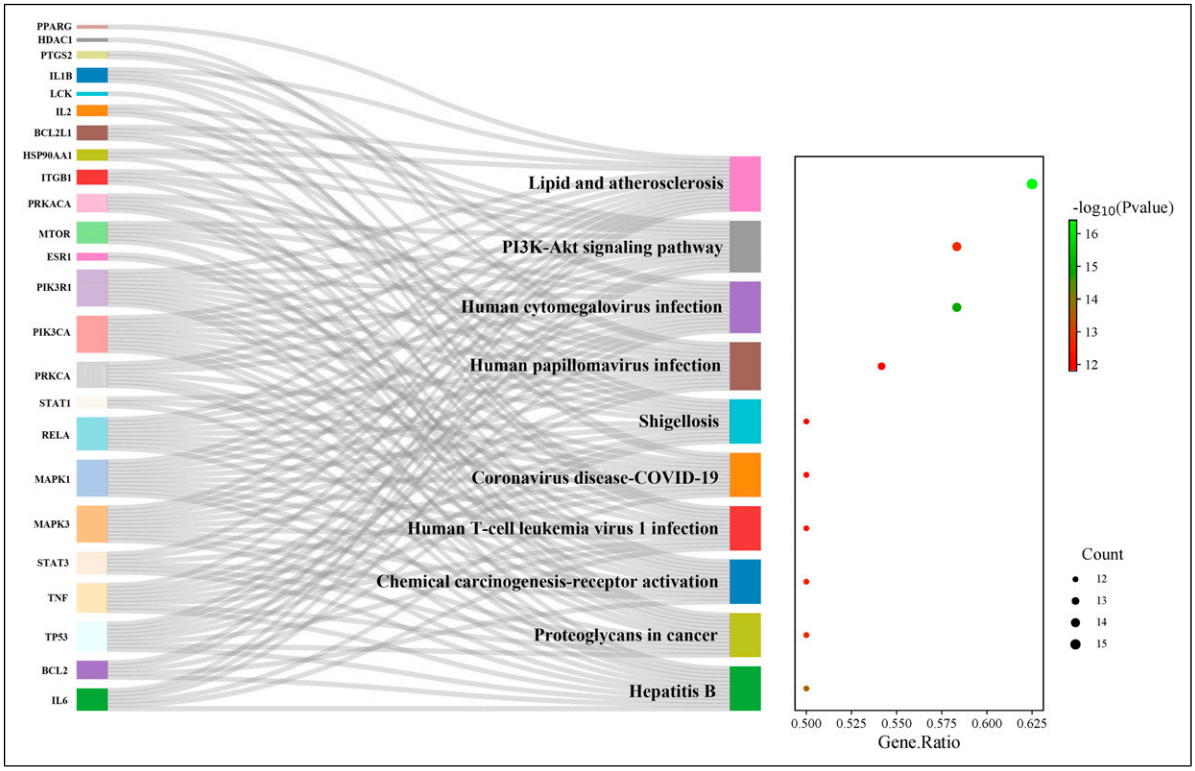

Figure 4. KEGG pathway enrichment analysis and visualization.

well as LYS-22, through 1 and 2 hydrogen bonds, respectively, in BCL2. The interaction between Tribuloside and STAT3 involved 1 hydrogen bond with PRO-333 and GLN-247, as shown in Figure 5C. Similarly, the hydrogen bond analysis revealed the interactions of Tribuloside with specific amino acids in different protein targets. In MAPK3, as depicted in Figure 5D, Tribuloside formed 1 hydrogen bond with GLU-194, one hydrogen bond with LYS-224, and two hydrogen bonds with GLN-372. The interaction between Tribuloside and TNF, represented in Figure 5E, involved LEU-43, ASN-30, and ARG-32, each connected through a single hydrogen bond. Lastly, Figure 5F illustrates that Tribuloside contributed 1 hydrogen bond each with LEU-62, SER-5, and LYS-88 in IL1B. Binding energy calculations were used to determine the affinity between the ligand (Tribuloside) and the protein target (receptor). The amount of the binding energy denotes the degree of their interaction, with values less than −7.0 kcal/mol indicating strong binding activity. A lower binding energy denotes higher affinity and greater conformational stability.[37] The docking results, presented in Table 1, demonstrated the favorable binding of Tribuloside to the active sites of the protein targets. Among them, the docking of MAPK3 and Tribuloside had the lowest binding energy. The average binding energy was −8.1 kcal/mol, indicating that all 6 candidate compounds exhibited high binding activity when docked with Tribuloside. These findings suggest that Tribuloside has potential as a therapy for ALI.

