

# Herbal Medicine for Treatment and Prevention of ALI/ARDS in Pre-Clinical Studies

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## Abstract.

Acute lung injury (ALI) and its aggressive form, the acute respiratory distress syndrome (ARDS), are a respiratory gas exchange disorder with several etiologies, including pneumonia, inhalation of toxic substances, sepsis, burns, pancreatitis, blood infections, etc. These etiologies cause injury to endothelial and epithelial lung cells leading to elevation of vascular and endothelial permeability, pulmonary edema, hypoxia, recruitment of inflammatory cells, and micro-vascular thrombosis. This review focuses on the ameliorative effects of some medicinal plants on different murine models of ALI.

## Introduction.

Acute lung injury (ALI) and its dangerous form, acute respiratory distress syndrome (ARDS), are a type of respiratory failure distinguished by extensive inflammation and severe hypoxemia due to ventilation/perfusion mismatching in the lung tissues. They are stimulated by direct bacterial, viral, or fungal infections of the lung and indirect infections from the urinary tract, peritoneum, skin, soft tissue, major trauma, and burns. Furthermore, aspiration of oral, gastric, and oesophageal contents that might be accompanied by infectious substances contributes to the excitement of ALI. Drug toxicity, acute pancreatitis, hemorrhagic shock, or ischemia-reperfusion injury, and smoke inhalation are etiologies less frequently correlated with the development of ALI/ARDS. Pulmonary edema from the neurogenic origin, high altitude, and that following lung transplantation are regarded as additional etiologies of ARDS. [1]

## The normal alveolus structure.

The healthy alveolar epithelium is a barrier comprised of two types of cells, type I cells (ATI) and type II cells (ATII). ATI cells are principally responsible for gas exchange, but they also regulate ion and water transport, peptide metabolism, macrophage function, cell proliferation, and signaling pathways. [2]

ATII cells synthesize the surfactant proteins (SPs) that help prevent airway collapse and have local defense functions of the respiratory tract. Moreover, ATII cells generate growth factors and cytokines and endogenous antimicrobial agents such as catelicidin lysozyme, lipocalin 2,  $\beta$ -defensin. Furthermore, ATII cells can self-regenerate and Trans-differentiate into ATI, in addition to their role in sodium transport that keeps alveoli remain relatively dry. [3]

The endothelium barrier lines the interior surface of blood vessels and regulates capillary permeability, angiogenesis, vasoconstriction, and vasodilation. It also has an antigen presentation capability. [4]

The interaction between the epithelial and endothelial barriers has a crucial role in managing fluid accumulation in the alveolar space. The alveolar epithelial cells are connected with tight junctions composed of transmembrane occludins and claudins and cytoplasmic zonula occludens (ZO). These tight junctions control the passage of fluid and ions beyond the epithelium. The epithelium also expresses adherens junctions composed of plasma membrane E-cadherin and  $\beta$ -catenin to maintain cell architecture. The endothelium is also

linked by tight junctions and adherens junctions that have a pivotal role in barrier function. The adherens junctions compose of vascular endothelial (VE) cadherin and form a complex with p120, alpha, and beta catenins. [5]

Alveolar macrophages (AM) are the primary resident mononuclear phagocytic cells in the bronchoalveolar space. There are three types of macrophages, namely pro-inflammatoryM1, anti-inflammatoryM2, and regulatory macrophages. [6]

Several lung fibroblast subtypes have been recognized, such as myofibroblasts, lipofibroblasts, and their precursors. Under normal conditions, fibroblasts produce a scant quantity of extracellular matrix. When an injury occurs, fibroblasts differentiate into secretory and contractile subtypes, migrate to the damage site, and secrete large amounts of extracellular matrix elements enhancing tissue repairing and wound healing. [2]

## **Models of ALI/ARDS.**

### **Lipopolysaccharide (LPS) induced ALI**

LPS is a pathogen-associated molecular pattern (PAMP), also known as endotoxin, and it is a virulence factor in the outer membrane of Gram-negative bacteria. LPS is extracted from bacteria and solubilized via LPS binding protein(LBP) and then transferred to glycoprotein CD14 that has two forms, membrane-bound CD14 (mCD14) in myeloid cells and soluble CD14 (sCD14) in the blood that responds to LPS in cells without mCD14 such as epithelial and endothelial cells, however CD14-independent binding of LPS exists. CD14, in turn, transfers LPS into myeloid differentiation 2 (MD-2) that facilitate the attachment to special pattern-recognition receptors (PRR) called Toll-like receptor 4 (TLR4). [7]

Upon binding to LPS, two copies of the TLR4-MD-2-LPS complex join to initiate the intracellular signaling pathways of TLR4. The first signaling pathway is myeloid differentiation primary response protein 88 (MyD88) dependent pathway. MyD88 and MyD88-adaptor-like protein (MAL), (MyD88/ MAL) recruit IL-1 receptor-associated kinase (IRAK4, IRAK1/2). IRAK4, IRAK1, and IRAK2 are activated via phosphorylation and separated from the MyD88/IRAK complex and bind to (TNF receptor-associated factor 6) TRAF6. TRAF6, in turn, activates the transforming growth factor B-activated kinase (TAK1) complex. The activated TAK1 forms a complex with TAK1 binding protein 1 (TAB1), TAB2, and TAB3 that activates two distinct pathways. The first pathway begins with activation of the I- $\kappa$ B kinase (IKK) complex that phosphorylates the NF $\kappa$ B inhibitor (I $\kappa$ B) for proteasomal degradation. Following the degradation of I $\kappa$ B, NF- $\kappa$ B translocates into the nucleus and induces inflammatory mediators. In the second pathway, TAK/TAB complex activates the MAPK family complex (c-Jun N-terminal Kinase (JNK) and p38, extracellular signal-regulated kinase (ERK)), which activates activator protein 1 (AP1) that enters the nucleus to excite gene expression. AP-1 can combine with several transcription factors to form complexes that synergistically mediate the transcription of fibrogenic genes. [8]

The activation of the NF- $\kappa$ B and MAPK pathways can regulate various inflammatory mediators, including Interleukin-1 $\beta$  (IL-1 $\beta$ ), IL1, IL12, IL-4, IL-10, IL6, IL-17, IL8, Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interferon gamma (IFN- $\gamma$ ), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), intercellular Adhesion Molecule 1 (ICAM-1), and chemokines such as macrophage inflammatory protein 3 (MIP-3) and monocyte chemoattractant protein 1 (MCP-1), ending in the elimination of bacterial pathogens. Though, exaggerated stimulation of inflammatory mediators results in septic shock. [9-12]

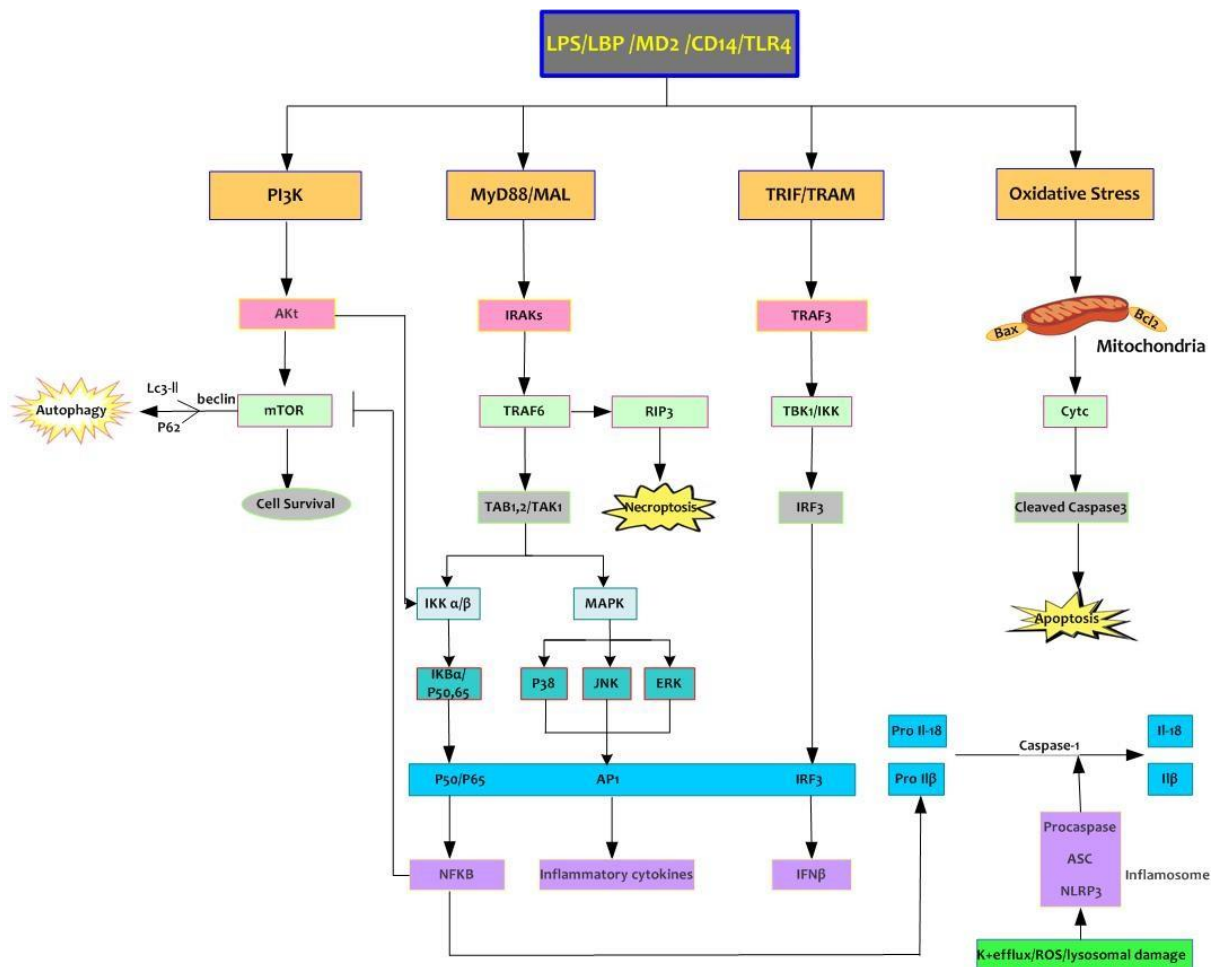
In the MyD88-independent pathway, Toll/IL-1R domain-containing adaptor-inducing IFN- $\beta$ -related adaptor molecule/Toll/IL-1R domain-containing adaptor-inducing IFN- $\beta$  (TRAM/TRIF) recruits TRAF3, T BK1, and IKK and interferon regulatory factor 3 (IRF3), conclusively provoking transcription of IFN1 (type I interferon). The myD88-independent axis also activates NF- $\kappa$ B signaling, but at a later time. [13]

