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# Journal Pre-proof



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**Molecular mechanisms of action of naringenin in chronic airway diseases**

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## **Abstract**

Chronic airway inflammatory diseases are characterised by persistent proinflammatory responses in the respiratory tract. Although, several treatment strategies are currently available, lifelong therapy is necessary for most of these diseases. In recent years, phytochemicals, namely, flavonoids, derived from fruits and vegetables have been gaining tremendous interest and have been extensively studied due to their low toxicological profile. Naringenin is a bioflavonoid abundantly found in citrus fruits. This substance has shown notable therapeutic potential in various diseases due to its promising diverse biological activities. In this review, we have attempted to review the published studies from the available literature, discussing the molecular level mechanisms of naringenin in different experimental models of airway inflammatory diseases including asthma, chronic obstructive pulmonary disease (COPD), lung cancer, pulmonary fibrosis and cystic fibrosis. Current evidences have proposed that the anti-inflammatory properties of naringenin play a major role in ameliorating inflammatory disease states. In addition, naringenin also possesses several other biological properties. Despite the proposed mechanisms suggesting remarkable therapeutic benefits, the clinical use of naringenin is, however, hampered by its low solubility and bioavailability. Furthermore, this review also discusses on the studies that utilise nanocarriers as a drug delivery system to address the issue of poor solubility.

**Keywords:** Naringenin; chronic airway inflammatory disease; asthma; chronic obstructive pulmonary disease; lung cancer; pulmonary fibrosis

## 1. Introduction

### 1.1. Chronic airway inflammatory diseases

Chronic airway inflammatory diseases are long-term pathological conditions affecting the respiratory system and the structures associated to it. With the continual advancements in Science, humans have been, from time immemorial, striving to discover effective therapies for such diseases. Nevertheless, there is a steady rise in the number of people who are newly affected from such diseases each year, leading to an immense global health burden. The most common chronic airway inflammatory diseases are, asthma, chronic obstructive pulmonary disease (COPD), lung cancer, pulmonary fibrosis and cystic fibrosis. There already exists a large body of research and extensive published literature on these diseases (Mehta et al., 2019a), leading to several significant ground-breaking scientific advancements.

Currently, there are over 358 million people who are globally affected by asthma alone. However, it has been predicted that, by the year 2025, this number may jump up to 400 million (Mehta et al., 2019b; Soriano et al., 2017). In a similar vein, the global prevalence of COPD was estimated to be a staggering 251 million cases, as reported by the '2016 Global Disease Burden Study' (Soriano et al., 2017). Among the major chronic respiratory diseases, lung cancer continues to remain as the leading cause of cancer-related deaths in humans (Barta et al., 2019). It is reported that, more than a billion people on the face of earth are exposed to polluted air and tobacco smoke, which are key detrimental factors directly associated with the mortality and incidences of lung cancer (Jemal et al., 2010; Thun et al., 2013; Youlten et al., 2008). Pulmonary fibrosis, a relatively rare form of chronic respiratory inflammatory disease is reported with an incidence rate of 7-16 cases per 100,000 population (Raghu et al., 2006). Cystic fibrosis, on the other hand has an incidence rate of 20 cases per 100,000 live births, as reported in a study conducted in California, among 2,282,138 newborns between the years 2005 to 2010 (Spoonhower and Davis, 2016).

Airway inflammation is a complex and complicated biological reaction that occurs as a defensive response, to protect body tissues. The entire process primarily involves various immune cells as well as, several molecular mediators. It is well known that inflammation is initiated when several reactive oxygen species (ROS) are formed during bodily defence mechanisms against invasion of pathogens in the respiratory tract (Mittal et al., 2014). Inflammation in any respiratory tissues will attract other inflammatory cells, after exposure to

oxidants, which eventually favour oxidative stress in the lungs. These myriad of reactions and processes lead to sustained inflammation and eventually chronic oxidative stress (Kirkham and Barnes, 2013).

## 1.2. Naringenin

For several decades now, the biological activities and therapeutic benefits of flavonoids have been gaining much interest in several diseases (Wilcox et al., 1999). In order to study their prospective impacts, many active compounds from various forms of folk medicine were separated and studied in detail. In the past few years, several experimental and clinical studies have also been conducted on polyphenols. Naringenin is a therapeutic flavone, which has gained attention in recent years from among them. It is a common active compound that is present in various citrus fruits, which include, grapefruit, orange and lemon (Zaidun et al., 2018).

Naringenin (4,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one), has a molecular weight of 272.257g/mol (Lin-Shiau et al., 2004). It falls under the subclass of flavanone, which is classified under the broad umbrella of flavonoids. It constitutes a common structure of two aromatic rings together with a linear 3-carbon chain (C6-C3-C6), forming an oxygenated heterocyclic nucleus. The heterocycle in naringenin includes a saturated 3-carbon chain and an oxygen atom at carbon-4 (Kumar and Pandey, 2013) (Fig. 1). Naringenin is found in high concentrations, especially, in grapefruit (43.5 mg/100ml), followed by orange juice (2.13mg/100ml) and lemon juice (0.38mg/100ml) (Erlund, 2004; Gattuso et al., 2007). Naringenin has been long investigated for its various pharmacological benefits. Its potential therapeutic effects include anti-inflammatory (Luo et al., 2012; Shi et al., 2015), antioxidant (Moon et al., 2011), anti-cancer (Chang et al., 2017), immunomodulatory (Du et al., 2009), hepatoprotective (Hermenean et al., 2014), nephroprotective (Borradaile et al., 2003), neuroprotective (Hartogh et al., 2019) and anti-diabetic (Kumar et al., 2013; Nguyen-Ngo et al., 2019; Seyedrezazadeh et al., 2016).