Tribuloside is a Promising Treatment for LPS-Induced ALI

In the control group, the lung tissue showed no signs of alveolar septum thickening, inflammatory cell infiltration, or fibrosis. In contrast, in the model group, the alveolar wall was severely damaged, resulting in an evident expansion of the alveolar septum. This was accompanied by a large infiltration of inflammatory cells, vascular congestion, and visible lung tissue fibrosis. Furthermore, on days 3 and 7, the alveolar septum suffered significant expansion, with a high prevalence

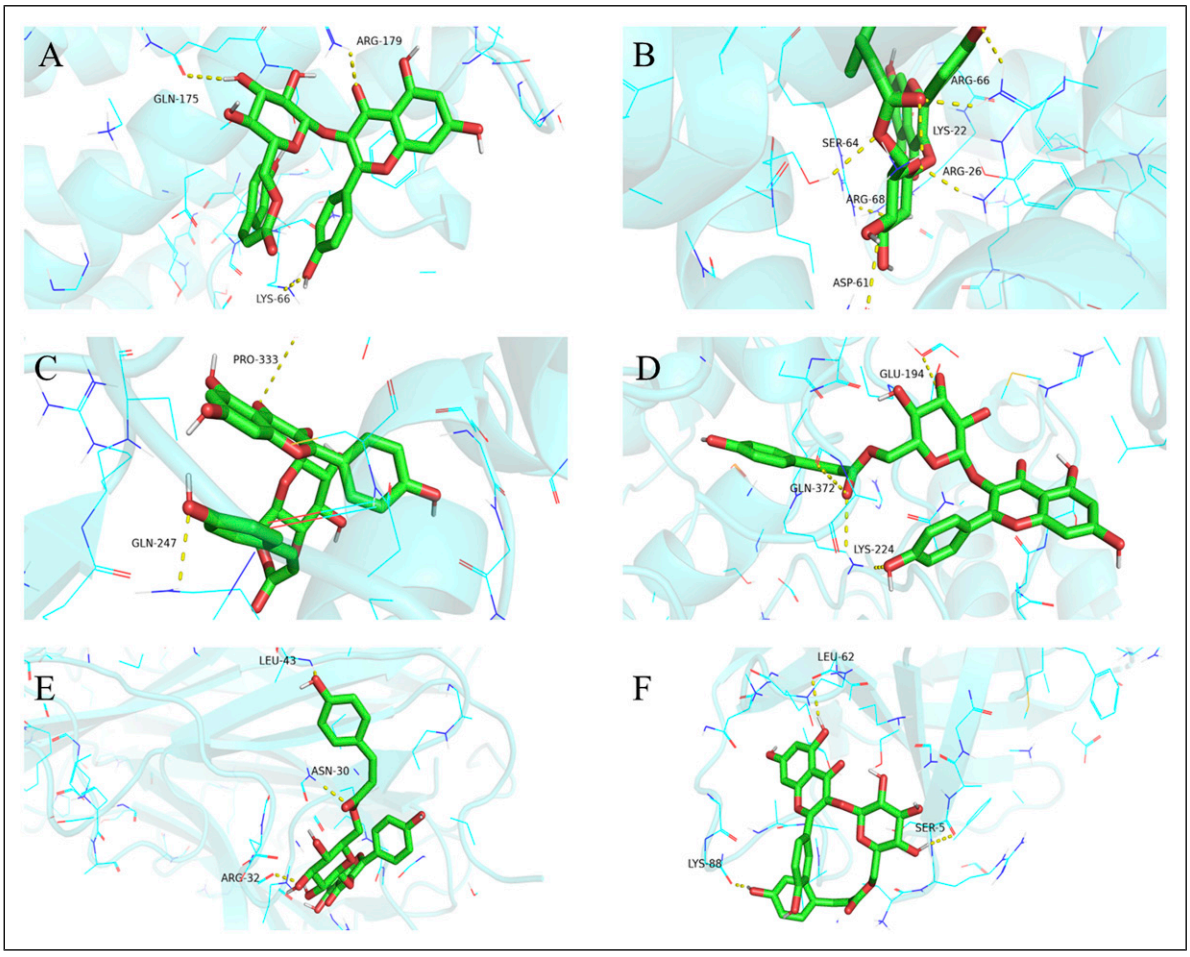

Figure 5. Molecular docking of Tribuloside with target proteins. (A) Interaction of Tribuloside with IL6; (B) Interaction of Tribuloside with BCL2; (C) Interaction of Tribuloside with STAT3; (D) Interaction of Tribuloside with MAPK3; (E) Interaction of Tribuloside with TNF; (F) Interaction of Tribuloside with IL1B. Hydrogen Bonds Are Shown as Dashed Lines.

Table 1. Docking Results for Tribuloside With Target Proteins.

Receptors	UniProt ID	PDB ID	Binding Energy (kcal/mol)	Coordinates
IL-1B	P01584	1HIB	−7.2	X: 24.699; Y: 2.659; Z: 73.44
TNF	P01375	1A8M	−8.4	X: 19.558; Y: 46.576; Z: 38.877
IL-6	P05231	1ALU	−7.7	X: −.09; Y: −21.154; Z: 9.752
BCL2	P10415	2W3L	−8.9	X: 34.941; Y: 34.908; Z: 4.686
MAPK3	P27361	2ZOQ	−9.7	X: 14.745; Y: −11.068; Z: 23.978
STAT3	P40763	6NJS	−7.6	X: −5.647; Y: 25.908; Z: 33.107

