SCIREA Journal of Clinical Medicine
ISSN: 2706-8870
http://www.scirea.org/journal/CM
June 4, 2022
Volume 7, Issue 3, June 2022
https://doi.org/10.54647/cm32844

Protective Mechanisms of Quercetin in Various Lung-induced Injuries

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ABSTRACT

Acute respiratory distress syndrome (ARDS) remains to be a paramount healthcare issue, frequently resulting in respiratory failure and death. Recently, the rapidly expanding knowledge about the pathophysiology of novel severe acute respiratory syndrome (nSARS-CoV-2) infection provides a significant insight regarding the implication of cytokines storm, which mainly causes an acute lung injury (ALI) in COVID-19 patients and directs the disease severity. As hypoxemia worsens, ALI can progress into ARDS leading to a high mortality rate. Despite advances in clinical care, the lungs of a subset of ARDS survivors show persistent fibrotic changes triggered by an imbalance between higher reactive oxidative species and lower anti-oxidative substrates. Clinical evidence have shown the pneumoprotective effects of quercetin in certain pulmonary conditions. Albeit many studies evaluating quercetin's anti-inflammatory action on bacterial lipopolysaccharide-induced models have been done, anti-inflammatory studies using viral-induced models or its surrogate are still lacking. In this review, the authors discuss the possible molecular mechanism of quercetin in targeting specific pathways in lung injury and its sequelae, including pulmonary fibrosis that is induced both by infectious and pneumotoxic agents.

Keywords: Quercetin, acute lung injury, acute respiratory distress syndrome, anti-inflammatory, pulmonary fibrosis, pneumoprotective

Background:

Acute respiratory distress syndrome (ARDS) remains to be a paramount healthcare issue, affecting more than 190,000 people in the United States per annum, with a mortality of 27-45%, determined by comorbidities and the severity of the illness [1]. Nowadays, the rapidly expanding knowledge about the pathophysiology of novel severe acute respiratory syndrome (nSARS-CoV-2) infection provides a significant insight regarding the implication of cytokines storm, which mainly causes an acute lung injury (ALI) in COVID-19 patients and directs the disease severity. As hypoxemia worsens, ALI can progress into ARDS leading to a high mortality rate among patients who progressed to a severe stage of ALI. Based on earlier studies, approximately 67-85% of ARDS mortality in the intensive care unit (ICU) patients due to COVID-19 was identified. Contrarily, general ARDS mortality and those attributable to SARS following ICU admission were estimated as 35.3% and 52.2% respectively [2]. Despite advances in clinical care, notably pulmonary protective strategies of mechanical ventilation, the lungs of a subset of ARDS survivors show persistent fibrotic changes. Hence, in many cases correlate with declining health-related quality of life (HRQoL) that last months to years after the acute illness [1].

Following the acute inflammatory phase of ARDS, another contributing factor in the development of fibroproliferative changes is an imbalance between the higher reactive oxygen species (ROS) production and the exhaustion of anti-oxidative substrates [1]. Similarly, a number of toxic substances are produced/secreted by the bacteria, like lipopolysaccharide (LPS), which is associated with the severity of lung injury and ultimately causes pulmonary fibrosis. Such a toxic effect of LPS can be alleviated with quercetin [3–5]. Bleomycin and Benzo-a-Pyrene are also well documented in some literatures as pulmonary fibrosis-inducing agents [4,6–8]. However, although many studies evaluating quercetin's anti-inflammatory action on bacterial lipopolysaccharide-induced models have been done, anti-inflammatory studies using viral-induced models or its surrogate are still lacking.

Quercetin ($C_{15}H_{10}O_7$) is a polyphenolic flavonol compound, one of six subclasses of flavonoids. Being one of the most abundant forms of flavonoids, it consists of 3 rings and 5 hydroxyl groups placed at the 3-, 3'-, 4', 5-, and 7- positions. It is ubiquitously found in

apples, berries and onions. Theoretically, quercetin posseses antiviral properties against nSARS-CoV-2 binding site of the viral spikes protein to ACE-2 receptors, main protease (M^{pro}) or $3CL^{pro}$, Papain-like protease (Pl^{pro}), and RNA-dependent RNA polymerase (RdRp). Hence, it interferes the release of cytokines (i.e. IL-6, IL-8, TNF-α, IL-1β, IL-10, CXCL10, IL-1RA, IL-18, NLRP3 inflammasome) which trigger inflammatory response, ROS, and pulmonary fibrosis in nSARS-CoV-2 infection [9]. Clinical evidence also shown the effectiveness of quercetin for cetain respiratory conditions such as chronic obstructive pulmonary diseases (COPD), upper respiratory tract infections (URTI), sarcoidosis, and as an adjuvant therapy of COVID-19 [9]. In this review, we discuss the current evidence of quercetin in alleviating lung injury and its sequelae which occur as a result of inflammatory process both from infectious and pneumotoxic agent.