Besides MAPKs and NF- $\kappa$ B, (phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling also contributed to the signal transduction of TLR4, controlling the expression of both inflammatory and anti-inflammatory cytokines. TLR4/PI3K/Akt pathway mediates the expression of Transient receptor potential canonical 6 (TRPC6) and TRPC6-dependent Ca<sup>2+</sup> influx that is involved in LPS-stimulated epithelial inflammatory reactions via the activation of ERK1/2, p38, and NF- $\kappa$ B, consequently stimulating the production of inflammatory cytokines. [14]

TLR4-independent mechanisms of ALI by LPS have also been proposed via the upregulation of caspase-11 in mice without TLR4. [15] Moreover, LPS can attach to the P2X7 receptor expressed by immune cells leading to enhanced cellular Ca<sup>2+</sup> permeability and alterations in ion homeostasis and consequently the discharge of pro-inflammatory mediators. [16]

The nucleotide-binding domain, leucine-rich repeat protein-3 (NLRP3) inflammasome, has a substantial effect on inflammatory lung injury. NLRP3 inflammasome is complex consists of sensor protein, the adaptor protein, an apoptosis-associated speck-like protein with caspase activation and recruitment domain (ASC), and procaspase-1. [17]

The activation process of NLRP3 inflammasome is a two-step mechanism. The priming step includes expression and post-translational modification of NLRP3 and pro-IL-1 $\beta$  and the activating step. In Canonical NLRP3 inflammasome activation, the priming step is usually enhanced by TLRs and NF- $\kappa$ B stimulation, mediating the expression of NLRP3. In response to damaged ROS-producing mitochondria, destabilized lysosomes, and the ion efflux and influx enhanced by DAMPs or PAMPs, inflammasomes are assembled, and consequent caspase-1 activation takes place in the activating step. Caspase-1 activates pyroptosis and cleaves pro-IL-1 $\beta$  and pro-IL-18 to their mature frames. In the non-canonical NLRP3 inflammasome activation, LPS enters the cytosol and is detected with Pro-caspase-11 that, in turn, stimulates the NLRP3 assembly and the consequent activation of pyroptosis and cytokines release. [18]



### Schematic overview of the Toll-like receptor (TLR) 4 signaling pathway, inflammasome activation, and multiple cell death modalities.

LPS interacts with LBP and binds to TLR4 with the aid of CD14 and MD2, activating MyD88-dependent and MyD88-independent TLR4 signaling pathways. In the MyD88-dependent pathway, MyD88/ MAL recruits IRAK4, IRAK1/2. IRAK4, IRAK1, and IRAK2 are phosphorylated and separated from the MyD88/IRAK complex and bind to TRAF6. TRAF6, in turn, activates the TAK1 complex that consequently forms a complex with TAB1, TAB2, and TAB3 and activates two distinct pathways. The first pathway begins with activation of the I- $\kappa$ B kinase (IKK) complex that degrades I $\kappa$ B to permit translocation of NF- $\kappa$ B into the nucleus and induces inflammatory mediators. In the second pathway, TAK/TAB complex activates the MAPK family complex (JNK, p38, ERK), in which AP1 enters the nucleus to excite gene expression. In the MyD88-independent pathway, TRAM/TRIF recruits TRAF3, TBK1, and IKK and IRF3 to stimulate IFN1. TLR4/PI3K/Akt pathway is also downstream of MyD88 that activates ERK1/2, p38, and NF- $\kappa$ B. Activation of the NLRP3 inflammasome, composed of NLRP3, ASC, and pro-CASP1, is managed by two-step signals. The priming step includes the expression of NLRP3 and pro-IL-1 $\beta$  via activation of NF- $\kappa$ B. The second "activation" signal promotes the assembly of inflammasome components via three major mechanisms, including ROS, lysosomal damage, and K<sup>+</sup> efflux leading to caspase-1 activation. Caspase-1 activates pyroptosis and cleaves pro-IL-1 $\beta$  and pro-IL-18 to their mature frames. If excessive, autophagy stimulates cell death. The deactivation of the PI3K/AKT/mTOR pathway activates autophagy. LC3, Beclin 1, and p62 are autophagy-related proteins. Apoptosis, necroptosis, and pyroptosis are programmed forms of cell death. Apoptosis relies on caspase activation and Cyt-C release under the control of the Bcl-2 and Bax. Necroptosis depends on RIP3.

## **Effect of LPS on the alveolus structure**

### **Effect of LPS on alveolar epithelial and endothelial cells**

ATI and ATII respond to LPS via TLRs and produce several pro-inflammatory mediators. ATI cells also express receptors for advanced glycation end products (RAGE) that also mediate LPS induced inflammatory effects. LPS via induction of oxidative stress can activate apoptosis, autophagy, and necroptosis of ATII and decreases their ability to generate lung surfactant. LPS interacts with the TLR4 receptor on endothelial cells and activates the consequent discharge of inflammatory molecules. LPS also activates extraordinary levels of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and complement factors such as C1s, C1R, and C3 that induce endothelial cell damage during ALI and sepsis. [19]

### **Effect of LPS on alveolar macrophages and Fibroblasts**

Stimulation of human macrophage THP-1 cells with LPS in vitro resulted in up-regulation of cytokine expressions, TLR4, NF- $\kappa$ B, MAPKs, and Janus kinase/STAT3 axes with consequent translocation of NF- $\kappa$ B, AP-1, and STAT3 into the nucleus where it induces their gene expression. [20]

LPS through TLR4 Signaling stimulates fibroblast differentiation into synthetic and contractile myofibroblasts and collagen production that results in lung fibrosis. [19]

### **Role of neutrophil in LPS-induced ALI**

Several chemokines in the primary site of lung damage, like IL-8 and IL-17, stimulate the neutrophil influx from blood circulation into the alveolar air space, increasing lung tissue destruction and endothelial hyperpermeability via aROS and proteolytic enzymes-dependent mechanisms. [21]

Neutrophils also express TLRs that recognize pathogen-associated molecular patterns (PAMPs) and respond to invading microbes. Pathogens binding to neutrophil receptors increases calcium release from the endoplasmic reticulum and the membrane channels that stimulate PKC and induce nitric oxide and the NADPH dependent ROS production resulting in the activation of neutrophil suicidal NETosis. NETosis is the mechanism by which neutrophils damage microbial virulence agents and begin cell rupture. This self-sacrificing activity of neutrophils towards the invading microbes, although advantageous concerning capturing microbes but its exaggeration leads to cell injury. Injury of the vascular endothelial cells by NETs can result in endothelial remodeling, allowing extravasation of platelets. [22, 23]

### **Autophagy and ALI**

Autophagy is a cellular mechanism that removes dysfunctional ingredients within lysosomes. The inhibition of the mammalian target of rapamycin (mTOR), the downstream of PI3K /Akt pathway stimulates autophagy. The role of autophagy in ALI persists unclear. Some studies showed that autophagy could protect against ALI, while others found that autophagy aggravated it. Consequently, the role of autophagy in ALI may depend on the pathophysiology of ALI and whether the autophagic process was over-activated. [24]

### **ALI and apoptosis, necrosis, necroptosis, fibrosis**

Apoptosis is one of the principal mechanisms that control normal cellular homeostasis by eliminating injured or dysfunctional cells. If apoptotic cells are not cleared, they eventually undergo secondary necrosis producing leakage of cellular contents, inflammatory reactions,

and severe tissue damage. Alveolar cell apoptosis results in impairment of endothelium and epithelium barrier. During the disease progress, oxidative stress leads to apoptosis via a caspase-dependent pathway that impairs mitochondrial energy metabolism and accelerates the release of Cytochrome c (Cyt-C). Cytochrome c release is under the control of the anti-apoptotic Bcl-2 and the pro-apoptotic Bax, and it activates a caspase-dependent pathway and conclusively causes apoptotic DNA fragmentation. LPS, pro-inflammatory cytokines, and oxidative stress activate MAPK-mediated apoptotic cell death via a caspase-independent pathway. [25]

ROS also activates the programmed and regulated form of necrosis, necroptosis via receptor-interacting protein kinase 3 (RIP3) /mixed lineage kinase domain-like protein (MLKL). [26]

The accumulation of protein-rich fluid and dead cell debris in the bronchoalveolar space diminish gas exchange capacity. However, type II alveolar cells differentiate to type I alveolar cells, besides new vascular endothelium develops to overcome lung injury and increase oxygenation.

Maintenance of inflammatory reaction may end in lung fibrosis. The principal cellular mediator of fibrosis is myofibroblasts. Transforming growth factor (TGF)  $\alpha$ , TGF- $\beta$ , IL-1 $\beta$ , VEGF, macrophage inflammatory protein 2(MIP2), Platelet-derived growth factor (PDGF) are important regulators of fibrosis via Promoting the deposition of excess collagen and other extracellular matrix components. [27]

### **Major cellular defense mechanisms**

AMPK is a serine/threonine-protein kinase comprising of catalytic  $\alpha$  ( $\alpha$ 1 and  $\alpha$ 2), and regulatory  $\beta$  ( $\beta$ 1 and  $\beta$ 2) and  $\gamma$  ( $\gamma$ 1,  $\gamma$ 2, and  $\gamma$ 3) subunits. Expanding research implies that anti-inflammatory effects exerted by AMPK activation in various immune cells, including neutrophils, T cells, macrophage, and mast cells. AMPK activation hindered NF- $\kappa$ B upregulation, MAPKs phosphorylation, and the generation of IL-6, IL-1 $\beta$ , iNOS, COX-2, and MCP-1, in LPS-induced macrophages. [28]

The activation of AMPK $\alpha$ 2 isoform, containing the catalytic  $\alpha$ 2 subunit of AMP, hindered IKK phosphorylation and I $\kappa$ B $\alpha$  ubiquitination in endothelial cells (ECs) from AMPK $\alpha$ 2 $^{+/+}$  but not in ECs from AMPK $\alpha$ 2 knockout mice. [29] AMPK activation also inhibited IKK phosphorylation in LPS-induced macrophages. [30]

Nrf2 is the central switch for the expression of most of the cellular antioxidant proteins. Under basal conditions, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1). Upon oxidative stress, Nrf2 is activated and translocated into the nucleus, dimerized with Maf protein, and bound to antioxidant response element (AREs). Nrf2/ARE binding regulates the anti-inflammatory, and antioxidant gene expression such as heme oxygenase 1 (HO-1), catalase and SOD, glutathione cysteine ligase regulatory subunit (GCLC), glutathione cysteine ligase modulatory subunit (GCLM), NADPH quinone oxidoreductase 1 (NQO1), thioredoxin-1 (Trx-1) etc. In this process, AMPK inactivates glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) to increase the nuclear accumulation of Nrf2. [31] Nrf2 activation is known to inhibit NF $\kappa$ B activation. [32] PI3K / Akt Mediate the activation of Nrf2 axis as well. [33]