of infiltrating inflammatory cells. In Figure 6A, there were notable findings of fibroblast proliferation and focal aggregation. Additionally, the inflammation and fibrosis scores were significantly higher in the LPS group compared to the control group ($P < .001$, Figure 6(B), (C)). Importantly, treatment with Tribuloside for 3 or 7 days after LPS exposure showed a significant alleviation of these effects ($P < .001$ and $P < .05$, respectively). Furthermore, the LPS group showed a substantial decrease in both the quantity and percentage of alveolar area when compared to the control group ($P < .001$, Figure 6(D), (E)). Tribuloside administration on days 3 or 7 significantly increased the number of alveoli and percentage of alveolar area ($P < .001$). These findings underscore Tribuloside's ability to effectively cure LPS-induced ALI.

Tribuloside Lowers Inflammatory Cytokine Levels in LPS-Induced ALI

To study Tribuloside's anti-inflammatory effects on ALI, we used LPS-induced ALI lung tissue as an inflammatory model and measured cytokine levels in bronchoalveolar lavage fluid (BALF). Compared to the control group, LPS treatment significantly increased IL-1β, TNF-α, and IL-6 expression levels ($P < .001$, Figure 7(A)–(C)). In contrast, the levels of TNF-α, IL-6, and IL-1β decreased after the administration of Tribuloside in comparison to the LPS group ($P < .001$).