### 1.2.1. Bioavailability

The large hydrophobic ring structure of naringenin contributes to its low water solubility and minimal bioavailability. Kanaze and co-workers demonstrated that the oral bioavailability of naringenin is approximately 5.81%, when they administered 135mg naringenin in humans. (Kanaze et al., 2007). The absorption of naringenin occurs through both passive and active transport and the process is not affected by a change in pH (Garg et al., 2001; Justesen et al., 1998; Xu et al., 2009; Zhang et al., 2015).

### 1.2.2. Anti-inflammatory properties

There is a strong published evidence to suggest the potential efficacy of flavonoids in inflammatory conditions, due to their wide range of mechanisms of action that work at the molecular level. At the early phase of inflammation, the pathogen- (PAMPs) and damage (DAMPs)-associated-molecular-patterns initiate the immune response. Resident macrophages generate more chemotactic molecules to attract leukocytes, mostly neutrophils. By generating superoxide anion, other ROS, along with nitrogen, neutrophils and activated macrophages eventually create oxidative stress. Naringenin inhibits the recruitment and the generation of superoxide anion (Manchope et al., 2016; Martinez et al., 2015), while increasing the ability of glutathione and antioxidants to quench free radicals. Naringenin also acts on macrophages that induce activation of the nuclear factor erythroid 2-related factor 2 (Nrf2), a nuclear factor that induces antioxidant and anti-inflammatory reactions, resulting in the expression of hemeoxygenase-1 (HO-1) (Manchope et al., 2016). PAMPs, DAMPs and ROS induce the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B) in macrophages, which lead to the synthesis of pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, IL-1 $\beta$  and tumour necrosis factor alpha (TNF- $\alpha$ ).

In addition, NF- $\kappa$ B activation has also been correlated with inflammatory activity in allergic airway illness (Hsu et al., 2017; Poynter et al., 2002). Naringenin has been proven to inhibit NF- $\kappa$ B activation *in vitro* (Conc. 30-1000nM) (Felipe A Pinho-Ribeiro et al., 2016) and *in vivo* (Conc. 50mg/kg) (Felipe A. Pinho-Ribeiro et al., 2016). NF- $\kappa$ B activation is widely implicated in inflammatory diseases (Chung, 2006). The inhibition of NF- $\kappa$ B pathway by naringenin is shown in Fig. 2.

There are two different pathways for NF- $\kappa$ B activation, i) the canonical pathway and ii) the alternate pathway. Naringenin inhibits only the canonical pathway, which is initiated by microbial products and pro-inflammatory cytokines such as IL-1 and TNF- $\alpha$  (Lawrence, 2009). This stimulation results in the activation of I $\kappa$ B kinase (IKK) complex (Ghosh and Karin, 2002), eventually phosphorylating the IKK  $\beta$ . The IKK complex then phosphorylates I $\kappa$ B that is bound to the p50/p65 subunits of NF- $\kappa$ B and inhibits its activity (Karin and Ben-Neriah, 2000). The phosphorylated I $\kappa$ B undergoes ubiquitinylation followed by proteosomal degradation. This permits NF- $\kappa$ B to translocate to the nucleus which leads to its expression in the genes, causing inflammatory responses. Naringenin may prevent degradation of I $\kappa$ B, thereby, inhibit NF- $\kappa$ B transcription activity (Lawrence, 2009).

## **2. Therapeutic potential of naringenin in chronic airway inflammatory diseases**

### *2.1. Asthma*

Asthma is a common airway inflammatory disease characterized by increased inflammatory cells, mucus production, remodelling, and inflammatory mediators in the airway, which may cause impairment in the lung function (Hansbro et al., 2017; Kim et al., 2020). In asthma, high oxidative stress stimulates intracellular signalling cascades. Inflammation is usually initiated via overexpression of pro-inflammatory markers which lead to expression of the inflammatory cytokines (Dua et al., 2019b; Liu et al., 2017). Such cytokines increase oxidative stress, stimulate immune responses, induce cytotoxicity, promote hyper-responsiveness of the bronchi, stimulate bronchospasm and also increase the secretion of mucin. The development of IgE has been correlated with the progression of asthma through interleukins, such as, IL-4 and IL-5. Besides, asthma induces oxidative stress due to a suppression in antioxidant defence mechanisms and an increase in ROS formation, that can lead to the damage of the lungs (Da Cunha et al., 2016; Fitzpatrick et al., 2012). Oxidative stress also induces mitochondrial dysfunction and inactivation of the electron transport chain in the lungs (Dua et al., 2019a; Mabalirajan et al., 2008; Silveira et al., 2019) (Fig. 3).

Despite the morbidity and mortality rate of asthma being on the decreasing side, with the current therapies, poor responses towards corticosteroids were reported in 5–10 percent of asthma patients. Moreover, adverse effects resulting from excessive use of steroids have exerted a significant negative impact on the patients' well-being. Consequently, there were also concerns that have been raised on the safety of prolonged use of corticosteroids (Silveira et al., 2019; Wadhwa et al., 2019b). However, recently reported evidence revealed the potential therapeutic effect of naringenin in experimental models of asthma (Guihua et al., 2016; Kim et al., 2020).