SIRT1 is nicotinamide adenine dinucleotide (NAD) dependent protein deacetylase that regulates cellular responses to stressors and energy metabolism and stimulates Nrf2 transcriptional activity and upregulate Nrf2 downstream gene expressions such as those encoding SOD and GSH so that the SIRT1/Nrf2 axis can ameliorate the oxidative stress injury by increasing the total antioxidant capacity (TAC). [34]

During cellular stress conditions, the Forkhead box O transcription factors (FOXOs) translocate into the nucleus, increase their protein expression, and consequently control cell cycle, improve cellular immunity, and combat oxidative stress. [35]

The forkhead box O3 gene (FOXO3), and its homologs, regulate genes implicated in DNA protection, longevity, metabolism, and overcoming oxidative stress, autophagy, and cellular apoptosis. [36]

### Schematic overview of the major cellular defense mechanisms

During oxidative stress, Sirt1 and FOXO3 create a complex that results in deacetylating FOXO3 to defend against oxidative stress. Sirt1 deacetylates FOXO1 and combat oxidative stress response also. Similarly, Sirt1 binds to FOXO1 and accumulates FOXO4 in the nucleus to defend against oxidative stress. Besides, FOXO3a and FOXO1 modulate and enhance Sirt1 expression. Upregulation of Sirt1 and FOXO1 via inhibition of miR-217 in endothelial cells enhances antioxidant capability and counteracts their dysfunction. Sirt1 deacetylates p65 and inhibits NF- $\kappa$ B transcription. Sirt1 diminishes NADPH oxidase (NOX) generation, upregulates both AMPK and manganese superoxide dismutase (MnSOD) expression. [35]

### Natural agents found to ameliorate LPS induced ALI

#### Thymol

Thymol is a natural phenol compound extracted from thyme. It has anti-inflammatory and antioxidant properties. Thymol inhibited LPS mediated inflammatory cytokines production in BALF such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in the LPS-induced mice model of ALI. The inhibition of these inflammatory cytokines was due to the suppression of the NF- $\kappa$ B signaling cascade. Furthermore, thymol significantly decreased lung tissue levels of myeloperoxidase (MPO) and malondialdehyde (MDA). Thymol antioxidant effect was due to the up-regulation of the (Nrf2/HO-1). Moreover, thymol reduced lung wet-to-dry (W/D) ratio and histopathological alterations of lung tissue mediated by LPS challenge. [37]

#### 3, 4, 5-Trihydroxycinnamic Acid

Trihydroxycinnamic acid (THC) efficiently downregulated the mRNA expression of IL-8 in LPS-challenged A549 airway epithelial cells in vitro. THC also enhanced the expression of HO-1 enzyme in A549 cells. THC also activated AMPK in A549 cells and RAW264.7 macrophages. The stimulation of the HO-1 enzyme and AMPK protected against the inflammatory process induced by LPS in vitro. In the LPS challenged mice model of ALI, THC significantly decreased LPS mediated neutrophil infiltration in BALF and levels of neutrophil-derived proteolytic elastase (NE) in BALF and serum. THC also diminished inflammatory mediators releases such as MCP-1 in the BALF and TNF- $\alpha$  and IL-6 in BALF and serum. Moreover, THC prevented iNOS expressions and NF- $\kappa$ B activation. [38]

#### Puerarin

Puerarin is an isoflavonoid extracted from Gegen, the Chinese herb. Puerarin significantly suppressed LPS-induced MPO activity in lung tissues of the mice model of LPS-induced ALI. Meanwhile, puerarin reduced histopathological injuries of the lung and lung W/D ratio. Treatment with puerarin inhibited the expression of inflammatory cytokines, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Puerarin also prevented LPS-stimulated TNF- $\alpha$  expression in RAW264.7 cells and IL-8 expression in A549 cells in vitro. [39]

#### Aucubin

Aucubin is a biological molecule extracted from plants, including *Eucommia ulmoides* and *Aucuba japonica*. It displayed anti-inflammatory and anti-oxidative properties. In the LPS-challenged mice model of ALI, Ai decreased LPS induced oxidative stress via decreasing MDA



and O<sub>2</sub>• activity and increasing major antioxidant defense systems, including Nrf2, HO, and NQO-1. Moreover, Ai reduced LPS induced inflammatory cytokines and down-regulated the expression of phosphorylated NF-κB (p-NF-κB). Also, in LPS-challenged macrophages, in vitro, Ai decreased ROS release but increased expression of NQO-1/ HO-1. [40]

### **Isoalantolactone**

Isoalantolactone (ISO) is a sesquiterpene lactone extracted from *Inula helenium* with anti-inflammatory properties. ISO histopathologically diminished lung injury generated by LPS in mice model of ALI. ISO also decreased MPO, MDA, lung W/D, and inflammatory cytokines production, including TNF-α and IL-1β, instigated by LPS. ISO increased the activities of SOD, catalase (CAT), and glutathione peroxidase (GPX). ISO also suppressed the PI3K / AKT pathway that explained the decrease in NF-κB activation. Moreover, ISO enhanced Nrf2/HO-1 signaling pathway. [41]

### **Pristimerin**

Pristimerin is a triterpenoid compound sourced from the Hippocrateaceae and Celastraceae families. Pre-treatment with Pris meaningfully diminished lung edema in a mice model of LPS-induced ALI. Moreover, Pris reduced LPS triggered inflammatory cell recruitment into mice lung tissue, inhibited lung MPO activity, and improved lung histopathological alterations. Pris also prevented oxidative stress and improved the antioxidant capacity of the lung tissue. Moreover, pris attenuated inflammatory cytokines such as TNF-α and IL-6, inhibited LPS-induced elevation of pro-apoptotic protein, Bax, and caspase-3, and up-regulated the antiapoptotic Bcl2 protein. [42]

### **Isoliquiritigenin**

Isoliquiritigenin (ISL) is a chalcone molecule with anti-inflammatory potential. ISL hindered inflammatory cytokine production in isolated mouse peritoneal macrophages challenged with LPS in vitro. ISL inhibited LPS-induced structural injury and inflammatory cell influx in the mice models of LPS challenge mediated ALI in vivo. These protective effects were due to the downregulation of the NF-κB cascade that mediated suppression of inflammatory reactions in vitro and in vivo. [43]

### **Barbaloin**

Barbaloin is an anthraquinone molecule extracted from Aloe leaf exudate. Barbaloin significantly ameliorated the pathological changes in lung tissues in LPS challenged mice. Moreover, barbaloin attenuated MPO activity, reduced the phosphorylated levels of IκBα and p65 subunit of NF-κB, lowered the content of MDA and the release of TNF-α, IL-1β, and IL-6, and improved SOD activity in lung tissues. Furthermore, barbaloin decreased release of TNF-α, IL-1β, IL-6 and the phosphorylated levels of IκBα, IKK, p65 subunit of NF-κB, in RAW264.7 macrophages challenged with LPS in vitro. Barbaloin also hindered LPS-mediated ROS generation via decreasing TLR4 expression and suppressed ROS/PI3K/Akt/NF-κB pathway in macrophages. [44]

### **Tabersonine**

Tabersonine is an indole alkaloid principally extracted from *Catharanthus roseus*. Tabersonine improved LPS-mediated ALI in vivo murine model and prevented LPS triggered macrophages

stimulation in vitro. Tabersonine reduced the K63-Linked Polyubiquitination of TRAF6, thus suppressed NF- $\kappa$ B and p38 MAPK and its downstream substrate MAP kinase-activated protein kinase (MK2) and the consequent release of inflammatory mediators in vivo and in vitro. Tabersonine also histopathologically improved lung damage, inhibited neutrophil influx, and reduced MPO in vivo murine model of LPS induced ALI. [45]

### **Hesperetin**

Hesperetin (HES) is a biological flavanone with powerful anti-inflammatory properties. Pre-treatment with HES dramatically diminished LPS-induced lung pathological damage in mice model of LPS- mediated ALI. HES also reduced TNF- $\alpha$ , IL-6, total protein concentration, the total number of neutrophils, and MPO levels. HES inhibited TLR signaling pathway via binding to MD2 accessory protein and preventing MD2/TLR 4 complex and consequently down regulated the activation of MAPKs, and controlled the degradation of I $\kappa$ B, which explained the anti-inflammatory action of HES in LPS induced ALI. [46]

### **Chicoric acid**

Chicoric acid (CA) significantly attenuated lung histological alterations and lung W/D ratio of LPS- mediated ALI in mice. Furthermore, CA diminished protein leak, inflammatory cell recruitment, MPO levels, and inflammatory cytokine discharge in BALF. Additionally, CA decreased levels of ROS and MDA but increased levels of GSH and SOD. Furthermore, CA markedly down-regulated LPS-mediated MAPK and NLRP3 activation and up-regulated the expression of Nrf2 and its downstream antioxidant enzymes, namely NQO1 SOD, glutamate-cysteine ligase catalytic/modifier (GCLC/GCLM) subunit, and HO-1, which explained the protective action of CA against LPS mediated ALI. [47]

### **Hyperin**

Hyperin is a natural flavonoid molecule found to have anti-inflammatory effects. Hyperin meaningfully prevented histopathological alterations and W/D ratio of lung tissue in LPS-mediated ALI in mice. Moreover, Hyperin decreased inflammatory cell recruitment, the activity of MPO. Furthermore, hyperin markedly reduced the generation of inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and suppressed NF- $\kappa$ B signaling cascade activation, which explained the potential protective effect of hyperin against LPS- mediated ALI in mice. [48]

### **Eugenol**

Eugenol is a phenolic monoterpene molecule found in clove oil with anti-inflammatory and antioxidant properties due to its ability to suppress NF- $\kappa$ B activation. Eugenol dose-dependently improved histopathological alterations and mechanical function of the lung induced by LPS in mice. Additionally, eugenol prevented inflammatory cytokines generation such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, down-regulated NADPH oxidase, increased CAT, SOD, GPX, and decreased protein oxidation in LPS-induced ALI in mice. [49]

### **$\alpha$ -Cyperone**

$\alpha$ -Cyperone is a volatile oil ingredient found in *Cyperus rotundus* L.  $\alpha$ -Cyperone showed a powerful protective effect in the mice model of LPS-induced ALI.  $\alpha$ -Cyperone decreased the lung W/D ratio, the MPO activity, the influx of inflammatory cells, and the release of inflammatory cytokines.  $\alpha$ -Cyperone also inhibited NF- $\kappa$ B and NLRP3 signaling cascades. Moreover,  $\alpha$ -Cyperone increased the expression levels of SIRT1. [50]

### **Glycitin**

Glycitin is a natural component extracted from leguminous seeds with potent anti-inflammatory effects. Glycitin improved lung histopathological alterations, W/D ratio, and activity of MPO in LPS-induced ALI in mice. Glycitin downregulated the expression of inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in a dose-dependent manner. Glycitin inhibited the TLR4 signaling pathway and consequently suppressed NF $\kappa$ B and MAPKs signaling cascades, which explained the ability of glycitin to ameliorate LPS mediated ALI. [51]