Basic mechanism directing fibro-proliferative response in ARDS:

For a few decades, our understanding of the patho-mechanism directing fibro-proliferative response in ARDS has progressed substantially **(Figure 1)**. ARDS is a heterogenous clinical syndrome characterized by acute inflammation, microvascular damage, and increased permeability of pulmonary vasculature and epithelium. This, sequentially, driving to series of events comprising plasma exudation into the alveolar spaces, which, together with compromised alveolar fluid clearance, results in alveolar edema [1]. Moreover, pro-inflammatory cytokines and chemokines release along with activation of coagulation system results in neutrophils, monocytes/macrophages, and lymphocytes influx into the alveoli following the defects in alveoli-capillary membrane. During acute inflammatory stage of ARDS, potent cytotoxic mediators generated from infiltrating leukocytes are released, including reactive oxygen and nitrogen species, elastase, matrix metalloproteases (MMPs), leads to alteration and damage in the pulmonary architecture. The interplay between ongoing damage and failure of reparative process in timely manner are essential factors which promote fibro-proliferative response [1,10].

In some ARDS patients, robust and perpetual accumulation of macrophages, fibrocytes, myofibroblast, and fibroblast in the alveoli, give rise to the excessive deposition of fibronectin, collagen type I and III, and other extracellular matrix (ECM) components [1,10]. This phenomenon occurs in parallel with an imbalance between antifibrotic (e.g. keratinocyte growth factor, hepatic growth factor, and prostaglandin E2) and profibrotic (e.g. transforming

growth factor (TGF-α and -β), IL-1 β, platelet-derived growth factor/PDGF, and lysophosphatidic acid) [1,10]. The initial injury owing to inflammatory process in the alveolar stucture may be amplified by damage from shear forces exerted by mechanical ventilation, denoting the importance of pulmonary protective ventilation approaches to limit ongoing epithelial injury which may direct fibroproliferative response. This ventilator-associated injury also interferes surfactant production [1,11].

Another key factor in ARDS is vascular lesions comprising endothelial injury, increased microvascular permeability, obliterative, thrombotic and fibroprolifertaive alteration which are associated with diffuse alveolar damage histologically [10]. Production of angiogenic substance owing to these alterations has also been reported to promote further injury and fibroproliferative response. Those include vascular endothelial growth factor (VEGF), macrophage inflammatory protein-2 (MIP-2), and angiopoietin-2 [10,12].