Discussion

ALI is a common respiratory illness characterized by a variety of pathological alterations caused by lung tissue injury.[38] The etiology of ALI/ARDS includes changes in pulmonary immune homeostasis, systemic inflammation, and functional apoptosis alterations, culminating in organ failure and negative consequences on overall vitality.[39] Traditional Chinese medicine (TCM) has long been used to prevent and treat a variety of ailments. Currently, various compounds such as baicalin, quercetin, curcumin, resveratrol, scopolamine, safranin, haustorium glycoside, and peonidin have been validated as beneficial therapeutic agents for acute lung injury within different chemical groups such as flavonoids, polyphenols, alkaloids, and saponins.[40-43] Tribuloside, a natural medicine, offers therapeutic potential; however, the bioactive components and mechanism of action in treating acute lung injury are unknown. As a result, the current study uses network pharmacology and molecular docking approaches to predict possible Tribuloside targets for ALI therapy. However, it is important to highlight that bioinformatics can only infer correlations and cannot provide information about the precise up- or down-regulation of a drug's biological activity. Thus, it is highly desirable to carry out experimental validation of the drug's activity and mechanism of action.[44] The LPS-induced mouse model is now the most often used approach for investigating acute lung damage. Endotoxins, or lipopolysaccharides (LPS), are substances found in bacteria's outer membranes. Upon bacterial infection, LPS can elicit an inflammatory immunological response in the body.[45]

In ALI, LPS stimulates immune cells in the lungs, leading to the release of inflammatory mediators such as TNF-α and IL-1. These chemicals can cause inflammation and lung tissue damage. Furthermore, LPS stimulates macrophages to produce chemokines, which attract additional inflammatory cells (such as neutrophils and monocytes) to the lung tissue, aggravating the inflammatory response. To summarize, LPS can cause acute lung injury via a variety of methods. In this study, we studied the influence of Tribuloside on an experimental mouse model of ALI caused by LPS. Tribuloside successfully reduced lung tissue inflammation and fibrosis, repaired damaged alveolar structure, lowered inflammatory cytokines (including IL-6, IL-1β, and TNF-α), and alleviated lung tissue damage in an LPS-induced ALI animal model. These findings were compatible with the predictions of network pharmacology and molecular docking analyses. Tribuloside's major targets for treating ALI include IL-6, BCL2, TP53, TNF, STAT3, IL-1β, and MAPK3. Notably, IL-6 emerged as the most important target among them, with the greatest number of target nodes in the core PPI network. IL-6, an inflammatory cytokine produced during numerous inflammatory processes, infection, and tissue injury, is critical in regulating acute-phase proteins. Furthermore, IL-6 induces the liver to create acute-phase response proteins, such as C-reactive protein (CRP), which aids in infection resistance and tissue regeneration. Given the importance of inflammatory reactions in the etiology of ALI,[46] strict control of IL-6 expression holds great importance. Furthermore, our findings showed that the LPS group had higher IL-6 expression than the control group. In contrast, the LPS + Tri group had lower levels of IL-6