### **Kaempferol**

Kaempferol (KPF) is a flavonoid compound presents in tomato, tea, grapes, broccoli, apples, beans, and strawberries with antioxidant, anti-diabetic, and anti-inflammatory properties. Pretreatment of LPS excited isolated mouse peritoneal macrophages with KPF inhibited LPS-mediated production of IL-6 and TNF- $\alpha$  by preventing nuclear translocation of NF- $\kappa$ B in vitro. Furthermore, pre-treated HEK293cell line stably expressing IL-1RI with KPF before its stimulation with IL-1b showed reduced levels of K63-linked polyubiquitinated IRAK-1 and TRAF6, TAK1, I $\kappa$ B $\alpha$ , and JNK. Moreover, KPF showed a protective effect in the mice model of LPS induced ALI via improving lung histopathological changes and lug W/D ratio. Besides, KPF reduced the influx of inflammatory cells, the release of TNF- $\alpha$  IL-1 $\beta$ , IL-6, iNOS, and COX2 in the lung tissue of mice excited with LPS.KPF also reduced total protein levels and neutrophil counts in BALF and diminished TNF- $\alpha$  and IL-6 in BALF and serum. [52]

### **Jaceosidin**

Jaceosidin is a flavonoid molecule with considerable anti-complement activity. Jaceosidin significantly decreased the W/D ratio of the lung and protein concentration in the BALF in LPS challenged mice model of ALI. Jaceosidin also reduced the generation of inflammatory cytokines, including (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ) and enhanced expression levels of IL-10 and IL-4 in BALF. Moreover, Jaceosidin significantly reduced MPO, COX-2, COX-2 mRNA, NF- $\kappa$ B, and NF- $\kappa$ B p65 mRNA but intensified CAT activity. Furthermore, Jaceosidin improved histopathological alterations of the lung and decreased the serum levels of complement3 and complement 3c. [53]

### **Nerolidol**

Nerolidol, found in essential oils of flowers and many plants, is a valuable anti-inflammatory and antioxidant. Nerolidol prevented the LPS-induced recruitment of PMNs, hindered Cytokine, chemokine, adhesion molecule expression, and down-regulated biomarkers of inflammatory protein stimulated by LPS inhalation in the lung of mice. Nerolidol blocked the phosphorylation of NF- $\kappa$ B p65, p38 MAPKs, ERK, and JNK, which explained the anti-inflammatory effects of nerolidol. [54]

### **D-carvone**

D-carvone is the main component of many essential oils such as caraway, spearmint, and dill, but is most plentiful in caraway seed oil. Pre-treatment of LPS challenged mice model of ALI with D-carvone relieved the lung injury via decreasing the lung W/D value and the count of total cells, neutrophils, and macrophages in BALF. D-carvone also reduced TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in serum and improved lung histopathological changes mediated by LPS. [55]

### **S-allyl mercapto cysteine**

S-allyl mercapto cysteine (SAMC) is an organosulfur molecule sourced from garlic with anti-inflammatory and antioxidant properties. Treatment of LPS mediated mice model of ALI with SAMC improved the lung histological alterations and reduced pulmonary edema. Additionally, SAMC exhibited an anti-inflammatory response via reducing the recruitment of inflammatory cells, the levels of MPO, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, iNOS, and COX2 through inhibiting the activation of NF- $\kappa$ B signaling cascade. Moreover, SAMC reduced MDA content, increased GSH, and SOD. Furthermore, SAMC increased HO-1 and NQO1 expressions through modulating the Keap1/Nrf2 signaling cascade. [56]

### **Tangeretin**

Tangeretin is a polymethoxylated flavone that exists abundantly in citrus peels. Tangeretin markedly reduced the Pathological changes in the lung and lung W/D ratio in LPS challenged mice model of ALI. It also decreased the number of total cells and neutrophils, TNF- $\alpha$  in BALF, and lung levels of MPO. Moreover, tangeretin reduced the Proportion of pulmonary IL-17+CD4+ T cells and BALF cytokines, namely IL17 and IL-22. Tangeretin suppressed Notch signaling in T helper 17(Th17) cells involved in the pathogenesis of ALI. [57]

### **Biochanin A**

Biochanin A is an isoflavone derivative, abundantly found in red clover, alfalfa, cabbage, and chickpea. Biochanin A has valuable antifibrotic, antioxidant, anti-apoptotic, and anti-inflammatory properties. Biochanin A reduced LPS induced lung pathology, pulmonary capillary leak, and lung high value of W/D in mice. Also, it decreased total protein content, inflammatory cell infiltration in the BALF, and pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in both BALF and lung. The anti-inflammatory effect of biochanin A was due to inhibition of the TLR4/NF- $\kappa$ B pathway, besides activation of the anti-inflammatory transcription factor, Peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ). [58]

### **Glycyrrhetic acid**

Glycyrrhetic acid (GA) is a triterpene saponin with many therapeutic characteristics. Pretreatment of LPS mediated ALI mice model with GA improved lung histopathological alterations, macrophage recruitment, and alveolar edema. Furthermore, GA down-regulated Cle-caspase-1 expression and inhibited Nlrp3 assembly and activation. Moreover, GA decreased ROS levels and downregulated PI3K /AKT signaling axis in macrophages. GA might be a hopeful agent for the amelioration of ALI. [59]

### **Bavachin**

Bavachin is a natural compound extracted from the Asian traditional plant, *Psoralea corylifolia*. Pre-treatment of LPS stimulated murine macrophage cell line J774A.1 with bavachin decreased LPS-stimulated NO, PGE2, iNOS, and mPGES-1 expression. Moreover, Bavachin diminished LPS- mediated IL-12p40 and IL-6 generation and downregulated the expression of NF- $\kappa$ B and MAPKs signaling pathways. Besides, bavachin inhibited NLRP3 inflammasome assembly, caspase-1 activation, IL-1 $\beta$  secretion, and consequently reduced the maturation and expression of IL-1 $\beta$ . Additionally, bavachin suppressed the release of IL-6, IL-12p40, and NO by LPS-activated murine peritoneal macrophages. The results confirmed that bavachin might be a powerful candidate in managing ALI. [60]

### **Ruscogenin**

Ruscogenin( RUS )is steroidal sapogenin extracted from Radix Ophiopogon japonicus. RUS prevented lung endothelial apoptosis in vivo mice model. This was confirmed with a dose-dependent reduction in the TUNEL positive endothelial cells, Bax and Cleaved caspase-3, and upregulation of Bcl-2. The protective effect of RUS against pulmonary endothelial apoptosis was due to inhibition of the TLR4/MYD88/NF- $\kappa$ B signaling pathway in pulmonary endothelium. This effect was absent inTLR4 knockout mice RUS also protected pulmonary endothelial cell apoptosis in vitro via down-regulating signaling of TLR4. [61]

### **Apigenin C-glycosides**

Apigenin C-glycosides (ACGs) extracted from *Microcos paniculata* suppressed inflammatory lung reactions in LPS mediated ALI in BALB/c mice. The protective effects of ACGs were due to the reduction of lung edema and endothelial hyper-permeability. ACGs also dose-dependently down-regulated the expression levels of IL-6 and IL-1 $\beta$  and TNF- $\alpha$  in BALF and lung tissue and decreased apoptosis. Furthermore, ACGs hindered LPS-mediated up-regulation of the three MAPKs signaling including, p38, ERK1/2, and JNK, and increased the expression level of Bcl-2 but suppressed cleaved caspase-3 and Bax. Additionally, ACGs inhibited the TLR4 / TRPC6 signaling axis, attributing the anti-inflammatory and anti-apoptotic effect of Apigenin C-glycosides. [62]

### **Icariin**

Icariin (ICA), the principal powerful ingredient of flavonoids of *H epimedii*, has a broad spectrum of pharmacological outcomes, such as anti-inflammatory, anti-tumor, anti-depressant, protection against neuronal damage, improving synapse development in neurons, supporting the bone growth, enhancing sexual performance. ALI was induced in mice by LPS and bilateral adrenalectomy. Treatment with ICA increased glucocorticoid receptor  $\alpha$  levels in lung tissues. It also decreased expression levels of NF- $\kappa$ B p65, STAT3, c-jun, TNF- $\alpha$ , and IL-6. [63]

### **Nigella sativa L**

*Nigella sativa L* is a plant with several medicinal properties. It has anti-inflammatory, antioxidant, antimicrobial, antiasthmatic, antianxiety, antiepileptic, anti-depressant, and anti-cancer. Besides, it has significant therapeutic effects on immunomodulation and cardio-protection. In the rat model of LPS induced ALI, *Nigella sativa L* (NS) extract decreased LPS mediated elevated levels of white blood cells, oxidative stress in BALF and serum, and levels of IFN- $\gamma$ , TGF- $\beta$ 1, IL-4, and PGE2 in the BALF. Furthermore, NS extract ameliorated lung histopathological alterations due to the LPS challenge. All of these effects were dose-dependently achieved. [64]

### **Orientin**

Orientin (Ori) is a flavonoid ingredient sourced from natural plants, with antioxidant and anti-inflammatory characteristics. Orientin dramatically mitigated LPS-mediated ALI in Male C57BL/6 mice via improving the lung histological alterations and reducing lungW/D, protein concentration, the discharge of inflammatory cells and cytokines into BALF. Orientin also diminished MDA, iNOS, COX-2, HMGB1, and ROS. Furthermore, Ori treatment significantly inhibited NF- $\kappa$ B and NLRP3 inflammasome but stimulated the expression levels of Nrf2, HO-1, GCLC, NQO1, GSH, and SOD. [65]

### **Sophoricoside**

Sophoricoside (SOP) is an isoflavone glycoside separated from the seed of *Sophora japonica* L. It has anti-allergy, anti-cancer, and anti-inflammatory activities. Pretreatment of a murine model of ALI with SOP significantly improved LPS-mediated lung pathological injury, tissue hyperpermeability, neutrophil leak, and the generation of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. Moreover, SOP diminished iNOS, NO, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in LPS-challenged RAW264.7 cells and BMM in vitro. Treatment with SOP didn't activate NF- $\kappa$ B and MAPKs in macrophages but substantially activated AMPK, the upstream regulator of Nrf2, and consequently increased Nrf2 expression and nuclear translocation. This was confirmed by using the AMPK inhibitor that suppressed Nrf2 expression stimulated by SOP, which proved that SOP ameliorated LPS-mediated ALI via activating the AMPK/Nrf2 axis. [66]