A study conducted by Ying *et al.*, have reported that naringenin attenuated airway hyper-reactivity (AHR) and airway inflammation in a murine model of asthma (Shi et al., 2009). The *in vivo* studies have shown reduced levels of IL-4 and IL-13 in bronchoalveolar lavage (BAL) fluid and total serum immunoglobulin E (IgE), after being treated with naringenin (25, 50, or 100mg / kg / body weight). In addition, pulmonary I $\kappa$ B $\alpha$  degradation and NF- $\kappa$ B DNA-binding activities have also been observed. Furthermore, naringenin significantly reduced the levels of chemokine (C-C motif) ligand 5 (CCL5), CCL11 and inducible synthase of nitric oxide (iNOS) (Shi et al., 2009). Theoretically, the inhibition of NF- $\kappa$ B along with a reduced gene expression may be the factors that are responsible for this phenomenon.



Further research by Ying and colleagues (2015) has shown that naringenin ameliorated chronic inflammation, continuous hyper-responsiveness and remodelling of the airways. Results revealed that the total T-helper 2 (Th2) cytokines, total serum and IgE levels in BAL fluid of mice had markedly reduced after the treatment with 50mg/kg dose of naringenin. This implies that naringenin may delay the airway remodelling progress (Shi et al., 2015).

Interestingly, hyperplasia in the airway smooth muscle (ASM) was found to be associated with an increased severity of asthma (Haitchi et al., 2005; Ramos-Barbón et al., 2010; Wang et al., 2008). In accordance with previous reports,  $\alpha$ -SMA areas were drastically increased in animal models after exposure to ovalbumin, while naringenin treatment had successfully caused a reduction in ASM hyperplasia (Shi et al., 2009). A study by Shi *et al.*, demonstrated a reduction in the resistance to airflow in respiratory tract after treatment with naringenin (Shi et al., 2015). As ASM contributes to the narrowing of the airways along with airway hyper-reactivity, it is presumed that naringenin could have reduced the thickness of the ASM layer and could have ameliorated AHR.

Furthermore, Seyedrezazadeh et al., attempted to critically examine the anti-inflammatory potential of flavonoids (hesperetin and naringenin) on asthma. The study revealed that inflammation and remodelling of the airways in murine chronic asthma models were markedly reduced after hesperetin-naringenin treatment. Histopathological examination highlighted the absence of goblet cells metaplasia as well as decreased intra-alveolar macrophages upon treatment with hesperetin-naringenin. This study also confirmed that the combination of hesperetin-naringenin led to the reduction in sub-epithelial fibrosis, hypertrophy of smooth muscle in airways, and atelectasis of lungs. These findings suggest that naringenin may ameliorate airway structural remodelling, which contributes to the treatment of asthma (Seyedrezazadeh et al., 2015).

Moreover, Iwamura et al., reported that the oral administration of naringenin chalcone significantly reduced Th2 production from the splenic cluster-of-differentiation-4 (CD4) T cells without affecting its proliferative ability (Iwamura et al., 2010). Total cell counts of infiltrating leukocytes decreased in mice with naringenin treatment. It is well known that IL-13 causes hyper-responsiveness in the airways and produces mucus in the absence of inflammatory cells (Wills-Karp, 1999). These symptoms are very typical of asthma and are further attributed to its fatality. IL-4, in addition, stimulates mucus production. IL-5 is known to improve the survival of eosinophils (Rothenberg and Hogan, 2006). The study also reported a reduction in the hyperproduction of mucus and cytokines (IL-4, IL-5, IL-13). Naringenin-chalcone may have inhibited the type I allergic reaction by suppressing the

production of histamine from mast cells. However, the mechanisms employed in the suppression of Th2 production by CD4 T cells and the dysregulation by mast cells are not fully known. More studies are needed to further explain the various mechanisms of action. Therefore, naringenin-chalcone could be proposed as a substitute for asthma treatment (Iwamura et al., 2010).

Moreover, thymic stromal lymphopoietin (TSLP) is known to play a vital role in allergic diseases. In a study conducted by Moon et al., the findings demonstrated that the synthesis and expression of TSLP in HMC-1 cells were inhibited with naringenin treatment (Moon et al., 2011). Naringenin (100 $\mu$ M) inhibited TSLP production at a maximal rate of 62.27 $\pm$ 10.79%. Naringenin also reduced the NF- $\kappa$ B luciferase activity (Moon et al., 2011). These findings suggest that naringenin may inhibit TSLP production and therefore, could be useful in the treatment of inflammation and allergic diseases (Table 1).

## 2.2. Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is a serious public health burden and is prevalent in smokers (Chellappan et al., 2020; Luo et al., 2012; Zaidun et al., 2018). The inflammation in COPD is known to be mediated by several factors such as pro-inflammatory cytokines, glucocorticoid receptor (GR) and NF- $\kappa$ B. Patients with COPD have been reported to respond poorly to corticosteroid therapy (Barnes, 2013; Wadhwa et al., 2019a).