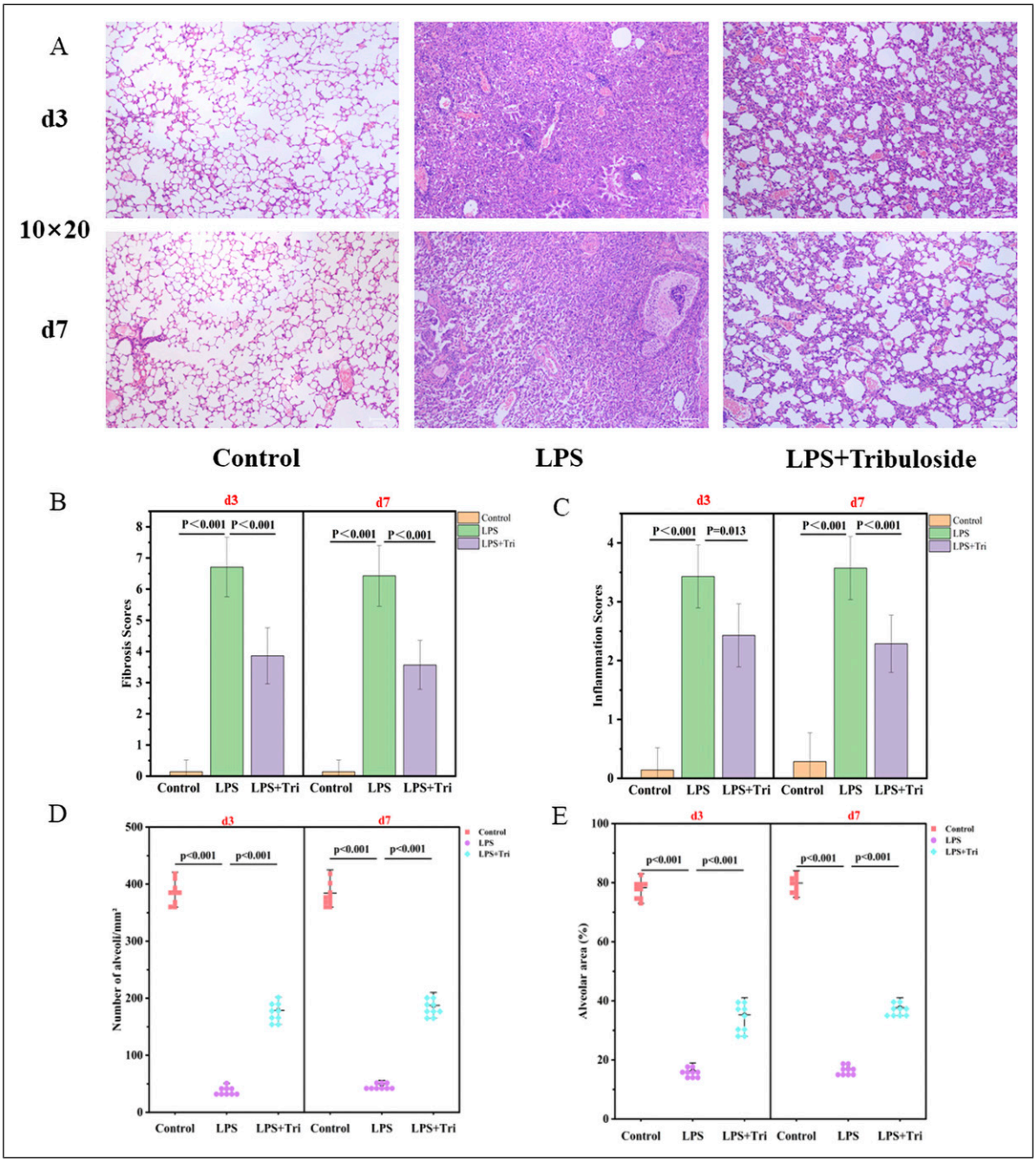

Figure 6. Histopathological changes in lung tissue. (A) Representative images showing the progression of lung injury in the control, model, and tribuloside-treated groups on days 3 and 7. (B) Inflammation scores in the control, model, and tribuloside-treated groups. (C) Fibrosis scores in the control, model, and tribuloside-treated groups. (D) quantification of the number of alveoli in the control, model, and tribuloside-treated groups. (E) analysis of the percentage of alveolar area in the control, model, and tribuloside-treated groups.

expression than the LPS group. These data lend credence to IL-6's critical role and putative involvement in Tribuloside's ALI protection. Our analysis of the core PPI network suggests that TNF and IL-1β, with higher degree values, may help mitigate ALI. Previous research has offered more support for our notion. TNF-α, an inflammatory cytokine, promotes leukocyte migration to infection or damage sites during inflammation. It also stimulates T-cell activity, contributes to antibody formation, and modulates cell-mediated immune responses.[47] Overactive TNF-α has been associated to numerous inflammatory disorders, such as rheumatoid arthritis, Crohn's disease, psoriasis, autoimmune diseases, and some cancers.[48] IL-1β is a key pro-inflammatory factor that causes localized symptoms like redness, swelling, discomfort, and