### **Mangiferin (MF)**

Mangiferin (MF) is a xanthone glucoside isolated from the fruits and bark of mango plants. It has anti-cancer, antimicrobial, antiviral, anti-inflammatory, and antioxidant characteristics. Pretreatment of mice model of LPS-mediated sepsis with MF diminished the pathological lesions of the lung and kidney, reduced vascular permeability of lung and kidney to albumin via increasing the expression of occludin and inhibiting matrix metalloproteinase-9 (MMP9) - mediated shedding of the cell surface proteoglycan, syndecan-1 (SDC-1). MF also alleviated oxidative injuries via hindering the generation of ROS and MDA and increasing the activity of SOD of the lung and kidney. Ultimately, MF downregulated the signaling of NF- $\kappa$ B and HMG $\beta$ 1 of the lung and kidney. [67]

### **Dihydroquercetin**

Dihydroquercetin (DHQ) is a well-known flavonoid with Anti-inflammatory, antioxidant, and anti-cancer activity. DHQ at a dose of 5  $\mu$ g/mL notably hindered LPS-mediated discharge of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and decreased their mRNA expression levels in lung epithelial cells (TC-1 cell) of mice in vitro. Furthermore, DHQ strongly inhibited apoptosis as shown by cytometry, the decreased levels of Bax protein, and the increased levels of Bcl-2 protein in TC-1 cells of mice in vitro. Pre-treatment of LPS induced Balb/c mice model of ALI in vivo with DHQ decreased lung histopathological alterations, aggregation of inflammatory cells, lung W/D ratio, alveolar hemorrhage, and MDA. Moreover, DHQ improved the oxygenation index and reduced the release of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. DHQ mitigated LPS-mediated ALI in vivo and in vitro via miR-132-3p/FOXO3/NF $\kappa$ B axis. [68]

### **Echinacea Polysaccharides**

Echinacea Polysaccharides (EP) have anti-apoptotic, antioxidant, and anti-inflammatory activity. Murine model challenged with LPS mediated ALI and treated with EP exhibited amelioration of LPS-induced lung pathological changes, a decrease of W/D ratio, MPO, inflammatory cells infiltration in BALF, and TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in LPS-challenged lungs. Furthermore, EP overcame LPS-mediated apoptosis, decreased Bax and cleaved caspase-3, and increased Bcl-2. Moreover, pre-treatment of LPS challenged RAW 264.7 cells in vitro with EP 100  $\mu$ g/ml decreased TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Besides, both in vivo and in vitro, EP suppressed the expression levels of TLR4, MyD88, NF- $\kappa$ B, p-NF- $\kappa$ B, and enhanced I $\kappa$ B $\alpha$  after LPS challenge. [69]

**Other models of ALI and herbal remedies found to attenuate lung injury induced by them.**

### **Lipoteichoic acid-induced ALI**

Lipoteichoic acid (LTA), the main component of Gram-positive bacterial cell wall, is glycolipids that could stimulate TLR2 and share many inflammatory characteristics of LPS in Gram-negative bacteria. After ligand recognizing and sequential TLR2 dimer rearrangement, MyD88/ MAL recruits the IRAK 4, IRAK1, which then initiates auto-phosphorylation. Phosphorylated IRAK1 dissociates from MyD88/IRAK complex and binds to TRAF6. Ubiquitinated TRAF6 triggers the activation sequence TAB2 – TAK1 – IKK complex. I $\kappa$ B phosphorylation and degradation by the IKK complex lead to the release of NF- $\kappa$ B, translocation to the nucleus for gene up-regulation. TAK1 also activates MKK6 leading to consequent upregulation of ERK, JNK, and p38 directing AP-1 activation that triggers gene transcription of cytokines and accessory molecules. [70]

### **Costunolide**

Costunolide, a sesquiterpene lactone, diminished the influx of neutrophil, the release of IL-6, TNF- $\alpha$ , and the neutrophil infiltration indicator, keratinocyte-derived chemokine (KC), in LTA challenged mice model of ALI. Costunolide also decreased lung edema induced by LTA. Moreover, Costunolide suppressed the release of iNOS and cytokines in bone marrow-derived macrophages (BMM) pretreated with costunolide and challenged with LTA in vitro. Furthermore, In LTA-challenged BMM, Costunolide inhibited the complexation of TAK1 with its binding protein TAB1 and decreased the phosphorylation of MAPKs such as p38 and ERK but not JNK. However, Costunolide didn't affect NF- $\kappa$ B upregulation. [71]

### **Whole bacteria-induced ALI model**

The installation of *E. coli* into mice lungs produces an ALI model related to that of human ALI, comprising severe pneumonia, sepsis, alveolar wall thickening, bilateral lung edema, alveolar hemorrhage, inflammatory reactions, and leukocyte influx [72]. *Staphylococcus aureus* (*S. aureus*) is an opportunistic, Gram-positive bacterium. Lipoteichoic acid and Peptidoglycan are considered the two principal constituents of the cell wall of the *S. aureus* that are capable of exciting inflammatory responses in vivo and in vitro via the activation of TLRs. When inhaled, these cell wall constituents lead to the infiltration of neutrophils and the generation of cytokines in the BALF. TLR2 is the principal receptor implicated in their recognition. Activation of endothelial cell TLRs by heat-killed *S. aureus* stimulates the activation of MAPKs (p38, ERK1/2, and JNK1/2), and NF- $\kappa$ B, and consequently, the production of the inflammatory cytokines that intensify lung edema and alveolar haemorrhage. [73]

### **Sinomenine**

Sinomenine (SIN) is a plant alkaloid mainly sourced from *Sinomenium acutum* with anti-inflammatory and anti-oxidant properties. *Escherichia coli* (*E.coli*)-challenged mice showed lung histological alterations and increased MPO activity, MDA level, W/D value, and inflammatory cytokines release in Lung tissues. While pre-treatment of mice with SIN reversed all these changes, SIN also down-regulated *E.coli*-mediated NF- $\kappa$ B activation and increased expression of HO-1, Nrf2, and NQO1 in lung tissues. [73]

### **Glycyrrhizin (GL)**

Glycyrrhizin is the main active ingredient of licorice root with antioxidant and anti-inflammatory properties. Intraperitoneal administration of Glycyrrhizin showed a powerful anti-inflammatory effect in mice models of *S. aureus*-mediated ALI. GL administration

markedly relieved inflammation via the inhibition of various cytokines in serum and lung, including (IL-6, TNF- $\alpha$ , IL-8, IL-1 $\beta$ , and HMGB1) and macrophage and neutrophil influx in the lung. Furthermore, GL suppressed NF- $\kappa$ B, MAPKs (p38, ERK) and pyroptosis. [74]

### **Ginsenoside Rb1**

Ginsenoside Rb1 is the principal bioactive ingredient obtained from *Panax ginseng* with many pharmacological properties. Rb1 significantly decreased the W/ D ratio and improved the histo-pathological alteration of lungs in the *S. aureus*-induced ALI mouse model. Moreover, Rb1 suppressed the release of IL-6, IL-1 $\beta$ , and TNF- $\alpha$  in vivo and in RAW 264.7 macrophage cells in vitro. Immuno-fluorescence assays have demonstrated that Rb1 inhibited the activation of TLR2 induced by *S. aureus*. Rb1 attenuated TLR-2-induced activation of NF- $\kappa$ B and the three MAPK (p65, ERK, JNK) signaling axes so that Rb1 protected against *S. aureus*-mediated ALI in mice. [75]

### **Salvinorin A**

Salvinorin A (SA) is a neoclerodane diterpene found in the dried leaves of *Salvia divinorum* with analgesic and anti-inflammatory characteristics. Mice challenged with methicillin-Resistant *Staphylococcus aureus* (MRSA)-mediated ALI and treated with SA displayed a considerable reduction in the lung influx of neutrophils, mRNA expression of TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . In vitro results exhibited that SA attenuated LTA-mediated oxidative stress apoptosis and inflammation in RAW264.7 cells. Furthermore, results showed that SA significantly increased mRNA and protein expression levels of Nrf2 and increased the mRNA levels of Nrf2-dependent genes such as HO-1, SOD1, SOD2, Gclm, and Trx-1. Knockout of Nrf2 in mice prevented the inhibition of SA on oxidative stress and neutrophil influx in MRSA-mediated ALI, which approved that SA ameliorated MRSA-induced ALI via the Nrf2 signaling axis. [76]

### **Models of ALI/ARDS secondary to peritonitis, cecal ligation and puncture.**

Surgical ligation and perforation of the cecum (cecal ligation and puncture (CLP)) induce peritonitis. The higher the numbers of cecum openings and the larger size of the needle utilized to make these openings, the more severity of CLP induced lung injury. The most commonly detected micro-organisms in the blood cultures after CLP-mediated peritonitis were the enteric gram-negative species. The effects of LPS and live bacteria models are almost fast. On the contrary, the onset of CLP is less abrupt and needs days to develop. The principal characteristics of CLP-mediated lung injury are neutrophilic inflammation, pulmonary edema, and hypoxemia. [72]

### **Gossypin**

Gossypin, a flavone mainly extracted from *Hibiscus vitifolius*, has been demonstrated to inhibit inflammation, angiogenesis, oxidative stress, and carcinogenesis. Administration of gossypin (GOS) to a rat model of CLP induced lung injury reduced oxidative stress and reinforced lung anti-oxidant system. GOS diminished the elevated tissue levels of inflammatory cytokines, including (IL-6, HMGB1, and IL-1 $\beta$ ) and suppressed TNF- $\alpha$  mRNA expression. GOS also down-regulated NLRP3 and NF- $\kappa$ B signaling pathways. [77]

### **Curcumin**

Pre-treatment with curcumin decreased lung IL-17A expression, MPO-releasing neutrophils, and expression of NF- $\kappa$ B p65 in mice model of CLP induced lung injury. It also regulated the



levels of M1/M2 macrophages via activating Treg cell differentiation and inducing IL-10 secreted from regulatory T cells. Curcumin in vivo augmented Treg proportions and increased IL-10 secretion in BALF and serum of CLP surgery challenged mice. Curcumin also enhanced the differentiation of Treg cells and the secretion of IL-10 in vitro. [78]

### **Emodin**

Emodin is a potent chemical compound extracted from many plants with antioxidant, anti-inflammatory, and anti-cellular apoptotic activity. Pre-treatment of CLP challenged rats with emodin significantly ameliorated lung histo-pathological alterations. Emodin increased the reduced levels of aquaporin AQP1 and AQP5, and tight junctions claudin-3 and ZO-1. Emodin decreased inflammatory cytokine releases, such as TNF- $\alpha$  and IL-6. Moreover, emodin reduced lung cellular apoptosis that was detected by measuring the reduction of TUNEL positive cells. In conclusion, emodin diminished inflammation, repaired pulmonary epithelial barrier, and decreased mortality rate in CLP-mediated, implying that emodin could be a potential therapeutic target for managing ALI/ARDS. [79]

### **Acute pancreatitis induced ALI**

Gram-negative infections consider the main risk factors that predispose to ALI /ARDS in acute pancreatitis due to their translocation from the gut to the circulation and distant organs such as lung and subsequent interaction to TLR4. Mice lacking TLR4 exhibited less severe acute pancreatitis and lung injury. [80]