Naringenin is thought to be beneficial in COPD due to its anti-inflammatory properties. A study carried out by Liu et al., investigated the effects of naringenin in both, laboratory-bred strain (BALB/c) mouse model induced with COPD using cigarette smoke (CS) and also with an *in vitro* model using A549 cells (Liu et al., 2018). The animals were pre-treated with naringenin in doses of 20, 40 and 80 mg/kg concentration, whereas, the *in vitro* cells were treated with naringenin along with CS extract exposure. Naringenin was found to significantly improve the pulmonary function, decrease inflammatory cells, and inhibit the pro-inflammatory cytokine production in BAL fluid and serum of CS animal group. Naringenin also inhibited the NF- $\kappa$ B pathway as revealed by reduced phosphorylation of NF- $\kappa$ B and I $\kappa$ B (Hämäläinen et al., 2007; Hua et al., 2016; Kumar R. and Abraham, 2017; Liu et al., 2018). Moreover, the levels of GR mRNA and protein were also significantly increased upon treatment with naringenin both in CS-exposed animals and cell culture. Therefore, naringenin could be an ideal therapeutic agent in ameliorating COPD-related inflammation (Liu et al., 2018).

Mucus hypersecretion is one of the common symptoms among COPD patients. In most of the cases, it is due to enlarged submucosal glands and an increased number of goblet cells in response to chronic airway irritation (Vestbo et al., 2013). A study conducted by Yang et al., related naringenin with mucus hypersecretion. It was found that naringenin reduced mucus hypersecretion by inhibiting the development of ROS and down-regulating NF- $\kappa$ B pathway via EGFR-PI3K-Akt / ERK MAPKinase signalling in the epithelial cells of human airways (Yang et al., 2011).

A further study conducted by Lin et al., revealed that naringenin exhibited an expectorant behaviour in the *in vivo* models. Naringenin (90 mg/kg) reduced the viscosity of mucus, which is caused by an increased airway volume secretion. This resulted in an improved mucus removal from the airway. In addition, the elevated airway secretion volume was attributed to the increased secretion of lysozyme (Lin et al., 2008). Moreover, naringenin also reduced the mucin secretion. These activities demonstrated naringenin's promising therapeutic benefits in ameliorating respiratory mucus hypersecretion (Table 1).

### 2.3. Lung cancer

Lung cancer is predominantly caused by long term exposures to carcinogens such as tobacco-smoke or inorganic agents, which manifest their carcinogenic effects primarily through oxidative stress (Finocchiaro et al., 2014; Sharma et al., 2019). Chronic and cumulative oxidative stress can cause continuous and sustained pulmonary inflammation which plays a vital role in cancer initiation and progression. Bodduluru et al., reported the chemopreventive effects of naringenin (50mg/kg). The study reported the suppression of oxidative stress, inflammatory and cell proliferation in benzo(a)pyrene (B[a]P) induced lung cancer bearing mice. The antioxidant and free-radical scavenging activity of naringenin is observed through a decreased lipid peroxidation by enhanced activity of enzymatic antioxidants, namely, glutathione peroxidase (GPx), catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD) and glutathione reductase (GR), in addition to non-enzymatic antioxidants like vitamin C and glutathione (GSH) Moreover, naringenin ameliorated inflammatory response through the down regulation of NF- $\kappa$ B and subsequently reduced the levels of inflammatory cytokines, namely, TNF- $\alpha$ , IL-1 $\beta$  and IL-6. Besides, decreased expression levels of proliferating cell nuclear antigen (PCNA) and CYP1A1 upon naringenin treatment have demonstrated the capability of naringenin in inhibiting cancer initiation and cell proliferation respectively (Bodduluru et al., 2016). Tundis et al., in their

study, also demonstrated that naringenin, which was isolated from *Salvia leriifolia* ethyl acetate extract, exhibited selective antiproliferative activity against human lung large cell carcinoma (COR-L23). Interestingly, naringenin (IC<sub>50</sub> values of 33.4  $\mu$ M) showed more potent antitumor properties compared to a Vinca alkaloid, vinblastine (IC<sub>50</sub> values of 50.0  $\mu$ M) (Tundis et al., 2011).

Metastasis remains a major contributing factor to poor prognosis and survival in lung cancer patients (Sangodkar et al., 2010). Chang et al., suggested that, treatment with 200 and 300 $\mu$ M naringenin for 48h markedly inhibited lung cancer A549 cells migration. Naringenin has shown to downregulate metalloproteinase (MMP)-2 and -9 proteolytic activity in the degradation of extracellular cell matrix. This, in particular, led to the inhibition of cancer cell invasion into the circulation. In addition, naringenin also intercepted the migration of A549 cells, via attenuation of AKT activities (Chang et al., 2017).