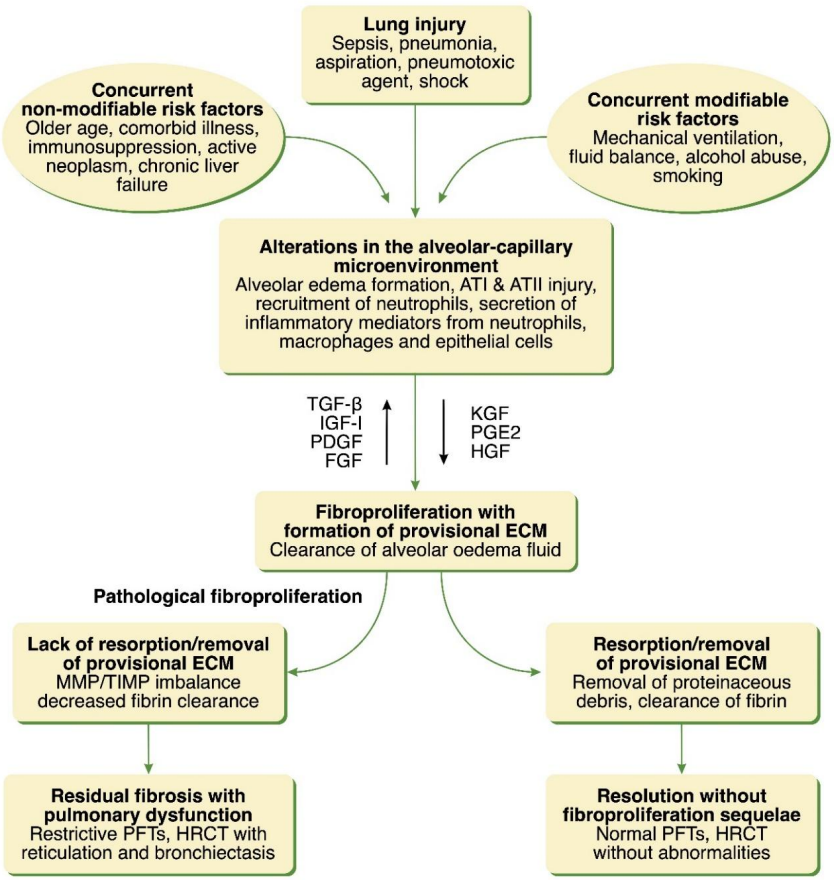

Figure 1. Pathophysiological processes that direct fibroproliferation changes in ARDS. Typical alterations associated with lung injury progress over time affected by environmental and individual-specific risk factors. A provisional extracellular matrix (ECM) is eventually

formed to promote repair and proteinaceous fluid causing pulmonary edema is then cleared from the alveolar space. Albeit the provisional ECM largely subsides after the restoration of pulmonary architecture, a subset of patients remains with ECM deposition and has remaining fibrosis sequelae. AT: angiotensin; TGF: transforming growth factor; IGF: Insulin-like growth factor; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; KGF: keratinocyte growth factor; PGE: prostaglandin E; HGF: hepatocyte growth factor; MMP: matrix metalloproteinases; TIMP: tissue inhibitor of metalloproteinase; PFT: pulmonary function test; HRCT: high-resolution computed tomography.

Mechanistic of Acute Lung Injuries and Quercetin Protection:

1. Quercetin protects lipopolysaccharide-induced acute lung injury;

Several *in vivo* studies have reported the promising potential of quercetin in ALI-induced mice models. Takashima and colleagues [3] reported that intratracheal administration of quercetin significantly reduced the wet lung-to-body weight ratio in mice challenged with lipopolysaccharide (LPS). The wet lung-to-body weight ratio is associated with the severity of lung injury and it is used as an index for pulmonary edema [13]. The number of total cells and neutrophils, thickening of alveolar wall and interstitial infiltration as observed in histological sections all of those showed a declining tendency in the presence of quercetin pretreatment. Consistently, quercetin in dose-dependent manner, was able to enhance the expression of heme-oxidase-1 (HO-1) both at the protein level and at the mRNA level. This expression enhancement was seen in AMJ2-C11 cells up to 3.5-fold for mRNA and 2.7-fold for protein compared to the control group [13]. On the other hand, quercetin-cultured AMJ2-C11 cells demonstrated significant decline in the mRNA expression and production of IL-1β, IL-6, and TNF-α production induced by LPS. Further exploration of quercetin suppression effect was done by *ex vivo* methods. It revealed that the expression of previously mentioned cytokines and both latent and active MMP-9 activities in bronchoalveolar lavage fluid (BALF) cells were also suppressed by quercetin pretreatment [13]. MMP-9 is predominantly generated by neutrophils and macrophages, and its activation mechanisms are incorporated to other MMPs, like MMP-3 and neutrophil elastase [14,15].

Consistent with previous studies, Huang and colleagues [4] evaluated the anti-inflammatory and antioxidant effects of quercetin on LPS-induced acute lung injury. LPS exposure evokes extensive pulmonary damage, profoundly manifested by interstitial and alveolar spaces

infiltration of inflammatory cells, and thickening of the alveolar wall. Intriguingly, quercetin was able to attenuate these changes. Attenuation of lung wet/dry ratio was exerted by quercetin, indicating that quercetin ameliorates the development of pulmonary edema. Other significant results demonstrated by quercetin pretreatment were reduction of myeloperoxidase activity, neutrophil count, BALF protein level and BALF level of IL-6 and TNF-α. Concerning the oxidative stress prevention occurs in the mice model, quercetin pretreatment emanated reduction in malondialdehyde (MDA) level and elevation in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [4]. Another study by Cui and colleagues focusing on septic-induced mice model supported these findings [6]. At the dose of 15 and 25 mg/kg to septic mice, quercetin administration significantly reduced in reactive oxygen species (ROS) level; reversed and reduced damage to pulmonary parenchyma; reduced the expression of HMGB1 (high mobility group box 1). Likewise, it significantly increased the level and activity of SOD, CAT, and Ascorbate peroxidase (APX). It is well known that the ROS [16] and the HMGB1 protein [17] play an essential role in the development of sepsis. Hence, its suppression is presumably able to ameliorate the subsequent deteriorated symptoms clinically during ALI-induced sepsis.