### **Apocynin**

Apocynin is a natural NADPH oxidase (NOX) inhibitor with significant antioxidant activity. Apocynin significantly diminished severe acute pancreatitis (SAP)-induced ALI in rats via inhibition of lung levels of NOX2, NOX4 and ROS. Furthermore, Apocynin prevented I $\kappa$ B $\alpha$  phosphorylation and degradation and NF- $\kappa$ B p65 nuclear localization. NOX inhibition attenuated the levels of NLRP3 ASC, pro-Caspase-1, and cleaved Caspase-1 in the lung and TNF- $\alpha$ , IL-6, and IL -1 $\beta$  in serum. Consequently, Apocynin might have ameliorative efficacy in SAP and SAP- mediated ALI. [81]

### **Diallyl disulfide**

Diallyl disulfide (DADS) is an organosulfur molecule contained in garlic. It was tested as an amelioration agent to prevent cerulein-induced AP and subsequent ALI in mice. In both the lung and pancreas, results exhibited that treatment with DADS decreased serum levels of amylase, MPO content, and histo-pathological change. Furthermore, DADS reduced TNF- $\alpha$  and the hydrogen sulfide-generating enzymes such as preprotachykinin A (PPTA) and cystathionine- $\gamma$ -lyase (CSE). DADS also suppressed the expression of the receptor of substance P, neurokinin-1-receptor (NK1R). Moreover, DADS suppressed the phosphorylation and degradation of I- $\kappa$ B, thus inactivated NF- $\kappa$ B in the lung and pancreas. [82]

### **Limb ischemia/reperfusion injury-induced ALI**

Limb ischemia is a rapid decrease in limb perfusion due to inadequate blood flow. Restoring blood flow to the limb is needed to save it and prevent amputation, but it may locally exacerbate the tissue I/R injury, systematically create severe inflammatory reactions, and induce ALI /ARDS. [83]

### **Tetrahydropalmatine**

Tetrahydropalmatine (THP) is an isoquinoline alkaloid present in several different plant species with anti-inflammatory, antioxidant, anti-coagulant properties. ALI was induced in rats via limb I/R. Treatment with THP significantly ameliorated lung histological alterations and W/D ratio that were mediated by limb I/R. THP significantly decreased MDA and MPO and increased SOD in lung tissues. Moreover, THP increased the activation of PI3K/AKT/mTOR and decreased cellular autophagic activity. [84]

### **Ventilator-induced lung injury**

Ventilator-induced lung injury (VILI) is a mechanical stretch injury that locally irritates the lung tissue leading to the generation of pro-inflammatory molecules and consequent damage of the endothelium/epithelium barrier and lung edema throughout the mechanical ventilation (MV). Locally released mitochondrial DNA (mtDNA) from injured mitochondria of damaged lung cells act as endogenous damage-associated molecular patterns (DAMPs) and activate neutrophils and provoke inflammation. [85]

### **Epigallocatechin gallate**

Epigallocatechin Gallate is a phenolic compound in plenty of plants such as green and black tea. It has antioxidant, anti-inflammatory, and chemo-preventive properties.

Epigallocatechin gallate (EGCG) significantly and dose-dependently ameliorated high tidal volume (HTV) - mediated local release of the pro-inflammatory factor, mitochondrial DNA (mtDNA), recruitment of local inflammatory cells, expression of inflammatory cytokines in BALF of rats. [85]

### **Herbal remedies found to attenuate lung injury induced in other uncommon models of ALI/ARDS.**

#### **Luteolin**

Acute mercury exposure is associated with lung injury due to oxidative stress, the release of NF- $\kappa$ B activity, and subsequent apoptosis and necrosis. Luteolin is a food-derived bioactive compound with numerous pharmacological properties. Administration of luteolin reduced pulmonary histologic change and apoptosis in mice challenged with mercuric chloride (HgCl<sub>2</sub>) induced ALI. The protective effects of luteolin might be due to the decrease of MPO enzyme activity, inflammatory cytokines, MDA, and the enhancement of SOD and GSH. Furthermore, luteolin up-regulated protein kinase B /nuclear factor E2-related factor 2 (Akt/Nrf2) signaling cascade and enhanced lung capacity to resist oxidative stress-mediated by HgCl<sub>2</sub> in mice. Luteolin also prevented NF- $\kappa$ B activation in HgCl<sub>2</sub>-mediated lung injury. [86]

#### **Oleanolic Acid Acetate**

Polyhexamethylene guanidine phosphate (PHMG-P), the commonly used disinfectant in humidifiers, causes lung injury and consequent extensive pulmonary fibrosis via increasing the influx of inflammatory cells, the release of many cytokines, such as IL 1 $\beta$ , IL-6, TGF  $\beta$ 1 and NF  $\kappa$ B. Oleanolic acid acetate (OAA) is a triterpenoid molecule extracted from *Vigna angularis* with anti-inflammatory effects. OAA administration efficiently and dose-dependently improved PHMG-P -triggered lung damage in mice via decreasing differential and the total number of cells in BALF, histopathological changes, and content of the marker of collagen destruction, hydroxyproline. Furthermore, the OAA administration significantly reduced IL-1 $\beta$ , IL-6, and TNF- $\alpha$ . Additionally, OAA administration significantly reduced markers of lung

fibrosis, transforming growth factor-beta 1(TGF- $\beta$ 1) and fibronectin. OAA also down-regulated NLRP3 inflammasome that participated in lung inflammation and fibrosis. [87]

### **Procyanidin B2**

Paraquat, the commonly used herbicide, can be inhaled and accumulates in the lungs, producing lung edema, hemorrhage, injury to the alveolar lining cells, and pulmonary fibrosis in acute toxicity. Paraquat upregulates oxidative stress, NLRP3 inflammasome, and cytokines releases such as IL-1 $\beta$  and IL-18. Procyanidin B2 is a natural compound present in many plants, particularly in grape seeds, apples, and cacao seeds. Procyanidin B2 possesses anti-inflammatory and antioxidant characteristics. Procyanidin B2 decreased poly-morphonuclear leukocyte count and MPO and MDA levels in BALF in rats model of paraquat-induced lung damage. Procyanidin B2 also increased the activity of SOD. Furthermore, procyanidin B2 reduced levels of IL-1 $\beta$ , IL-18 mRNA, and IL-18 protein. Moreover, procyanidin B2 dose-dependently suppressed NLRP3, apoptosis-associated speck-like protein (ASC), and caspase-1. [88]

### **Chrysin**

The injection of carrageenan into a rat's pleural cavity induced neutrophils influx, oxidative stress, inflammatory reactions with subsequent pleurisy, and lung injury. Chrysin is a natural polyphenol compound abundantly found in honey, propolis, fruits, and vegetables. Chrysin decreased carrageenan-induced lung pleurisy through the prevention of neutrophils influx and oxidative stress response. The effect of chrysin was due to enhancing the SIRT1 /NRF2 signaling cascade that augmented lung resistance to oxidative stress damage mediated by carrageenan. [89]

### **Maslinic acid**

Particulate matter (PM) is one of the most prominent indicators of air pollution. It is composed of many different components, such as oxygenated volatile organic compounds, insoluble metals, and polycyclic aromatic hydrocarbons. PM holds in the respiratory tract because of its small diameter that is less than or equal to 2.5  $\mu$ m, and it is associated with many inflammatory lung diseases. PM<sub>2.5</sub> activates the TLR4 signaling axis and stimulates inflammatory molecules release, oxidative stress, subsequent apoptosis, and autophagy.

Maslinic acid (MA) is a plant triterpenoid contained in olive and diverse plant species. Treatment with MA attenuated diesel particulate matter - induced lung pathology, a high value of W/D, pulmonary capillary leak, and lymphocyte infiltration in mice. MA also decreased lung MPO content, inflammatory cytokines production, namely TNF - $\alpha$  and IL -1 $\beta$ , and BALF contents of total protein and NO. The anti-inflammatory effects of maslinic acid were due to the inhibition of TLR4 and MyD88. MA also stimulated mTOR and decreased the autophagy marker proteins such as LC3-II and beclin-1. [90]

### **Conclusion and future directions**

ALI/ARDS is a lung disease that is caused by bacterial, viral, and other germs pneumonia, chest injury, and secondary to other medical conditions such as acute pancreatitis and ischemia-reperfusion injury, with high mortality rates. Its pathogenesis involves multiple inflammatory signaling cascades and oxidative stress caused by a diversity of cell types in the lung tissues. Up till now, this condition is being controlled by mechanical ventilation and accurate fluid control in high-risk patients with limited focus on the inflammatory process in lung tissues.

In multiple preclinical studies, herbal remedies have been found to be effective against ALI/ARDS. These herbal remedies could control multiple inflammatory signaling cascades and enhance antioxidant enzymes and pathways; therefore, they need to be examined by clinical trials. Novel routes of administration for these herbal products could be developed, including to be formulated as inhalable nanoparticles to allow their direct delivery to the lung with fewer systemic side effects than oral therapy and to increase their bioavailability or to be loaded in lipid vesicles and administered via inhalation that would improve the opportunity to fight this critical medical condition.

## References

1. M. Matthay, R. Zemans, G. Zimmerman et al., "Acute respiratory distress syndrome", *Nature Reviews. Disease primers*, vol. 5(1), pp.18, 2019.
2. E. L. Herzog, A. R. Brody, T. V. Colby, R. Mason, and M. C. Williams, "Knowns and unknowns of the alveolus", *Proc Am Thorac Soc*, vol. 5(7), pp. 778-82, 2008.
3. R. J. Mason, "Biology of alveolar type II cells", *Respirology (Carlton, Vic.)*, vol. 11(S1), pp. S12-S15, 2006.
4. D. Bergey, "The Endothelium: Global Integrator of Vascular-Immune Interactions", *Journal of Cellular Immunology*, vol. 1 (1), (2019).
5. S. Herold, N.M. Gabrielli, and I. Vadász, "Novel concepts of acute lung injury and alveolar-capillary barrier dysfunction", *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 305(10). "pp. L665-L681, 2013.
6. A. Naeem, S.N. Rai, and L. Pierre, "Histology, Alveolar Macrophages", *Book from StatPearls Publishing, Treasure Island (FL)*, 2020.
7. C. Alexander and E.T. Rietschel, "Invited review: Bacterial lipopolysaccharides and innate immunity", *Journal of Endotoxin Research*, vol. 7(3), pp. 167-202, 2001.
8. H. Heine and S. Riekenberg, "Chapter 31 - Toll-like receptor recognition of lipoglycans, glycolipids, and lipopeptides in Microbial Glycobiology", *Academic Press: San Diego*, pp. 623-635, 2010.
9. C.C. Lin, C.H. Shih, Y.L. Yang et al., "Thrombin induces inducible nitric oxide synthase expression via the MAPK, MSK1, and NF- $\kappa$ B signaling pathways in alveolar macrophages", *European Journal of pharmacology*, vol. 672, pp. 180-7, 2011.
10. Y. Liu, W.W. Su, S. Wang, P. B. Li, "Naringin inhibits chemokine production in an LPS-induced RAW 264.7 macrophage cell line", *Mol Med Rep*, vol. 6(6), pp. 1343-1350, 2012.
11. W. Fang, S.X. Cai, C.L. Wang et al., "Modulation of mitogen-activated protein kinase attenuates sepsis-induced acute lung injury in acute respiratory distress syndrome rats", *Mol Med Rep*, vol. 16 (6), pp.9652-9658, 2017.
12. Y. Feng, Z. Fang, B. Liu, X. Zheng, " p38MAPK plays a pivotal role in the development of acute respiratory distress syndrome", *Clinics (Sao Paulo, Brazil)*, vol. 74, pp.e509-e509, 2019.
13. L. A. J. O'Neill and A. G. Bowie, "The family of five: TIR-domain-containing adaptors in Toll-like receptor signaling", *Nature Reviews Immunology*, vol. 7(5): p. 353-364, 2007.
14. L.F. Zhou, Q. Z. Chen, C. T. Yang et al., "TRPC6 contributes to LPS-induced inflammation through ERK1/2 and p38 pathways in bronchial epithelial cells", vol. 314(3), pp.C278-C288, 2018.
15. J. A Hagar, D.A Powell, Y. Aachoui, R. K. Ernst, and E. A Miao, "Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock", *Science (New York, N.Y.)*, vol. 341 (6151), pp.1250-1253, 2013.