In addition, resistance to chemotherapy is also one of the major obstacles to treat lung cancer effectively. Interestingly, naringenin has been reported to enhance the anticancer effect of chemotherapeutic agents and thereby, sensitize the drug-resistant cancer cells to chemotherapy. It has also been reported that resistance to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) treatment has been demonstrated in human non-small-cell lung cancer (NSCLC) cell line A549 (Zhuang et al., 2010). TRAIL is a potent apoptosis inducing cytokine which selectively kills cancer cells over normal cells upon binding with the death receptor 4 (DR4) and DR5. Previous findings revealed that poor expression and functional signalling of DRs could contribute to TRAIL resistance in tumour cells (Zhang and Fang, 2005). A recent study by Jin et al., showed that co-treatment with naringenin (100  $\mu$ M) and TRAIL specifically induced and enhanced the activity of TRAIL-mediated apoptosis by upregulating DR5 expression in TRAIL-resistant A549 cells, which further induced caspase-8 and the cleavage of BH3 protein Bid (Jin et al., 2011). Furthermore, resistance to gefitinib, a first line agent in treating metastatic NSCLC has emerged in most of the patients and has caused death due to cancer metastasis from the resistant tumour cells (Qi et al., 2018). Li et al., suggested that Four Gentlemen Decoction, a Chinese poly-herb formulation which contains naringenin as one of the major components, has shown to enhance anti-cancer activity of gefitinib and inhibit metastasis in mice bearing lung cancer. Naringenin acted on multi-drug resistance-associated protein 1 (ABCC1), glutathione reductase (GSR) and 4-aminobutyrate aminotransferase (ABAT) which regulated taurocholic

acid, oxidized glutathione and oxygultaric acid respectively. Thereby, it was suggested that naringenin can alleviate TRAIL and gefitinib resistance (Li et al., 2019).

Instead of targeting tumour cells as treatment of lung cancer, Lian et al., reported that the combined use of naringenin and asiatic acid (AA), a natural herb-derived compound, has shown to be a promising immunotherapy, that acts by targeting the tumour microenvironment. Transforming growth factor  $\beta 1$  (TGF- $\beta 1$ ) promotes tumour growth and suppresses immune system through down-regulation of Smad7 and hyper-activation of Smad3 signalling in the tumour microenvironment. Naringenin (50mg/kg) acted as a Smad3 inhibitor whereas, AA (10mg/kg) acted as a Smad7 inducer in regulating Smad3/Smad7 signalling to suppress tumour progression by inducing cytotoxicity against cancer cell production and natural killer (NK) cell production in tumour-bearing mice. The Down-regulation of TGF- $\beta 1$ -mediated inhibition of Id2/IRF2 is believed to be accountable for the elevated NK cell production (Lian et al., 2018) (Table 1).

#### 2.4. Pulmonary fibrosis

Pulmonary fibrosis (PF) is characterized by chronic and progressive tissue repair responses that lead to excessive accumulation of extracellular matrix (ECM) and remodelling of the lung (Dua et al., 2018a; Kolahian et al., 2016). Recent studies have identified that naringenin exerted antifibrotic properties, as a potential agent to alleviate PF which is triggered by infection, radiotherapy, environmental and occupational pollutants.

TGF- $\beta$  is a major pro-fibrotic mediator which mainly is involved in pulmonary fibrosis, by promoting ECM accumulation. Du *et al.*, reported that oral administration of naringenin at 100mg/kg/day inhibited the pro-fibrotic activity in bleomycin-induced murine models of PF, through a reduction in the level of TGF-  $\beta 1$ , which further led to decreased CD4<sup>+</sup> CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (Du et al., 2009; Saito et al., 2018).

IL-1 $\beta$  is a proinflammatory cytokine that has been shown to contribute to PF progression (Kolb et al., 2001). Zhang and co-workers demonstrated that naringenin exerted protective effect in radiation-induced lung injury. The production of IL-1 $\beta$  was reduced upon treatment with naringenin and it has shown a similar efficacy with IL-1 $\beta$  neutralizing antibody in mice that received irradiation. Interestingly, naringenin was able to restore the homeostasis of

irradiation-induced inflammatory factors by recovering 22 inflammatory factors to normal levels in the unaffected rats (Zhang et al., 2018).

The induction of pulmonary fibrosis in pneumonia caused by *Mycoplasma pneumoniae* (MP) is associated with elevated pro-inflammatory cytokines through autophagy (Shimizu, 2016). Lin et al., conducted a study in MP infected mice, which showed significant inhibition of inflammatory mediators (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) and TGF- $\beta$  expression upon administration of naringenin at 100mg/kg/day. The mechanism involved in reversing MP-induced inflammatory response and PF was demonstrated by inhibiting autophagy-related protein Beclin-1 and LC3 in addition with reducing expression of p62 (Lin et al., 2018).

Studies have provided sufficient evidence that naringenin exhibits a protective action against occupational exposure and hazardous agents that cause fibrotic lung damage. According to a study done by Ali et al., naringenin given at a dose 100mg/kg/day alleviated inflammation and oxidative stress in B[a]P-induced rats (Ali et al., 2017). Naringenin suppressed the expression of COX-2 through down-regulation of NF- $\kappa$ B which led to decreased inflammatory response. Furthermore, naringenin treatment also attenuated B[a]P-induced oxidative stress by enhancing the activity of antioxidant enzymes (CAT, SOD, GST, GPx, GR and GSH) and suppressing ROS-mediated damage in lung tissues. A study conducted by Podder et al., on paraquat-exposed human bronchial epithelial BEAS-2B cells, demonstrated that naringenin at 100 $\mu$ M exerted cytoprotective effect via upregulation of Nrf2 antioxidant pathway. Nrf2 further activated its downstream antioxidant proteins HO-1 and NAD(P)H:quinone oxidoreductase 1 (NQO1) (Podder et al., 2014) (Table 1).