More recent study revealed that quercetin, in a dose-dependent manner, has positive effects on LPS-induced ALI in mice model focusing on blocking neutrophil recruitment, inhibiting proinflammatory cytokines release, and decreasing albumin leakage [5]. The percentage of neutrophil counts were significantly reduced by administration of lower, high doses of quercetin at 25 and 50 mg/kg respectively compared to control model, and dexamethasone treated group. Simultaneously, quercetin significantly elevated the macrophage percentage in BALF. LPS-stimulated cytokines released like IL-6, Keratinocyte Chemokine (KC), and IL-1β were also significantly reduced by quercetin [5]. LPS induction was found to significantly decrease the expression of Exchange Protein Activated by cAMP (Epac), but not PKA and phosphorylated PKA. Intriguingly, this effect was counter-acted by quercetin administration and the inhibition effect was further confirmed by using PKA/Epac activator (6nBz/8-CPT) and inhibitor (H89/ESI-09) to treat LPS-induced MLE-12 cells. Administration of Epac activator, 10 μM 8-CPT, significantly blocked KC release at 12 hours similar to quercetin while the same concentration of PKA activator, 6nBz could not block KC release from LPS-induced Mouse Lung type II Epithelial (MLE-12) cell line. Quercetin plus PKA inhibitor (H89) synergistically acted to decrease the release of KC from LPS-induced MLE-12 cells. On the contrary, Epac inhibitor (ESI-09) antagonized the inhibitory action of quercetin, thus

KC release was significantly elevated. These results suggest that quercetin protects LPS-induced ALI in MLE-12 cells through a Epac-dependent signaling pathway, which directs to the Epac activation and KC release suppression [5].

Another *in vivo* study supports the evidence of beneficial effects of quercetin on LPS-induced ALI in mice. It showed that quercetin pretreatment raised IL-10 secretion and abolished pulmonary pathological changes, MDA, myeloperoxidase activity, serum TNF-α , IL-6, IL-1β, and NO levels; reduced mortality and increased survival time [18]. Inhibition of crucial cytokines by LPS-induced murine macrophages (RAW264.7 cells) like TNF-α, IL-6, and IL-1β was also observed during *in vitro* and *in vivo* administration of isorhamnetin, a 3-O-methylated metabolite of quercetin [19]. In the ALI model, isorhamnetin significantly reduced neutrophil infiltration and pulmonary edema. Additionally, the protein level in BALF, the wet weight to dry weight ratio, and iNOS secretion were also lessened by isorhamnetin. Consequently, isorhamnetin demonstrated protective effect on LPS-induced ALI model by suppressing ERK1/2, JNK, P38, IκBα, and NF-kB/P65 phosphorylation [19].

2. Anti-inflammatory effect of quercetin on dsRNA analogue-induced lung inflammation;

Albeit many studies evaluating quercetin's anti-inflammatory action on bacterial lipopolysaccharide-induced models have been done, anti-inflammatory studies using viral-induced models or its surrogate are still lacking. Activation of macrophages induced by Polyinosinic-polycytidylic Acid (Poly (I:C)), a synthetic analogue of double-stranded RNA (dsRNA), is regarded as a viral-induced inflammation model *in vitro* [20]. RAW 264.7 mouse macrophages were incubated with Poly (I:C) and/or quercetin in different concentrations. The results of this study showed that quercetin at a concentration of 50 μM significantly suppressed calcium release and the production of NO, IL-6, MCP-1, IP-10, RANTES, GM-CSF, TNF-α, LIF, LIX, and VEGF. Likewise, quercetin at a concentration of 50 μM significantly suppressed the expression of Signal Transducer and Activated Transcription (STAT1 and STAT3) in the model [20]. Since endoplasmic reticulum (ER) calcium stores are depleted and intracellular calcium concentrations are elevated during oxidative stress-mediated STAT1 activation, inhibition of STAT is of importance in modulating the inflammatory process. During this process, the formed pro-apoptotic ER stress induces inflammation on macrophages [21].