16. E. Leiva-Salcedo, C. Coddou, F. E Rodríguez et al., " Lipopolysaccharide Inhibits the Channel Activity of the P2X7 Receptor", *Mediators of Inflammation*, pp.152625, 2011.
17. N. Hosseinian, Y. Cho, R. F. Lockey, N. Kolliputi, " The role of the NLRP3 inflammasome in pulmonary diseases", *Ther Adv Respir Disvol.* 9(4), pp.188-97, 2015.
18. I. Hafner-Bratkovič and P. Pelegrín, "Ion homeostasis and ion channels in NLRP3 inflammasome activation and regulation", *Current Opinion in Immunology*, vol. 52: p. 8-17, 2018.
19. Z. Nova, H. Skovierova, and A. Calkovska, "Alveolar-Capillary Membrane-Related Pulmonary Cells as a Target in Endotoxin-Induced Acute Lung Injury", *International Journal of molecular sciences*, vol. 20(4), pp.831, 2019.
20. X. Liu, S. Yin, Y. Chen et al., "LPS-induced pro-inflammatory cytokine expression in human airway epithelial cells and macrophages via NF- $\kappa$ B, STAT3 or AP-1 activation", *Mol Med Rep*, vol. 17(4), pp.5484-5491, 2018.
21. J. Grommes and O. Soehnlein, "Contribution of neutrophils to acute lung injury", *Mol Med* vol. 17(3-4), pp.293-307, 2011.
22. L. R. Prince, M. K. Whyte, I. Sabroe, L. C. Parker, "The role of TLRs in neutrophil activation", *Current Opinion in Pharmacology*, vol. 11(4): pp.397-403, 2011.
23. A.C. Ma and P. Kubes, "Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis", *J Thromb Haemost*, vol. 6(3), pp.415-20, 2008.
24. S. X. Liao, P.P. Sun, Y. H. Gu, X. M. Rao, L. Y. Zhang, and Y. Ou-Yang, "Autophagy and pulmonary disease", *Ther Adv Respir Dis*, vol. 13, pp.1753466619890538, 2019
25. K. Xie, Y. Yu, Y. Huang et al., " Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis", *Shock*, vol. 37(5), pp.548-55, 2012.
26. K. Mizumura, S. Maruoka, Y. Gon, A.M, Choi, S. Hashimoto, " The role of necroptosis in pulmonary diseases", *Respir Investig*, vol. 54(6), pp.407-412, 2016.
27. C. C. Dos Santos, "Advances in mechanisms of repair and remodeling in acute lung injury", *Intensive Care Medicine*, vol. 34(4), pp.619-630, 2008.
28. H. W. Jeong, K.C. Hsu, J.W Lee et al., " Berberine suppresses pro-inflammatory responses through AMPK activation in macrophages", *American Journal of Physiology-Endocrinology and Metabolism*, vol. 296(4), pp.E955-E964, 2009.
29. E Bess, B Fisslthaler, T Frömel, I Fleming, "Nitric oxide-induced activation of the AMP-activated protein kinase  $\alpha$ 2 subunit attenuates I $\kappa$ B kinase activity and inflammatory responses in endothelial cells", *PLoS One*, vol. 6(6), pp. e20848, 2011.
30. N. Kim, P. Lertnimitphun, Y. Jiang et al., "Andrographolide inhibit inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK", *Biochemical Pharmacology*, vol. 170, pp. 113646. , 2019.
31. K. Kim and S. Ki, "Nrf2: a key regulator of redox signaling in liver diseases, in *Liver Pathophysiology*", Elsevier, pp. 355-374, 2017.
32. A. L. Stefanson and M. Bakovic, "Dietary regulation of Keap1/Nrf2/ARE pathways: focus on plant-derived compounds and trace minerals", *Nutrients*, vol. 6(9), pp. 3777-801, 2014.
33. Y. Liu, P. Liu, Q. Wang, F. Sun, F. Liu, "Sulforaphane Attenuates H<sub>2</sub>O<sub>2</sub>-induced Oxidant Stress in Human Trabecular Meshwork Cells (HTMCs) via the Phosphatidylinositol 3-Kinase (PI3K)/Serine/Threonine Kinase (Akt)-Mediated Factor-E2-Related Factor 2 (Nrf2) Signaling Activation", *Medical Science Monitor*, vol. 25, pp. 811-818, 2019.

34. Z. Ren, H. He, Z. Zuo, Z. Xu, Z. Wei, and J. Deng, "The role of different SIRT1-mediated signaling pathways in toxic injury", *Cellular & molecular biology letters*, vol. 24, pp. 36-36, 2019.
35. W. Zhang, Q. Huang, Z. Zeng, J. Wu, Y. Zhang, and Z. Chen, "Sirt1 Inhibits Oxidative Stress in Vascular Endothelial Cells", *Oxidative Medicine and Cellular Longevity*, p. 7543973, 2017.
36. V. Sorrenti, S. Davinelli, G. Scapagnini, B. Willcox, R. Allsopp and D. Willcox, "Astaxanthin as a Putative Geroprotector: Molecular Basis and Focus on Brain Aging", *Marine Drugs*, vol. 18 (7), pp. 351, 2020.
37. L. Yao, G. Hou, L. Wang, X.-s. Zuo, and Z. Liu, "Protective effects of thymol on LPS-induced acute lung injury in mice", *Microbial Pathogenesis*, vol. 116, pp. 8-12, 2018.
38. J.-W. Lee, W. Chun, O.-K. Kwon et al., "3, 4, 5-Trihydroxycinnamic acid attenuates lipopolysaccharide (LPS)-induced acute lung injury via down-regulating inflammatory molecules and up-regulating HO-1/AMPK activation", *International Immuno-pharmacology*, vol. 64, pp. 123-130, 2018.
39. X. Wang, J. Yan, X. Xu et al., "Puerarin prevents LPS-induced acute lung injury via inhibiting inflammatory response", *Microbial Pathogenesis*, vol. 118, pp. 170-176, 2018.
40. Y.-L. Qiu, X.-N. Cheng, F. Bai, L.-Y. Fang, H.-Z. Hu, and D.-Q. Sun, "Aucubin protects against lipopolysaccharide-induced acute pulmonary injury through regulating Nrf2 and AMPK pathways", *Biomedicine & Pharmacotherapy*, vol. 106, pp. 192-199, 2018.
41. C.-b. Yuan, L. Tian, B. Yang, and H.-y. Zhou, "Isoalantolactone protects LPS-induced acute lung injury through Nrf2 activation", *Microbial Pathogenesis*, vol. 123, pp. 213-218, 2018.
42. A. A. Shaaban, D.H. El-Kashef, M.F. Hamed, and D.S. El-Agamy, "Protective effect of pristimerin against LPS-induced acute lung injury in mice", *International Immuno-pharmacology*, vol. 59, pp. 31-39, 2018.
43. X. Chen, X. Cai, R. Le et al., "Isoliquiritigenin protects against sepsis-induced lung and liver injury by reducing inflammatory responses", *Biochemical and Biophysical Research Communications*, vol. 496, pp. 245-252, 2018.
44. K. Jiang, S. Guo, C. Yang et al., "Barbaloin protects against lipopolysaccharide (LPS)-induced acute lung injury by inhibiting the ROS-mediated PI3K/AKT/NF- $\kappa$ B pathway", *International Immuno-pharmacology*, vol. 64, pp. 140-150, 2018.
45. D. Zhang, X. Li, Y. Hu et al., "Tabersonine attenuates lipopolysaccharide-induced acute lung injury via suppressing TRAF6 ubiquitination", *Biochemical Pharmacology*, vol. 154, pp. 183-192, 2018.
46. J. Ye, M. Guan, Y. Lu et al., "Protective effects of hesperetin on lipopolysaccharide-induced acute lung injury by targeting MD2", *European Journal of Pharmacology*, vol. 852, pp. 151-158, 2019.
47. H. Ding, X. Ci, H. Cheng, Q. Yu, and D. Li, "Chicoric acid alleviates lipopolysaccharide-induced acute lung injury in mice through anti-inflammatory and anti-oxidant activities", *International Immuno-pharmacology*, vol. 66, pp. 169-176, 2019.
48. X. Hu, H. Li, L. Fu et al., "The protective effect of hyperin on LPS-induced acute lung injury in mice", *Microbial Pathogenesis*, vol. 127, pp. 116-120, 2019.
49. C. B. Magalhães, N.V. Casquilho, M.N. Machado et al., "The anti-inflammatory and anti-oxidative actions of eugenol improve lipopolysaccharide-induced lung injury", *Respiratory Physiology & Neurobiology*, vol. 259, pp. 30-36, 2019.