### 2.5. Cystic fibrosis

Cystic fibrosis (CF) is a progressive inherited disorder which is attributed to the mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene, that causes build-up of abnormally thick, sticky mucus linings in the lungs (Rowe et al., 2005). Nonetheless, there is currently no cure for CF. However, treatments are available to slow down lung disease as much as possible. A study by Shi et al., showed that naringenin (50 $\mu$ M and 100  $\mu$ M) enhanced CFTR mRNA expression in LPS-induced CFTR down-regulated Calu-3 cells (Shi et al., 2017). Increased CFTR expression via cAMP pathway stimulated Cl<sup>-</sup> secretion, resulted in improved electrolyte and fluid secretion that led to the removal of sputum from the airway. The expectorant function of naringenin might be a promising treatment strategy for sputum removal and reduction (Table 1).

### 3. Clinical trials involving Naringenin

There are very few clinical trial studies on potential therapeutic outcomes with naringenin, and no clinical studies have been carried out yet on the anti-inflammatory effect of naringenin in airway inflammatory diseases (Salehi et al., 2019).

Till date, there are only 8 clinical trials in the entire database that were conducted using naringenin, which have been successfully registered at clinicaltrials.gov. The focus areas include, hepatitis, safety, pharmacokinetics of naringenin, bioavailability of naringenin and vascular protection effect of naringenin (“A Pilot Study of the Grapefruit Flavonoid Naringenin for HCV Infection - Full Text View - ClinicalTrials.gov,” n.d.). The clinical trial data with the ClinicalTrials.gov identifier (NCT03582553) evaluated the safety and pharmacokinetics of naringenin in healthy adults using an entire orange extract (*Citrus sinensis*). Results showed that, intake of naringenin at dose of 150mg to 900mg is safe in healthy adults. Serum levels were proportional to the dose given (“Safety and Pharmacokinetics of an Extract of Naringenin - Full Text View - ClinicalTrials.gov,” n.d.).

Most clinical studies investigated naringenin along with a complex food, instead of pure naringenin. For instance, whole orange juice which constitutes several polyphenols was studied instead of naringenin alone. This led to the difficulty in assessing the contribution of the single phytochemical (“Bioavailability of Carotenoids and Flavonoids From Fresh Oranges and Orange Juice. - Full Text View - ClinicalTrials.gov,” n.d.; Silveira et al., 2014). Clinical trials on naringenin to justify its anti-inflammatory effect in airway inflammatory diseases are highly recommended, as naringenin has been proven for its anti-inflammatory effect in both *in vivo* and *in vitro* studies. Thus, naringenin could be an alternative to the conventional treatment of airway inflammatory diseases (Salehi et al., 2019).

### 4. Drug delivery systems of naringenin

The poor aqueous solubility and minimal bioavailability of naringenin, owing to its large hydrophobic ring structure remains a major concern despite its promising therapeutic benefits.

Several studies have utilised nanocarriers as drug delivery system for naringenin in order to modulate its pharmacokinetics and pharmacodynamics profile, thus enhancing the therapeutic index of the drug. The reported drug delivery systems of naringenin and their key outcomes are summarized in Table 2.

#### *4.1. Polymeric nanoparticles*

Polymeric nanoparticles (NPs) are colloidal-shaped solid particles made of biodegradable and biocompatible polymers or copolymers, in which the drug can be encapsulated with the carrier (Dua et al., 2018b; Wadhwa et al., 2019c). The polymers that are commonly used to synthesise polymeric NPs include natural polymers such as alginate, chitosan, and gelatin in addition to synthetic polymers such as poly(lactide-co-glycolide) copolymers (PLGA), poly(lactide) (PLA), polyvinylpyrrolidone (PVP) and poly( $\epsilon$ -caprolactone) (PCL).

Kumar and coworkers studied on the anti-inflammatory and antioxidant activities of PVP-coated NPs on lipopolysaccharide induced RAW264.7 cell lines. The formulation was prepared by nanoprecipitation technique with an entrapment efficiency of 99.93% (Kumar and Abraham, 2016). PVP coated-naringenin NPs were found to be non-cytotoxic at any concentration below 200ug/mL. Furthermore, the anti-inflammatory effect in PVP-coated naringenin NPs has been found to be more pronounced, when compared to pure naringenin. Formulated naringenin has shown to suppress the inflammatory activities through down regulation of NF- $\kappa$ B via P38MAPK signaling pathways. This resulted in COX-2 and iNOS blockade, and thereby inhibited the inflammatory mediators (IL-6, IL-1 $\beta$ , TNF- $\alpha$  and MCP-1) and nitric oxide production (Kumar R. and Abraham, 2017).

Kumar et al., also reported that encapsulation of naringenin into chitosan NPs (CS-NPs) demonstrated improved antioxidant and anticancer activities of naringenin in A549 lung cancer cells. Naringenin-CS-NPs was prepared by ionic gelation method mediated by tripolyphosphate as a cross linker. The nanoparticles demonstrated an entrapment efficiency of approximately 70-80%. Cytotoxic test suggested that naringenin-CS-NPs exhibited selective cytotoxic effect against A549 cells. Moreover, the free radical scavenging activity in naringenin-CS-NPs is significantly higher than pure naringenin. Formulated naringenin is capable to inhibit hydroxyl radical, react with nitric oxide to inhibit production of nitrites and reduce the DPHH radical to non-radical form (Kumar et al., 2015).