3. Pneumo-protective effects of quercetin against Benzo-a-Pyrene induced lung injury;

Recently, the pneumoprotective effect of quercetin against Benzo-a-Pyrene (BaP)-induced lung injury has been reported [22,23]. *In vivo* study by Alzohairy *et al*. showed that quercetin was able to protect lung injury induced by BaP by modulating proinflammatory cytokine releases, oxidative stress, and maintaining pulmonary tissue architecture [22]. Pretreatment with quercetin 50 mg/kg mice body weight prior to BaP induction significantly reversed the serum level elevation of LDH, IL-1β, IL-6, TNF-α, and the reduction of antioxidant enzymes (CAT, GST, GSH) in pulmonary tissues. Pretreatment with quercetin also increased the total antioxidant capacity following BaP-induced lung injury, while malondialdehyde (MDA) levels were reduced. Based on histological analysis, some changes occurred following BaP induction including severe collagen deposition indicating interstitial fibrosis, major destruction of alveolar tissue, infiltration of inflammatory cells, hemorrhage, and congestion. Less collagen deposition and architectural distortion were observed in the pretreatment quercetin group. Likewise, the expression of VEGF and COX-2 in quercetin-treated mice was reduced [22]. Similar to the previous study by Chan *et al*. [23], quercetin supplementation (100 mg/kg) given to Mongolian gerbils exposed to BaP only or BaP plus β-carotene significantly reduced the inflammatory cell infiltration, the levels of TNF-α and IL-1β in the broncho-alveolar lavage fluid (BALF) and plasma. The suppressive effects of quercetin were followed by a down-regulation of phospho-c-jun and phospho-JNK expression induced by BaP only or BaP plus β-carotene in the pulmonary models [23].

4. Quercetin alleviating the pulmonary fibrosis induced by bleomycin;

Induction of lung fibrosis by bleomycin is an established and widely applied surrogate model of human lung fibrosis [7]. Administration of single intratracheal bleomycin (6.5 U/Kg) to rats model exerted a marked decrease in antioxidant capacity indicated by increase in the malondialdehyde (MDA) level, significant reduce in superoxide dismutase activity, low catalase activity. Likewise, bleomycin-induced fibrosis model showed elevated total leukocyte count in BAL, elevation in inflammatory cytokines notably TNF-α, elevated MMP-7 expression, excessive deposition of collagen in the lung as depicted by high concentration of hydroxyproline, and fibrotic changes which included moderate to severe hemorrhages, thickening of alveolar septa, fibroplasia, leukocytic infiltration in alveolar walls, and emphysema [7]. Nevertheless, oral quercetin administration is rendered to have ameliorative effect on the inflammatory changes and protective effects against local oxidative stress evolved by bleomycin [7].

Another *ex vivo* study in bleomycin-induced pulmonary fibrosis using human bronchial epithelial cell line BEAS-2B cell also supports the antioxidant and anti-inflammatory effect of quercetin by upregulating Nrf2 expression and Nrf-2 responsive genes as well as suppressing pro-inflammatory cytokines including TNF-α and IL-8 [8]. In a dose dependent manner, quercetin pretreatment of 50 μM showed 50% reduction in ROS production following bleomycin exposure. The results also revealed that bleomycin induced a slight, yet not significant elevation in the Nrf2 expression, a 50% significant reduction in the HO-1 expression, a not significant reduction in γ-GCS, no alteration in catalase (CAT) expression and no effect on Nrf2-ARE binding activity. Intriguingly, quercetin pretreatment enhanced Nrf2 expression and Nrf2-regulated genes HO-1 and γ-GCS, but not CAT; and a 30% significant increase in Nrf2-ARE binding activity. As stated earlier, inflammation and ROS are closely intertwined in pathogenesis of pulmonary fibrosis indicated by elevation of IL-8 and TNF-α. Pretreatment with 25 μM quercetin significantly reduced IL-8 production after bleomycin exposure (91). To further evaluate the anti-inflammatory effect of quercetin, LPS-stimulation of the blood of both IPF patients and control group was done. Concordantly quercetin was able to reduce the IL-8 and TNF-α level in the IPF group [8].

Molecular Mechanistic of Quercetin:

1. Quercetin upregulates Nrf2 expression in the pulmonary fibrosis

Upregulation of Nrf2-regulated HO-1 expression is known to confer protective mechanism in multiple models of lung injury and other diseases, including asthma, oxidative and/or inflammatory lung injury, vascular endothelial injury, and ischemia-reperfusion injury [24]. *In vitro* study by Nakamura et al. revealed that, quercetin suppressed collagen production in mRNA and protein levels via NIH3T3 mouse embryonic fibroblast cells, and that the suppressive mechanism deemed to be HO-1-dependent. Likewise, in a concentration-dependent manner, it was evident that quercetin at 15-30 μM, upregulated both the mRNA and protein expression of HO-1 as well induced the translocation of Nrf2 into the nuclei. Further evaluation demonstrated that HO-1 metabolites, CO, but not bilirubin, was able to decrease to near basal levels in the TGF-β-stimulated production of type I collagen mRNA and total soluble collagen in NIH3T3 cells. In other cell types; Human Lung Fibroblast (HLF), the level of mRNA and protein of HO-1 were induced 9.9-fold and 2.5-fold, respectively, hereon treatment with quercetin. Addition of quercetin at 30 μM in NHLFs cells