50. X. Liu, X. Jin, D. Yu, and G. Liu, "Suppression of NLRP3 and NF- $\kappa$ B signaling pathways by  $\alpha$ -Cyperone via activating SIRT1 contributes to attenuation of LPS-induced acute lung injury in mice", *International Immuno-pharmacology*, vol. 76, p. 105886, 2019.
51. Y. Chen, S. Guo, K. Jiang, Y. Wang, M. Yang, and M. Guo, "Glycitin alleviates lipopolysaccharide-induced acute lung injury via inhibiting NF- $\kappa$ B and MAPKs pathway activation in mice", *International Immunopharmacology*, vol. 75, p.105749, 2019
52. J. Qian, X. Chen, X. Chen et al., "Kaempferol reduces K63-linked polyubiquitination to inhibit nuclear factor- $\kappa$ B and inflammatory responses in acute lung injury in mice", *Toxicology Letters*, vol. 306, pp. 53-60, 2019.
53. X. I. Huang, X. c. Wei, L.-q. Guo et al., "The therapeutic effects of Jaceosidin on lipopolysaccharide-induced acute lung injury in mice", *Journal of Pharmacological Sciences*, vol. 140, pp. 228-235, 2019.
54. S. P. Chen, C. H. Su, R. Huang-Liu et al., "Protective effect of nerolidol on lipopolysaccharide-induced acute lung injury through the inhibition of NF- $\kappa$ B activation by the reduction of p38 MAPK and JNK phosphorylation", *Journal of Functional Foods*, vol. 51., p.103943, 2020.
55. M. Zhao and J. Du, "Anti-inflammatory and protective effects of D-carvone on lipopolysaccharide (LPS)-induced acute lung injury in mice", *Journal of King Saud University - Science*, vol. 32, pp. 1592-1596, 2020.
56. M. Mo, S. Li, Z. Dong et al., " S-allylmercaptocysteine ameliorates lipopolysaccharide-induced acute lung injury in mice by inhibiting inflammation and oxidative stress via nuclear factor kappa B and Keap1/Nrf2 pathways", *International Immunopharmacology*, vol. 81, p. 106273, 2020
57. M. Li, Y. Zhao, D. Qi, J. He, and D. Wang, "Tangeretin attenuates lipopolysaccharide-induced acute lung injury through Notch signaling pathway via suppressing Th17 cell response in mice", *Microbial Pathogenesis*, vol. 138, p.103826, 2020.
58. X. Hu, H. Qin, Y. Li et al., "Biochanin A protect against lipopolysaccharide-induced acute lung injury in mice by regulating TLR4/NF- $\kappa$ B and PPAR- $\gamma$  pathway", *Microbial Pathogenesis*, vol. 138, pp. 103846, 2020.
59. K. Wang, Y. Zhang, Y. Cao et al., "Glycyrrhetic acid alleviates acute lung injury by PI3K/AKT suppressing macrophagic Nlrp3 inflammasome activation", *Biochem Biophys Res Commun*, vol. 532(4), pp. 555-562. , 2020.
60. Y. L. Hung, S. C. Wang, K. Suzuki et al., "Bavachin attenuates LPS-induced inflammatory response and inhibits the activation of NLRP3 inflammasome in macrophages", *Phytomedicine*, vol. 59, pp. 152785, 2019.
61. Y. Wu, Y. Wang, S. Gong et al., "Ruscogenin alleviates LPS-induced pulmonary endothelial cell apoptosis by suppressing TLR4 signaling", *Biomed Pharmacother*, vol. 125, pp. 109868, 2020
62. Y. Liu, Z. Li, X. Xue, Y. Wang, Y. Zhang , and J. Wang, "Apigenin reverses lung injury and immunotoxicity in paraquat-treated mice", *International Immunopharmacology*, vol. 65, pp. 531-538, 2018.
63. X. Sun, H. Cheng, B. Liu, Y. Du, J. Dong, and J. Huang, "Icariin reduces LPS-induced acute lung injury in mice undergoing bilateral adrenalectomy by regulating GR $\alpha$ ", *European Journal of Pharmacology*, vol. 876, p.173032, 2020.
64. A. Mokhtari-Zaer, F. Norouzi, V.R. Askari et al., "The protective effect of Nigella sativa extract on lung inflammation and oxidative stress induced by lipopolysaccharide in rats", *Journal of Ethnopharmacology*, vol. 253, p. 112653, 2020.

65. Q. Xiao, Y. Cui, Y. Zhao, L. Liu, H. Wang, and L. Yang. "Orientin relieves lipopolysaccharide-induced acute lung injury in mice: The involvement of its anti-inflammatory and anti-oxidant properties", *International Immunopharmacology*, pp. 107189, 2020.
66. Y. X. Wu, S. Zeng, B.B. Wan et al., "Sophoricoside attenuates lipopolysaccharide-induced acute lung injury by activating the AMPK/Nrf2 signaling axis ", *International Immunopharmacology*, pp. 107187, 2020.
67. D. Zhang, S. Han, Y. Zhou, B. Qi, and X. Wang, "Therapeutic effects of mangiferin on sepsis-associated acute lung and kidney injuries via the downregulation of vascular permeability and protection of inflammatory and oxidative damages", *European Journal of Pharmaceutical Sciences*, vol. 152, pp. 105400, 2020.
68. J. H. Liu, L. Cao, C.H. Zhang, C. Li, Z.H. Zhang, and Q. Wu, "Dihydroquercetin attenuates lipopolysaccharide-induced acute lung injury through modulating FOXO3-mediated NF- $\kappa$ B signaling via miR-132-3p", *Pulmonary Pharmacology & Therapeutics*, vol. 64, pp. 101934, 2020.
69. H. Zhang, W. Lang, S. Wang, B. Li, G. Li, and Q. Shi, "Echinacea polysaccharide alleviates LPS-induced lung injury via inhibiting inflammation, apoptosis, and activation of the TLR4/NF- $\kappa$ B signal pathway", *International Immunopharmacology*, vol. 88, pp. 106974, 2020.
70. S. Mukherjee, S. Karmakar, S. P. Babu, "TLR2 and TLR4 mediated host immune responses in major infectious diseases", *Braz. J. Infect. Dis*, vol. 295 20, pp. 193–204, 2016.
71. Z. Chen, D. Zhang, M. Li, and B. Wang, "Costunolide ameliorates lipoteichoic acid-induced acute lung injury via attenuating MAPK signaling pathway", *International Immunopharmacology*, vol. 61, pp. 283-289, 2018.
72. Matute-Bello, G., Frevert, C. W. & Martin, T. R, "Animal models of acute lung injury", *Am. J. Physiol. Lung Cell Mol. Physiol.*, vol. 295, pp. L379-399, 2008.
73. S. Liu, Q. Chen, J. Liu, X. Yang, Y. Zhang, and F. Huang, "Sinomenine protects against E. coli-induced acute lung injury in mice through Nrf2-NF- $\kappa$ B pathway", *Biomedicine & Pharmacotherapy*, vol. 107, pp. 696-702, 2018.
74. L. Yao and T. Sun, "Glycyrrhizin administration ameliorates Streptococcus aureus-induced acute lung injury", *International Immunopharmacology*, vol. 70, pp. 504-511, 2019.
75. A Shaukat ,Y. F Guo, K. Jiang et al., "Ginsenoside Rb1 ameliorates Staphylococcus aureus-induced Acute Lung Injury through attenuating NF- $\kappa$ B and MAPK activation", *Microb Pathog*, vol. 132, pp. 302-312, 2019.
76. S. Zeng, D. Chen, G. Liu et al., "Salvianorin A protects against methicillin-resistant staphylococcus aureus-induced acute lung injury via the Nrf2 pathway", *International Immunopharmacology*, vol. 90, pp. 107221. , 2020.
77. I. Cinar, B. Sirin, P. Aydin et al., "Ameliorative effect of gossypin against acute lung injury in an experimental sepsis model of rats", *Life Sciences*, vol. 221, pp. 327-334, 2019.
78. Y.-s. Chai, Y.-q. Chen, S.-h. Lin et al., "Curcumin regulates the differentiation of naïve CD4+T cells and activates IL-10 immune modulation against acute lung injury in mice", *Biomedicine & Pharmacotherapy*, vol. 125, p. 109946, 2020.
79. R. Guo, Y. Li, M. Han, J. Liu, Y. Sun, "Emodin attenuates acute lung injury in Cecal-ligation and puncture rats", *International Immunopharmacology*, vol. 85, pp. 106626, 2020.
80. M.T. Zhou, C.S. Chen, B.C. Chen, Q.Y. Zhang, R. Andersson, "Acute lung injury and ARDS in acute pancreatitis: mechanisms and potential intervention", *World journal of gastroenterology*, vol. 16(17), pp. 2094-2099, 2010.



81. H.-z. Jin, X.-j. Yang, K.-l. Zhao et al., "Apocynin alleviates lung injury by suppressing NLRP3 inflammasome activation and NF- $\kappa$ B signaling in acute pancreatitis", *International Immunopharmacology*, vol. 75, p. 105821, 2019.
82. M. Mathan Kumar and R. Tamizhselvi, "Protective effect of diallyl disulfide against cerulein-induced acute pancreatitis and associated lung injury in mice", *International Immunopharmacology*, vol. 80, p.106136, 2020.
83. C. Koxsal, A. K. Bozkurt, N. Ustundag et al., "Attenuation of acute lung injury following lower limb ischemia/reperfusion: the pharmacological approach ", *J Cardiovasc Surg (Torino)*, vol. 47(4), pp. 445-9, 2006.
84. H. Wen, H. Zhang, W. Wang, Y. Li, " Tetrahydropalmatine protects against acute lung injury induced by limb ischemia/reperfusion through restoring PI3K/AKT/mTOR-mediated autophagy in rats ", *Pulmonary Pharmacology & Therapeutics*. 64: p. 101947. , 2020.
85. C.-y. Qin, J. Gu, J.-x. Fan et al., "Epigallocatechin gallate attenuates mitochondrial DNA-induced inflammatory damage in the development of ventilator-induced lung injury", *Phytomedicine*, vol. 48, pp. 120-128, 2018.
86. B. Liu, H. Yu, R. Baiyun et al., "Protective effects of dietary luteolin against mercuric chloride-induced lung injury in mice: Involvement of AKT/Nrf2 and NF- $\kappa$ B pathways", *Food and Chemical Toxicology*, vol. 113, pp. 296-302, 2018.
87. M.-S. Kim, J.-Y. Han, S.-H. Kim et al., "Oleanolic acid acetate attenuates polyhexamethylene guanidine phosphate-induced pulmonary inflammation and fibrosis in mice", *Respiratory Physiology & Neurobiology*, vol. 252-253, pp. 1-9, 2018.
88. Y. Jiang, W. Yang, and S. Gui, "Procyanidin B2 protects rats from paraquat-induced acute lung injury by inhibiting NLRP3 inflammasome activation", *Immunobiology*, vol. 223, pp. 555-561, 2018.
89. Z. Yang, Y. Guan, J. Li, L. Li, and Z. Li, "Chrysin attenuates carrageenan-induced pleurisy and lung injury via activation of SIRT1/NRF2 pathway in rats", *European Journal of Pharmacology*, vol. 836, pp. 83-88, 2018.
90. S.Y. Jeong, J. Kim, E.K. Park, M. C. Baek, and J -S. Bae, "Inhibitory functions of maslinic acid on particulate matter-induced lung injury through TLR4-mTOR-autophagy pathways", *Environmental Research*, vol.183, p. 109230, 2020.