Polymeric NPs such as PCL lack of target specificity, hence surface modification with specific ligands was attempted for tumour targeting (Cabeza et al., 2017). Parteek et al., showed that co-administration of biotin decorated polymeric nanoparticles of naringenin and gefitinib, enhanced the therapeutic efficacy and antitumor effect in lung cancer both *in vitro* and *in vivo*. The biotin-conjugated PCL-PEG NPs were synthesised using oil in water emulsion method. It showed minimal toxicity and facilitated targeted drug delivery to tumor cells. Co-administration of gefitinib and naringenin NPs promoted enhanced cell apoptosis while regulating the serum metabolites and biochemical parameters to normal levels. The co-therapy caused a remarkable increase in the pro-apoptotic proteins (BAX and caspase-9) and reduced anti-apoptotic proteins (MMP-9, P-16 and Bcl-2) (Parashar et al., 2018b). Parteek and coworkers also indicated that, entrapment of naringenin into hyaluronic acid-chitosan-PCL NPs, by utilizing layer by layer technique, is effective in targeting lung cancer cells. Cytotoxic study of the formulated naringenin on A549 cells depicted selective and enhanced anticancer effects. Increased drug uptake by cancer cells has been shown with formulated naringenin, in addition to active targeting and enhanced cytotoxic effect (Parashar et al., 2018a).

#### 4.2. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are lipid-based drug delivery systems that are produced from solid lipid matrix which are stabilized with nontoxic emulsifiers (Mehta et al., 2014; Naahidi et al., 2013). The solid lipid matrix can protect drug molecules against chemical degradation. Ji et al., demonstrated that naringenin SLNs possessed sustained drug release activity with enhanced stability and increased bioavailability upon pulmonary administration. Naringenin was incorporated into SLNs by emulsification and low-temperature solidification method using glycerol monostearate as solid lipid matrix. Naringenin-SLNs was reported to have an entrapment efficiency of 79.11% with sustained drug release properties. Furthermore, studies in A549 cells demonstrated that naringenin-SLNs were non-toxic. Upon administration of naringenin-SLNs via pulmonary instillation in rats, the findings indicated that the relative bioavailability was 2.53-fold greater compared to pure naringenin treatment (Ji et al., 2016).

## 5. Conclusion

Several studies have demonstrated the clinical potential of naringenin in regulating various biochemical pathways in airway inflammatory diseases by both preventive and therapeutic measures. Despite evidences proving that naringenin could offer a novel strategy on chronic respiratory disease, the available data on safety and efficacy in human studies remain limited due to its poor aqueous solubility and minimal bioavailability. Further studies in developing optimal drug delivery system are needed to better address the bioavailability, efficacy and safety of naringenin.

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**Figure legends:**

**Fig. 1.** The chemical structure of naringenin

**Fig. 2.** Cellular/molecular mechanisms and pathways involved in inflammation through inhibition of NF- $\kappa$ B by naringenin

**Fig. 3.** Summary of the therapeutic effects of naringenin in chronic inflammatory diseases and their (\**In-vitro*/#*In-vivo*) mechanisms

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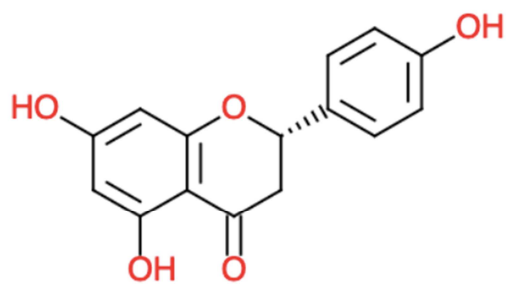
**Table 1** Summary of the therapeutic effects of naringenin in airway inflammatory diseases.

Type of disease	Study method	Study model / subject	Dose	Findings	Ref.
<b>Asthma</b>	<i>In vivo</i>	Female BALB/c mice	25, 50, or 100mg/kg	Attenuated airway inflammation and AHR.	(45)
	<i>In vivo</i>	Female BALB/c mice	50mg/kg <i>i.p.</i>	Decreased total serum Th2 and IgE cytokines in BAL fluid and slowed down the progression of airway remodelling.	(17)
	<i>In vivo</i>	Male BALB/c mice	9mg/100ml	Histopathological examination highlighted absence of goblet cells metaplasia, decreased intra-alveolar macrophages, sub-epithelial fibrosis, smooth muscle hypertrophy in airways and lung atelectasis	(49)
	<i>In vivo</i>	BALB/c mice	0.8 mg/kg <i>p.o.</i>	Attenuated AHR, eosinophilic airway inflammation and Th2 cytokine production.	(50)
	<i>In vitro</i>	HMC-1 cell line	100µM	Regulated the inflammatory responses in mast cells. Inhibited TSLP production through the inhibition of RIP2/caspase-1 as well as NF-κB pathway.	(44)
<b>COPD</b>	<i>In vivo</i>	BALB/c mice	20, 40 and 80 mg/kg	Improved the pulmonary function and attenuated the inflammation in the lung tissues. Inhibited IL-8, TNF-α, and MMP9 significantly. Decreased NF-κB p65 and IκB-α phosphorylation and improved GR level.	(53)
	<i>In vitro</i>	A549 cell line	2, 20 and 50mM	Inhibited IL-8, TNF-α and MMP9 expression. Upregulated GR expression.	(53)
	<i>In vivo</i>	Male and female Kunming mice male/female Pigeons Male and female SD rats	90mg/kg	Enhanced removal of mucus through increased airway volume secretion and reduced mucin secretion.	(59)
<b>Lung cancer</b>	<i>In vivo</i>	Male Swiss albino mice	50mg/kg	Decreased level of lipid peroxidation. Enhanced enzymatic and non-enzymatic antioxidants.	(61)