also markedly reduced the levels of type I collagen mRNA and total soluble collagen by 78.7% and 78.6% inhibition, respectively [25]. Contrariwise, quercetin in concentration-dependent manner showed stimulatory effect rather than suppressive on the TGF- β-induced phosphorylation of Smad/MAPK pathways. Quercetin also significantly stimulated phosphorylation of ERK and JNK, but not p38 in NIH3T3 cells. Moreover, specific inhibitors for ERK and JNK, PD98059 and SP600125 respectively, were used to evaluate whether both these signaling were involve in the suppressive effects of quercetin on TGF- β-induced collagen production. Surprisingly, no changes in the suppressive activity of quercetin were found. These findings suggested that the neither ERK nor JNK activation play a role in these suppressive effect, and different mechanism of suppression remains to be elucidated [25]. Similarly, *in vitro* study by Hayashi *et al.* revealed an increased nuclear localization of endogenous Nrf-2 and HO-1 expression in the quercetin-treated group following H_2O_2-induced lung epithelial cell line (LA-4 cell) [26]. At molecular levels, quercetin not only upregulates the expression of Nrf-2 protein and mRNA but also inhibits the proteasomal turnover and ubiquitination of Nrf-2. At the same time, reducing the level of Keap1 protein at the posttranslational level by quercetin was reported to be able to increase Nrf-2-dependent ARE activity [27].

2. Quercetin role in pulmonary senescent fibroblasts;

Some evidence signify that senescent cells may also cause and/or contribute in remodeling of tissue and many age-related disease, notably Chronic Obstructive Pulmonary Disease (COPD) and Idiopathic Pulmonary Fibrosis (IPF). Indeed, it was pointed that senescent myo-fibroblasts predominantly found in fibroblast foci. Secretome generated by the senescent fibroblasts known as Senescence Associated Secretory Phenotype (SASP) strongly stimulates a fibrotic phenotype in healthy human fibroblast which may elucidate the correlation of its build up to the progression of fibrosis [28,29]. Recently, some studies have demonstrated the therapeutic potential of combination of quercetin and Dasatinib (Tyrosine Kinase Inhibitor) in mice model of bleomycin induced pulmonary fibrosis. The treatment of this combination during the fibrotic phase reduced deleterious effect of fibrosis and senescence biomarkers in the mice lungs [28]. Likewise, *ex vivo* study demonstrated that the combination of quercetin and dasatinib induced apoptosis and diminished SASP factors in primary fibrotic mice alveolar epithelial type II cell induced by bleomycin, hence senescent cells were depleted [29].

Another investigation by Hohmann et al. revealed that senescent fibroblast cultures from stable and rapidly progressive IPF patients were extremely resistant to Fas Ligand (FasL)-induced and TNF Related Apoptosis Inducing Ligand (TRAIL) induced apoptosis. In addition to low expression of FasL and TRAIL receptors detected in senescent IPF fibroblast, there was a low expression of caveolin-1 and an increased activation of AKT, in comparison to senescent normal lung fibroblast cultures. Albeit quercetin alone was not pro-apoptotic, the resistance to FasL or TRAIL-induced apoptosis could be abrogated by quercetin. It induced an approximately 2-fold increase in Fas mRNA expression. Combination between quercetin plus FasL or TRAIL in the culture significantly induced caspase-3 activity, decreased cell viability and elevated LDH level in normal and IPF fibroblast. It was conspicuous despite 1.5-fold increase in Caveolin-1 (CAV1 mRNA) expression, quercetin did not fully restore CAV1 expression in stable or rapid IPF fibroblast as compared to normal lung fibroblast cultures. Intriguingly, as upregulation of phosphorylated AKT (p-AKT) has been implicated in low CAV1 expression in this study, addition of quercetin was able to downregulate the p-AKT expression in both groups [30]. Moreover, *in vivo* quercetin treatment at 30 mg/kg in bleomycin-induced pulmonary fibrosis of aged mice reversed the fibrosis by reducing hydroxyproline levels and collagen formation. Significant attenuation in the expression of pulmonary senescence markers p19-ARF and p21 was observed. In line with these results, quercetin also reduced the expression of Cdkn1a, Cdkn2a, Cdkn2d; SASP-related transcripts Mcp1, Mmp12, and Il6. These results collectively suggested that quercetin works by attenuating the senescent cells burden in the fibrotic lung models [30].