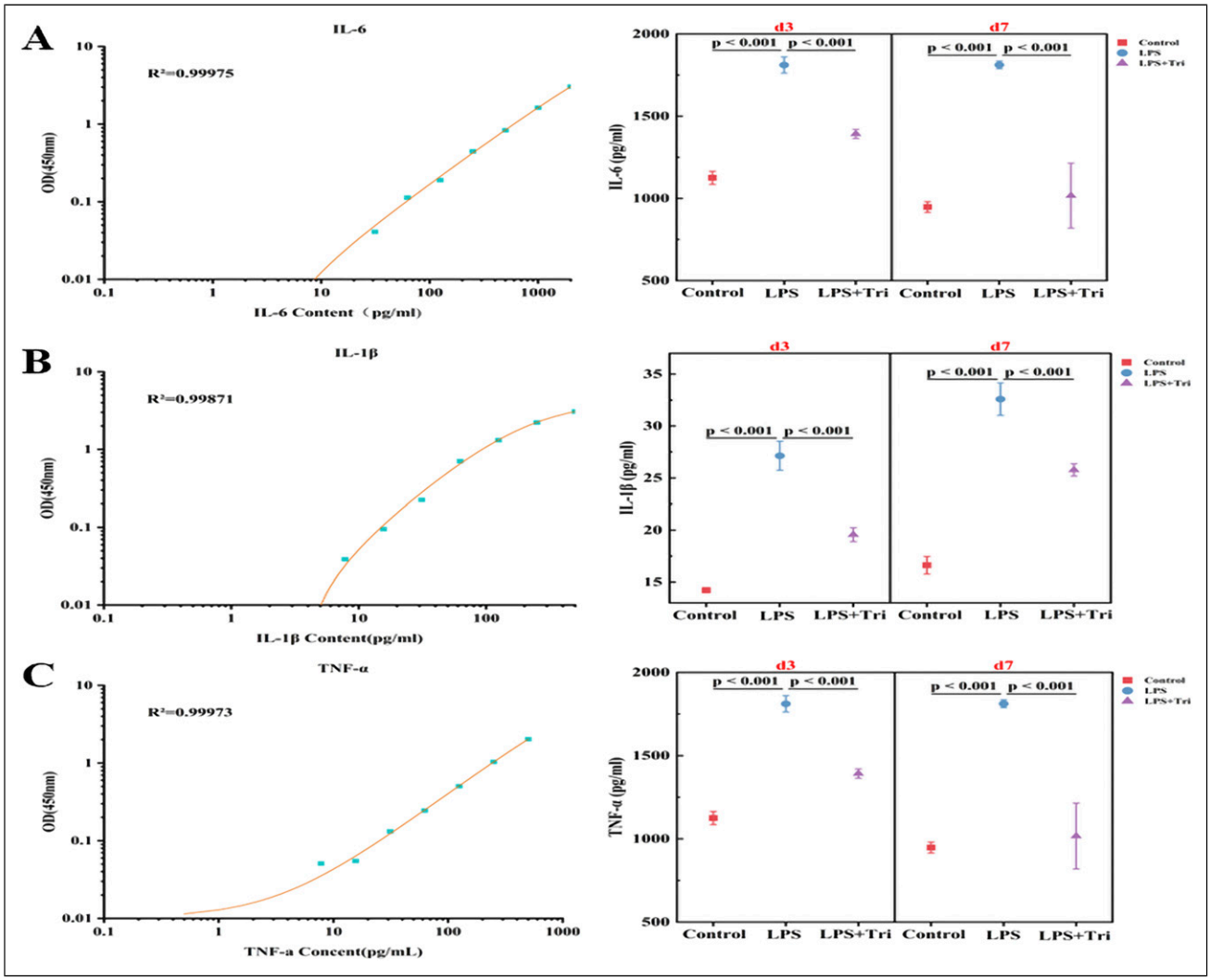

Figure 7. Effects of tribuloside on cytokine levels in LPS-induced ALI lung tissue. (A) Effect of tribuloside on IL-1β expression in LPS-induced ALI lung tissue. (B) Effect of tribuloside on TNF-α expression in LPS-induced ALI lung tissue. (C) Effect of tribuloside on IL-6 expression in LPS-induced ALI lung tissue.