				Down-regulated NF- $\kappa$ B and reduced TNF- $\alpha$ , IL-1 $\beta$ and IL-6 expression. Down-regulated CYP1A1 and PCNA.	
	<i>In vitro</i>	COR-L23 cell line	33.4 $\mu$ M	Exhibited more potent anti-tumor activity than vinblastine.	(62)
	<i>In vitro</i>	A549 cell line	25 - 300 $\mu$ M	Reduced AKT activities Down-regulated MMP-2 and MMP-9 activities.	(19)
	<i>In vitro</i>	A549 cell line	100 $\mu$ M	Enhanced the activity of TRAIL-induced apoptosis by upregulation of DR5.	(66)
	<i>In vivo</i>	Male C57BL/6 J mice	Not available	Enhanced anti-cancer efficacy of gefitinib and inhibited lung carcinoma metastasis.	(68)
	<i>In vivo</i>	C57BL/6 mice	50mg/kg	Suppressed lung carcinoma growth through rebalancing Smad3/Smad7 signalling.	(69)
<b>Pulmonary fibrosis</b>	<i>In vivo</i>	Female C57BL/6 and female BALB/c mice	100mg/kg	Inhibited profibrotic activity by down-regulating TGF- $\beta$ 1 expression.	(20)
	<i>In vitro</i>	L929 fibroblast cell line	100 mg/kg and 200 mg/kg	Ameliorated RILI through down-regulation of IL-1 $\beta$ and maintained the homeostasis of inflammatory factors.	(74)
	<i>In vivo</i>	Female BALB/c mice and female Wistar rats			
	<i>In vitro</i>	BEAS-2B cell line	100 $\mu$ M	Inhibited IL-6, IL-1 $\beta$ , TNF- $\alpha$ and TGF- $\beta$ expression.	(76)
	<i>In vivo</i>	BALB/c mice	100mg/kg		
	<i>In vivo</i>	Male Wistar rats	100mg/kg	Suppressed COX-2 expression through down-regulation of NF- $\kappa$ B pathway. Enhanced activity of antioxidant enzymes.	(77)
	<i>In vitro</i>	BEAS-2B cell line	5 - 100 $\mu$ M	Exerted cytoprotective effect via modulation of Nrf2-mediated antioxidant gene regulation.	(78)
<b>Cystic fibrosis</b>	<i>In vitro</i>	Calu-3 cells	50 $\mu$ M and 100 $\mu$ M	Enhanced CFTR mRNA expression.	(80)

**Table 2:** Summary of studies that report the different drug delivery systems for naringenin.

<b>Nanoparticle formulation</b>	<b>Treatment</b>	<b>Experimental model</b>	<b>Outcomes</b>	<b>Ref.</b>
<b>PVP-coated nanoparticles</b>	25 µg/mL  1-50 mg/kg, <i>i.v.</i>	<i>In vitro</i> , LPS-induced RAW264.7 cell line <i>In vivo</i> , male Sprague Dawley rats	Down regulated NF-kB expression via P38MAPK signaling pathways. Inhibited iNOS and COX-2 and reduced levels of TNF-α, IL-6, MCP-1 and IL-1β. Nanoparticles were nontoxic. Nanoparticles exerted enhanced anti-inflammatory effect than pure naringenin.	(55,82)
<b>Chitosan nanoparticles</b>	1-100 µg/mL	<i>In vitro</i> , A549 cell line	Inhibited production of hydroxyl radical, nitrites and DPHH radical. Exhibited cytotoxic effect towards A549 cells only. Improved antioxidant and anticancer effects with nanoparticles.	(83)
<b>Biotin-conjugated PCL-PEG nanoparticles</b>	NA  30 mg/kg, <i>i.p.</i>	<i>In vitro</i> , A549 cell line <i>In vivo</i> , urethane-induced male and female Albino Wistar rats	Up-regulated pro-apoptotic proteins (caspase-9 & BAX) and down-regulated anti-apoptotic proteins (P-16, MMP-9 & Bcl-2). Reported minimal toxicity and facilitated targeted drug delivery. Co-therapy with gefitinib enhanced antitumor effect.	(84)
<b>Hyaluronic acid conjugated chitosan-PCL nanoparticles</b>	1.5-50µM  50mg/kg, <i>p.o.</i>	<i>In vitro</i> , A549 cell line <i>In vivo</i> , urethane-induced male Albino Wistar rats	Possessed sustained drug release and increased tumor targeting. Exhibited cytotoxic effect towards A549 cells only. Enhanced antitumor effect with modified nanoparticles.	(85)
<b>Solid lipid nanoparticles</b>	1-50 µg/mL  20 mg/kg, intratracheal instillation	<i>In vitro</i> , A549cell line <i>In vivo</i> , male Sprague Dawley rats	Possessed sustained drug release, with enhanced stability and increased bioavailability. Nanoparticles were nontoxic.	(86)



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