3. Quercetin's inhibitory effects on other signaling pathways;

Intraperitoneal administration of quercetin showed a protective mechanism on acute lung injury (ALI) in mice with sepsis [31]. Low and high doses of quercetin at 30 and 50 mg/kg respectively rendered significant effects on ALI parameters and markers as compared to the sepsis-induced group by CLP procedure; and a high dose showed to generate greater improvement. Quercetin administration gave rise to elevated PaO_2 level and PaO_2/FiO_2 value, reduced lung water content and protein content in BALF as compared to the control model group. Intriguingly, the results also showed that expression of ICAM-1 and MIP-2 was suppressed by quercetin [31]. Intracellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein which presents on the cell surface such as neutrophils, leucocytes, endothelial cells, epithelial cells, and fibroblasts. It belongs to the immunoglobulin superfamily which has pivotal action in sepsis by mediating cell-cell

interaction and outside-in cell downstream signaling [32]. Macrophage inflammatory protein-2 (MIP-2) has a pivotal role also in the pathogenesis of sepsis. It belongs to the CXC chemokines family which recruits to infection sites [33]. Consequently, quercetin presumably protected against ALI in septic mice by regulating these chemokines.

Inhibitory effect of quercetin against Intracellular Adhesion Molecule-1 ICAM-1 expression and its association with Mitogen Activated Protein Kinase (MAPK) have been evaluated through *in vitro* study using A459 cells model by Ying et al. [34]. The IL-1β-induced expression of ICAM-1 mRNA and protein was attenuated by quercetin in a dose-dependent manner. It was suggested that quercetin actively blocked NF-κB activity and enhanced degradation of p65 and p50 subunits of I-κB. NF-κB. Following NF-κB activation, there will be a dissociation of I-κB from the complex resulting in translocation of p65 and p50 to the nucleus and binding to the DNA recognition site, hence initiating gene transcription [35]. The inhibitory activity of ICAM-1 expression demonstrated by quercetin was also facilitated by sequential attenuation of c-jun and c-fos mRNA expressions, components of AP-1 transcription factor which have pivotal role to induce ICAM-1 expression [34]. The authors further found that p38, ERK1/2, and JNK were involved as major MAPK signaling pathways which regulate AP-1 activity. Specific inbitors for p38, ERK, and JNK (SB203580, PD98059 and SP600125 respectively) were used to evaluate their individual action against IL-1β-induced c-jun and c-fos mRNA expression. PD98059 and SB203580 were able to inhibit c-fos mRNA expression while SP600125 was able to inhibit c-jun mRNA expression. Moreover, these specific inhibitors were administered to test their individual action in regulating the inhibitory actions of quercetin against IL-1β-induced expression of ICAM-1. The results demonstrated inhibitory effects of quercetin were partially inhibited by a specific inhibitor of p38 MAPK, SB203580, but not by a specific inhibitor of ERK and JNK, PD98059 and SP600125 respectively. It means that pre-incubation of cells with SB203580 allows quercetin to exhibit up-regulatory effects on ICAM-1 expression in IL-1β-induced A549 cells. This finding suggests that activation of the p38 MAPK pathway partially contributes to the process of ICAM-1 expression inhibition by quercetin [34].

Tripathi et al. found out the prophylactic efficacy of quercetin in improving vascular leakage in lung of mice under hypobaric hypoxic conditions [36]. The results showed that 50 mg/kg of quercetin supplementation was the optimum dose which is able to reduce ROS and MDA levels, elevate SOD, GSH and GPx levels, and reduce trans-vascular leakage and lung water content compared to non-treated hypoxia mice group. Reduction of LDH content and

albumin extravasation in BALF of hypoxic mice were also observed following quercetin prophylaxis. Furthermore, quercetin prophylaxis significantly downregulated the expression of IKKα/β and NFB in BALF of hypoxic mice driving to reduction in the level of TNF-α and INF-γ, pro-inflammatory cytokines. However, the level of anti-inflammatory cytokines, IL-4 and TGF-β, were upregulated by quercetin. The levels of Hif-1α and VEGF as well as ICAM-1, VCAM-1 and P-selectin in the lungs of hypoxia-induced mice displayed a significant increase [36]. Intriguingly, quercetin prophylaxis of 50 mg/kg as the optimum dose again significantly reduced the level of Hif-1α, VEGF, ICAM-1, VCAM-1 and P-selectin. Quercetin administration prior to hypoxia significantly reduced the detrimental changes on lung tissue under hypoxic conditions, including infiltration of inflammatory cells, collapsed alveoli, thickening of inter-alveolar walls and evidence of red blood cells in alveolar spaces [36].