fever. Excess IL-6, TNF-α, and IL-1β expression may indicate ALI-associated inflammation, according to studies.[49] KEGG data analysis revealed that Tribuloside treatment for ALI significantly enriched 49 target proteins over 162 signal pathways, indicating that it has therapeutic potential across numerous pathways. The PI3K-AKT signaling pathway, which had the highest gene count enrichment among pathways linked with ALI, was identified as a relevant pathway in the therapy of ALI. Inhibiting PI3K prevents multiplication and specialization of human lung fibroblasts into myofibroblasts.[50] Several studies have highlighted the significance of the PI3K-Akt pathway in ALI, which may contribute to pathological events such apoptosis, inflammatory responses, and increased alveolar permeability.[51] As a result, Tribuloside may control BCL2 via the PI3K-Akt pathway, which inhibits apoptosis-related signaling pathways, promotes cell survival, and reduces acute lung injury. The findings corroborate our hypothesis that the PI3K-AKT pathway is critical to Tribuloside's possible anti-ALI action. Furthermore, the GO enrichment analysis indicates that the key biological systems involved are associated with inflammatory reactions. The TNF signaling system, which is important in immunological and inflammatory processes, has been hyperactivated in a variety of illnesses, including cancer.[47] The TNF signaling pathway includes the core target proteins IL6, TNF, and IL1B. As a result, Tribuloside may reduce inflammation by regulating this mechanism. Six representative targets were discovered after doing network pharmacology analysis. Molecular docking was then used to validate the predictions produced by network pharmacology analysis. Docking findings demonstrated that Tribuloside binds well to IL6, IL1B, TNF, MAPK3, BCL2, and STAT3. The lowest binding energy was discovered between Tribuloside and MAPK3, indicating the most stable interaction. The MAPK pathway has been linked to the

progression of ALI/ARDS.[46,52] Tribuloside has anti-inflammatory properties and the ability to modulate the expression of inflammatory proteins and cell signaling pathways, presumably including the MAPK signaling pathway, which is critical in the inflammatory response. Tribulosides have also been investigated as anticancer drugs, with the potential to inhibit tumor growth by influencing cell proliferation, apoptosis, and metastasis. These effects may involve the MAPK signaling system due to their function in cell survival and apoptosis. It is important to recognize the limits of this work. There is currently no defined constraint for screening conditions of diseases and pharmacological targets in public network pharmacology databases. Furthermore, variations in data update speed and inclusion procedures between databases make it difficult to provide comprehensive and consistent data searches. Besides, The brief period of this experiment limits its ability to see how the medicine works in the long run. The study found that Tribulosides prevented Acute Lung Injury in mice. However, differences in metabolic rates between the 2 species make it unclear whether this effect can be recreated in humans.

Conclusion

In conclusion, a network pharmacology analysis was used to investigate the effects of Tribuloside on ALI. The research revealed IL6 as the most important target and the PI3K-AKT signaling pathway as critical. Furthermore, the findings were validated using molecular docking analysis, lending credence to the network pharmacology-based method. Furthermore, in vivo tests were used to validate Tribuloside's molecular mechanism of action against ALI. Using a multidisciplinary approach, this study provides solid data supporting Tribuloside's therapeutic potential in treating ALI.

Appendix

Abbreviations

ALI	Acute lung injury
PPI	Protein-protein interactions
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPS	Lipopolysaccharides
TNF-α	Tumor necrosis factor-α
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-1β	Interleukin-1β
IL1B	Interleukin-1B
MAPK3	Mitogen-activated protein kinase 3
BCL2	B-cell lymphoma 2
STAT3	Signal transducer and activator of transcription 3

Authors Contribution

Z.Y. and M.Q. conceived and designed the study. T.H. performed the experiments. T.H. analyzed the data. T.H. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Statement

Ethical Approval

All experiments involving mice were approved by the Animal Ethics Committee of Laboratory Animals at Baotou Medical College in Baotou, China. (No. [2023]38).

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Data Availability Statement

The data that supports the findings of this study is available from the corresponding author upon reasonable request.

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