Aforementioned protective mechanisms of quercetin are summarized in figure 2.

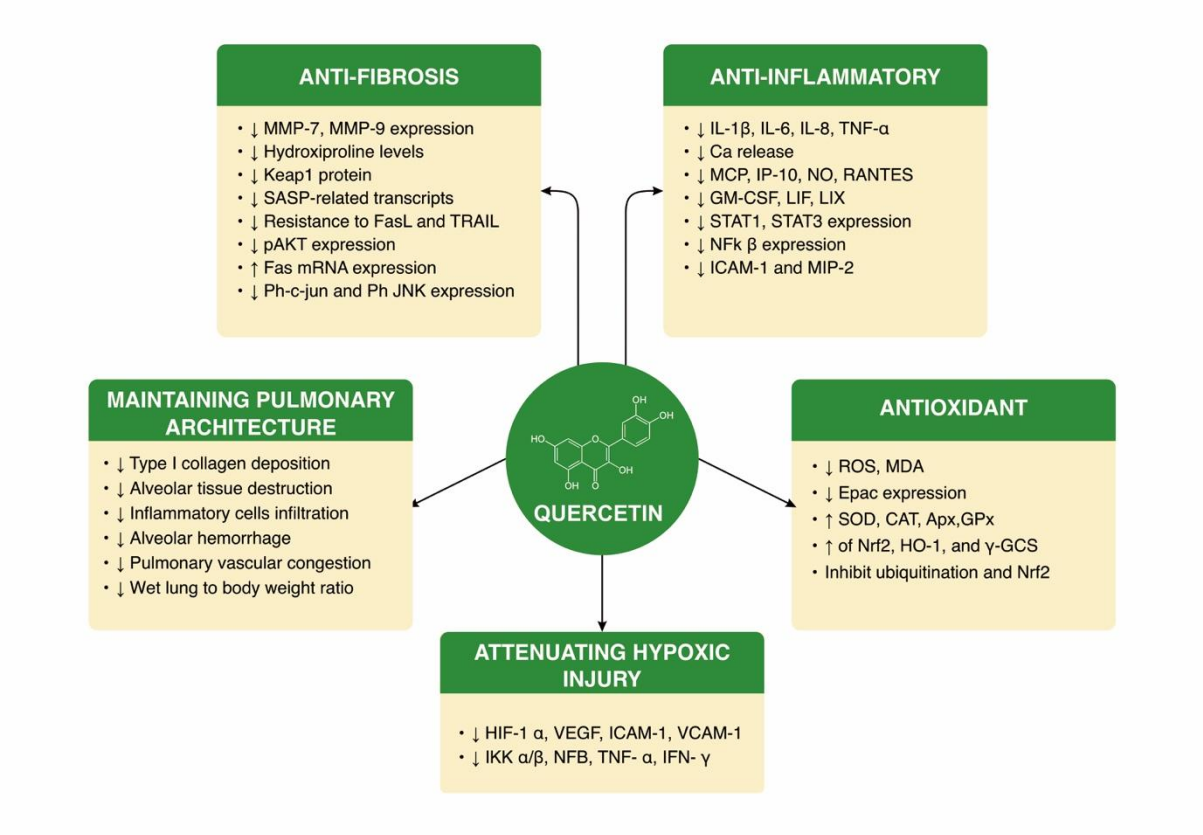

Figure 2: Several potential pneumo-protective mechanisms of quercetin.

CONCLUSION

The pathophysiology of specific pulmonary disease where pulmonary parenchyma is predominantly infected, and as the disease progresses, ALI and pulmonary fibrosis development may ensue. A comprehensive understanding of the prime balance pertaining to the physiological and pathological effects of the various profibrotic mediators may allow targeted therapy. Quercetin has been considered a significant bioflavonoid compound in the human diet and potentially benefits human health, specifically in alleviating inflammation and injury in the lungs. Collectively, many preclinical studies have reported the potential benefits of quercetin in inhibiting proinflammatory cytokines release and its downstream signaling, stimulating the transcription of antioxidant genes, and ameliorating pathological changes in pulmonary parenchyma which leads to fibrosis, especially during a hypoxic state. Understanding the multifaceted actions of quercetin, we consider quercetin as a good candidate for development and optimization of quercetin both as prophylaxis and adjuvant therapy in pulmonary infections, inflammation, and fibrosis.

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